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EFFECT OF EXOGENOUS APPLICATION OF ZINC AND SELENIUM ON QUALITY CHARACTERISTIC FOR SUNFLOWER PLANT UNDER WATER STRESS

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Abstract

Two field experiments were conducted during the spring season of 2016 and 2017 at the experimental field of the Agricultural College, University of Diyala in order to understand the effect of zinc and selenium on physiological properties of sunflower under different water stresses. Three treatments were used including water tension as the main factor followed by Zinc and Selenium application as the secondary and sub-secondary treatments respectively. The sunflower plant was irrigated after reaching 25%, 50% and 75% of available water. Zinc was applied at three rates (0, 20, 40) mg.L^{-1} while Selenium was applied at four rates (0, 2, 4, 6) mg.L^{-1}. both zinc and selenium were sprayed on the plant three times per season. The results indicated that the concentration of zinc in the grain decreased as the soil moisture content decreased, whereas the concentration of selenium in the grains, the concentration of glutathione, the efficacy of glutathione peroxidase and proline in the leaves increased. For zinc at 40 mg.L^{-1}, the concentration of zinc and selenium in grains, concentration of glutathione, glutathione peroxidase enzyme and proline in plant leaves increased by 18.5%, 133.3%, 41.6% , 59.1% and 101.3%, respectively compared to the control (0 mg.L^{-1}). The concentration of zinc in the grain and concentration of glutathione, and efficacy of glutathione peroxidase enzyme in plant leaves were higher in the selenium treatment at 4 mg.L^{-1} compared to other treatments. While the concentration of selenium in grain and concentration of proline acid in leaves were higher in selenium treatment at 6 mg.L^{-1} compared to other treatments. The overlap treatment (W3 + 40 mgZn.L^{-1} + 4mg Se.L^{-1}) exceeded the other treatments in terms of the concentration of glutathione and the efficacy of Glutathione peroxidase enzyme in plant leaves. Moreover, the overlap treatment (W3+40 mgZn. L^{-1}+6 mgSe. L^{-1}) exceeded the other treatments in terms of selenium concentration in grains and concentration of proline acid in leaves. The increase in plant quality characteristics is due to the role of these two elements (zinc, selenium) in improving the plant's ability to absorb water and nutrients, improving the water system and increasing the ability of the plant to adapt to the low moisture content of the soil.

Key words : Zinc, selenium, moisture, glutathione, glutathione peroxidase enzyme, proline acid.

Introduction

Sun flower is one of the most important oil crops belonging to the *Asteraceae* family. It is a source of natural oil containing 25- 48% oil and 20-27% protein (Dawood *et al*., 2012). It is a highly adaptive crop which can be cultivated in a wide range of temperature, humidity, soil type and variety of farming methods. However, its cultivation in Iraq is still low in terms of area and productivity.

Water stress is one of the most serious environmental stresses affecting plant growth. It is defined as the lack of water available to the plant or the inability to absorb water naturally from the root environment due to the power of water molecules (Akinci, 2013). Water constitutes 95-85% of the live plant tissue of most plants. There are three types of water. The first type is bound water which is chemically linked with most compounds such as salts and organic acids and it is difficult to move when needed or when the plant exposures to stress. The second type is surface water which exists on the surfaces of some double-polar molecules such as proteins, oils and most sugary fats. Surface water accounts for 10-5% of the total water content in plant tissue cells, this type is rare when needed because it enters basic structures in the protoplast, such as cellular membranes. Water molecules in this type (water surface) are linked to each other by capillary properties, hydrogen bonds, and imbibtion foreces (Taiz and Zeiger, 2010). The third type is water that moves between the plant tissues when needed and be free and represents more than 50% of the total water content in the plant. This type of water depends on the cells' osmotic forces to move (Mansouri and Radhouane, 2015).

Zinc is one of the eight most important micro-nutrients in plant nutrition, as it has a significant morphological, physiological and biochemical effect on the plant. It is a constituent component of a number of enzymes including alcohol dehydrogenase, copper-zinc superoxide dismutase (Cu/Zn-SOD), carbonic anhydrase (CAH), RNA Polymerase (Gokhan *et al*., 2003). Zinc is an enzyme adjunct to aldolase, isomerase, and carboxy-peptidase (Kim *et al*., 2006). Broadly *et al*. (2006) noted that the zinc element has precise physiological functions in living systems and that it is the primary component of thousands of proteins in the plant. A large number of these proteins are needed to resist climatic stresses. Zinc is a key component in plasma membrane synthesis and helps to protect plant tissues from oxidation. Zinc is involved in many physiological functions within the plant, it contributes to the formation of Tryptophan (the origin of the plant's Auxin), which is necessary for elongation of the cells. It is also an important component of phosphorylation and glucose synthesis. When plants suffer deficiency of this element, the process of starch formation stops (Akhtar *et al*., 2009). Zinc is also actively involved in other vital functions including its role in the metabolism of nucleic acids, DNA and RNA. It also increases vitamin C and the complex vitamin B group, as well as its contribution to pollen formation and chlorophyll formation and increases plant susceptibility to absorption of several soil elements (Alloway, 2009). The lack of zinc in the crop grain causes serious problems in human nutrition, especially the nutrition of children and leads to loss of appetite, delayed growth, underdevelopment, slow healing wounds and disorders of the immune system. One of the most important physiological roles of this component in biological systems is its role in protein synthesis, about 2800 human proteins have the ability to bind to zinc. Therefore, the reduction of zinc in the plant directly decreases zinc in humans (Gibson, 2006) this has been observed in different parts of the world such as India, Pakistan, China, Iran and Turkey (Hotz and Brown, 2004). Harvest Plus (2007) reports that in Africa, there are 500-600 million people at risk due to zinc deficiency. Sillanpaa (1990) in his studies of the presence of zinc in the world reported that the taken soil samples from different parts of Iraq showed that all of them suffer from zinc deficiency to a degree there is no difference between them, and pointed out that the soil of Iraq is the lowest in terms of containing zinc from the soil of 30 countries covered by the study. He explained that agricultural crops produced in all regions of Iraq suffer a clear lack of this element. This is a major problem in the agricultural field and its implications for public health, which requires great attention.

Selenium is not commonly found in nature, Volcanic activity is its main source, So, it lacks in most of the soil of the world. It is similar to sulfur in terms of the state of oxidation, which is absorbed by the plant in the form of Selenite SeO_3^{-2} or Selenate SeO_4^{-2} (Kasar *et al*., 2011). It is classified as one of the most powerful non-enzymatic antioxidants, in addition to being an adjunct to the enzyme antioxidant system (Ilkhani *et al*., 2016). The persistent deficiency of this element in human diets leads to myocardial infarction, angina and stroke (Chen *et al*., 2013). The lack of selenium in animal diets also causes a disease called white muscle. This disease causes an imbalance in muscle growth and loss of hair or fur to animals (Al-Hidary *et al*., 2015). Selenium has a role in activating the Aquaporins system by increasing the plant's ability to absorb water. Aquaporins are waterways that contribute to water moving and other molecules through cellular membranes, this theory is considered as one of the most recent theories of transport (Afzal *et al*., 2016). Aquaporins consists of a complex amino acids system that enters Cysteine and Methionine. Theses amino acids participated in the formation of Selenocysteine and Selenomethionine when selenium is involved. Four cysteine units form a cysteine loop containing an effective thiol group, These lattices are formed in a system with other atoms to form waterways that connect the cell membrane to the water gaps water flowing through the osmosic potential. The difference in the charge with the thiol group, and the hydrogen bonds helps water movement (Soveral *et al*., 2016). Selenocysteine (fig. 1) is one of the most recently discovered amino acids. Selenocysteine becomes the 21st amino acid in which selenium replaces sulfur in this acid (Hatfield *et al*., 2016). This acid is widely used in the treatment of various types of cancer, including breast cancer, because it is highly effective in resisting oxidative stress and sold at high prices (Guo *et al*., 2015). Selenium has a unique property: it emits electrons, then compensates lost electrons from the electronic spills that leak from Reactive Oxygen Species generated by the stress of the cell and lose its destructive capacity (Masuzawa *et al*., 2013). A rare feature of selenium is the diamagnetism, it is a property that causes the creation of a field that is opposed to any other external magnetic field. Since the Reactive Oxygen Species possess a harmful magnetic torque and an electron balance destroyer, the magnetic momentum of the Reactive Oxygen Species decrease as the selenium acts to extinguish Reactive Oxygen Species (Pal *et al*., 2013).

This study was established to investigate the role of the zinc and selenium elements under water stress on growth of sunflower plant and determine the suitable concentration of these two elements in arid and semi-arid climate.

Materials and Methods

The study was conducted during the spring period of 2016 and 2017 at the experimental fields of agricultural college, University of Diyala, Diyala Province, Iraq. The soil texture is silty clay deriving from sedimentary soil which is classified as Typic Torrifluvent. Table 1 shows some of the chemical and physical properties of the soil (Page, 1982). Sunflower experiment was divided into 108 parts (1.5m × 1.5m each) in which the distance between part and another was 0.75 m. In order to prevent water leakage and fertilizer transfer between treatments, 0.5 m left fallow between treatments. The trial site was cultivated with sunflower seeds (*Helianthus annuus* L.) on the day of 10th of March 2016 and 2017. The split-split plot design was used in order of a Randomized Complete Block Design (RCBD) with a three replicates for each treatment. Three treatments were used in which water tension was the main factor followed by application of Zine and Selenium as the secondary and sub secondary treatments, respectively. The water tension treatment included three levels, irrigation after reaching 25% (W1), 50% (W2) and 75% (W3) of available water.By using Gravimetric method. Zinc treatment was added at three rates 0 mg.L^{-1} (ZnO), 20 mg.L^{-1} (Zn_2O) and 40 mg.L^{-1} (Zn_4O) by spraying on plants as Zn-EDTA (14% Zink). Selenium was applied at four rates 0 mg.L^{-1} (Se0), 2 mg.L^{-1} and 4 mg.L^{-1} L (Se4) and 6 mg.L^{-1} (Se6) by spraying on plants as $NaHseO_3$ (80% Selenium). The plants were sprayed by Zinc and Selenium three times during the plant season.

Different types of fertilizers were added to the site of cultivation including Urea (46% N), Trisuperphosphate (20% P) and Potassium sulphate (41% K) at 200 Kg N/h, 75 Kg P/h and 150 Kg K/h, respectively. Nitrogen and potassium fertilizers were added to the soil three times per season while trisuperphosphate was added to the soil once per season at the day of cultivation. The concentration of zinc and selenium in grains was estimated

Table 1 : Some of the chemical and physical characteristics of the soil before cultivation.

The characteristics	Measurement units	The values
pH 1:1	-	7.60
EC 1:1	ds.m^{-1}	2.94
Available Nitrogen	mg.Kg^{-1} soil	65.50
Available potassium	mg.Kg^{-1} soil	206.00
Available Phosphorus	mg.Kg^{-1} soil	16.50
Available zinc	mg.Kg^{-1} soil	0.65
Available Selenium	mg.Kg^{-1} soil	0.33
Gypsum	g.Kg^{-1} soil	Nil
Lime	g.Kg^{-1} soil	290
% for moisture when Available water consumption	25%	22.85
	50 %	18.70
	75%	16.00
Soil texture	Clay g.Kg$^{\times 1}$ soil	240
	Silt g.Kg$^{\times 1}$ soil	193
	Sand g.Kg$^{\times 1}$ soil	567
Texture	Silty clay	
Field capacity	%	29
Permanent wilting point	%	13
Avilable water	%	16
Bulk Density	micagram.m$^{\times 3}$	1.45

using the Atomic Absorption Spectroscopy (Haynes, 1980). Glutathione was estimated in plant leaves by using Trichloro Acetic Acid and Dithiobs-2-nitrobenzoic acid using Spectrophotometer along a 412 nm wavelength (Moron *et al*., 1979). The efficacy of Glutathione Peroxidase (GPX) in plant leaves was estimated using the Sodiumazide, Trichloroacetic acid, Dithiobs-2-nitrobenzoic acid, as described in (Flohé and Günzler, 1984) using a Spectrophotometer at a wavelength of 420 nm. The method of Bates *et al*. (1973) was used to determine the extraction proline using Aqueous Sulfosalicylic acid.

Results and Discussion

Zinc concentration in grains

The results in table 2 showed that irrigation at the level of W3 significantly reduced the average concentration of zinc in sunflower plant seeds, reaching 35.1 mg. Kg^{-1} dry matter compared to the level of irrigation W1 and W2, with an average of 43.1 and 39.2 mg. Kg^{-1} dry matter, respectively. The reduction in soil moisture content result in lower turgor pressure stiffening of cellular

Fig. 1 : shows the entry of selenium atom in stead of sulfur in Selenocysteine formation (24)

membranes and thus the inability of cells to widen and elongate (Lipiec *et al*., 2013), leading to a decrease in zinc concentration in grains. This is in line with what Mansouri and Radhouane (2015) point out that the low moisture content of the soil leads to a decrease in the accumulation of dry matter in the leaves, a decrease the soluble nutrients in the water and inhibition of their transfer to the reproductive parts. This leads to abortion of the flower contract and poor fruit growth. This property was significantly affected by zinc treatment, in which the average of this property increased with the increase of zinc treatment. The third level of spraying (Zn40) exceeded the non-spray level (Zn0) and (Zn20) of this element with a significant increase of 18.5% and 6.8% respectively. Increasing zinc concentration within the plant by increasing the rate of application is normal as the element of zinc involved in the processes of metabolism of carbohydrates through its direct impact on the efficiency of the process of photosynthesis. It has been observed that the lack of this element leads to a reduction in the net photosynthesis by 50 - 70%. This effect may resulted from a low performance efficiency of the enzyme (carbonic anhydrase), which contains zinc into its structure (Akhtar *et al*., 2009). The level of the selenium treatment Se 4 was significantly higher than the levels (Se 0), (Se 2) and (Se 6) with a significant increase of 53.4%, 23.7% and 30.7% respectively, this is in line with Jalal and Jing-Quan (2016), who indicated that the selenium component plays a role in preventing the oxidation of the growth regulator (Gibberellic acid) by GAoxidase enzyme, leading to increasing its concentration. This increased the fruit yield due to the increases in the numbers division and plant growth.

The effect of the interaction between the two elements significantly increased the concentration of zinc in the grains. The level of treatment (Zn40 + Se4) exceeded the other levels of interference with an average of 53.8 mg. Kg^{-1} dry matter. While the lowest mean was 27.4 mg. Kg^{-1}dry matter at the non-spray level (Zn0 + Se0). The zinc content in the seed is an important factor in obtaining healthy plants especially in soils that are poor in zinc element. The interaction between irrigation periods and zinc concentrations was significantly different within levels. The highest mean was 46.2 mg. kg^{-1} dry matter for Zn40 concentration + W1 treatment, while the lowest mean at the third irrigation period (W3) and non-zinc spray (Zn0), which was 32.4 mg.Kg^{-1} dry matter. This is consistent with Rashid and Rayan (2004), who indicated the plant has low zinc content in arid and semi-arid regions. The treatment of plants with selenium under the influence of different moisture levels differed in their effect on the concentration of zinc in grains, the level of interference (W1 + Se4) exceeded the other levels of interference between these two factors, recording an average of 52.8 mg. Kg^{-1} dry matter. While the lowest mean was 29.0 mg. Kg^{-1} dry matter. for interference (Se0 + W3). By comparing plants with low levels of moisture (W3) with different levels of selenium, we observe that the W3 + Se4 treatment increased compared to the W3+Se0 treatment by 54.1%. The contribution of selenium to increase the effectiveness of enzymatic antioxidants (Superoxid dismutase, Peroxides, Catalase and Glutathione peroxidase) and the ability of this element to increase the activity and vitality of non-enzymatic antioxidants (Ascorbic acid, Carotenoids, Phenolic compound, Flavonoids and Vitamen E) reduce the effect of water stress intensity by inhibiting oxidative Reactive Oxygen Species production and reducing the effectiveness of the enzymes analyzed (Chai *et al*., 2016).

When examining the effect of the three factors on zinc concentration in grains, the highest concentration of this element was observed at the interference level (W1 + Zn40 + Se4) with an average of 59.0 mg. Kg^{-1}, the lowest average was obtained at the level of non-spraying of these two elements and at the level of the third moisture (W3), reaching 25.5 mg.Kg^{-1}. This is in line with UN-Water (2015), who reported that water stress has the physiological, biological and chemical processes of the

Table 2 : Effect of zinc, selenium and moisture levels on zinc content in grains (mg.kg^{-1} dry weight) (The average two seasons).

Zn*Se	Water Stress			Selenium	Zinc
	W3	W2	W1		
27.4	25.5	26.0	30.8	Se 0	Zn 0
37.1	31.2	37.9	42.2	Se 2	
42.4	40.0	41.5	45.8	Se 4	
35.9	33.0	35.7	39.0	Se 6	
32.8	30.0	33.1	35.5	Se 0	Zn 20
39.0	32.0	39.1	46.0	Se 2	
49.5	46.0	48.9	53.6	Se 4	
36.7	33.2	37.0	40.1	Se 6	
34.8	31.7	35.9	37.0	Se 0	Zn 40
41.7	35.3	42.0	47.8	Se 2	
53.8	48.1	54.3	59.0	Se 4	
38.8	36.0	39.2	41.3	Se 6	
2.65	3.05			LSD 0.05	
Average Zn					
35.6	32.4	35.2	39.4	Zn 0	Zn * W
39.5	35.3	39.5	43.8	Zn 20	
42.2	37.7	42.8	46.2	Zn 40	
2.50	2.00			LSD 0.05	
Se Average					
31.6	29.0	31.6	34.4	Se 0	Se * W
39.2	32.8	39.6	45.3	Se 2	
48.5	44.7	48.2	52.8	Se 4	
37.1	34.0	37.3	40.1	Se 6	
2.50	1.95			LSD 0.05	
	35.1	39.2	43.1	W Average	
	2.50			LSD 0.05	

Table 3 : Effect of zinc, selenium and moisture levels on selenium content in grains (mg .kg^{-1} dry weight) (The average two seasons).

Zn*Se	Water Stress			Selenium	Zinc
	W3	W2	W1		
0.13	0.18	0. 13	0.10	Se 0	Zn 0
0.34	0.45	0.30	0.28	Se 2	
0.45	0.61	0.45	0.30	Se 4	
0.64	0.82	0.63	0.48	Se 6	
0.21	0.29	0.20	0.15	Se 0	Zn 20
0.47	0.62	0.47	0.33	Se 2	
0.68	0.87	0.75	0.44	Se 4	
1.00	1. 50	0.82	0.69	Se 6	
0.32	0.40	0.29	0.27	Se 0	Zn 40
0.66	0.82	0.65	0.51	Se 2	
1.14	1.83	0.87	0.72	Se 4	
1.53	2.15	1.40	1.05	Se 6	
0.33	0.40			LSD 0.05	
Average Zn					
0.39	0.51	0.37	0.29	Zn 0	Zn * W
0.59	0.82	0.56	0.40	Zn 20	
0.91	1.30	0.80	0.63	Zn 40	
0.17	0.10			LSD 0.05	
Average Se					
0.22	0.29	0.20	0.17	Se 0	Se * W
0.49	0.63	0.47	0.37	Se 2	
0.75	1.10	0.69	0.48	Se 4	
1.06	1.49	0.95	0.74	Se 6	
0.17	0.25			LSD 0.05	
	0.87	0.57	0.44	W Average	
	0.17			LSD 0.05	

plant, leading to the breathing problem, as the drought affects the amount of CO_2 flowing through the leaves. As a result, the leaves growth become weak and the accumulation of dry matter in the plant decrease. Moreover, the drought was negatively affected the process of photosynthesis, metabolism, flowers and nodes. Furthermore, plant under water stress resulted in reducing the level of plant hormones regulating the growth of IAA, CK and GA and the absorption of metal ions from the soil, increasing viscosity and concentration of protoplasm and reducing the number of cells.

Selenium concentration in grains

The results show a significant increase in selenium concentration at the third moisture level (W3) compared to W1 and W2 by 97.72%, 52.63% respectively, and no significant difference was observed between the first moisture level (W1) and the second moisture level (W2) (table 3). The suitable levels of moisture improve many physiological and biochemical processes during plant growth, including elongation, cell division, membrane work, nitrogen metabolism and photosynthesis. Therefore, the plant's yield and the concentration of selenium in the plant increased. Increasing the concentration of selenium in the plant with low moisture content may be due to a reaction of plants to face drought stress. The increased concentration of selenium during stress is linked to amino acids. This formation of amino acids associated with selenium with high decomposition capacity such as phenylalanine acids is involved in the representation of salicylic acid. It is also believed that selenium oxide combined with water plays a role in the metabolism of Benzoic acid in the plant when the stress is transformed into salicylic acid by the enzymatic of Hydrolase Benzoic Acid. Salicylic acid plays an important role in water stress situations (Hayat and Ahmad, 2011). Naz *et al*. (2015) also indicated that the stress drives selenium to connect with proteins containing steroids in the smooth internal endoplasmic network and protecting it against oxidation. The table 3 shows the superiority of the third level of spraying with zinc element 40 mg.L^{-1}, recording an average of 0.91 mg. Kg^{-1} while the mean non-spray was 0.39 mg.Kg^{-1}. The zinc component is an important nutrient and the deficiency in its quantity inside the plant leads to morphological and physiological changes such as changes in the membrane, changes in carbohydrate representation, cytochromes formation, nucleotide and chlorophyll, as well as reduction in zinc-containing enzymes. These negatively affected plant yield and components (Gokhan *et al*., 2013). The concentration of selenium in the grains increased with increasing spraying of this element, in which the average of this element for four application rates (Se0, Se2, Se4, Se6) was 0.22, 0.49, 0.75 and 1.06 mg. Kg^{-1} respectively. These concentrations of selenium are in line with the normal concentration of this element in the plant, as the increase of this element is stored in other parts of the plant such as root (Golob *et al*., 2016).

The two elements interface significantly increased the concentration of selenium in grains. The highest mean was 1.53 mg. Kg^{-1} at the spray level (Se6 + Zn40) and the lowest mean was 0.13 mg. Kg^{-1} at the non-spray level of these two elements (Se0 + Zn0). The same table shows significant differences at the interaction between the levels of moisture and spray with zinc. The interference treatment (W3 + Zn 40) was the highest mean 1.30 mg.Kg^{-1} while the lowest mean was 0.29 mg. Kg^{-1} at not spraying zinc and first moisture level W1. By comparing the zinc concentrations with the low levels of moisture, it is noticed that the interference level (W3 + Zn 40) exceeded an average of 1.30 mg. Kg^{-1} while the lowest average was observed at(W3 + Zn0) with an average of 0.51 mg.Kg^{-1}. This is consistent with Taiz and Zeiger (2010), who state that zinc has a significant role in protecting the plant tissue from oxidation. They continued to say that zinc exists in the formation of transcription factors that responsible for the production of proteins in the translation, transcription process and the formation of mRNA. In terms of the overlap between the levels of moisture and selenium treatments, the highest mean of selenium concentration was 1.49 mg. Kg^{-1} at the level of interference (W3 + Se 6) while the lowest mean was 0.17 mg.Kg^{-1} at level of interference (W1 + Se0). The irrigation spacing increased the concentration of selenium in the grains beyond the interference level (W3 + Se 6) recording an average of 1.49 mg.Kg^{-1} while the lowest was 0.29 mg.Kg^{-1} at level (W3 + Se 0), Selenium is associated with amino acids to form Seleno-amino acid compounds to protect them from destruction, especially Glycine. It also enter into the synthesis of Glycine betaine, which increases the root-cell osmotic as it moves with water and nutrients to the root cells of the plant. Therefore, this leads to an increase in the solubility of the dry matter and the smooth flow of it into the growth fruit areas (Manaf, 2016).

The triple interference (Zn × Se × W) significantly influenced the selenium concentration in the grains. The highest mean of selenium concentration in the grain was 2.15 mg.Kg^{-1} for W 3+Se 6 + Zn 40 treatment, while the lowest mean was 0.10 mg.Kg^{-1} for W1 + Se0 + Ze0 treatment. The addition of nutrients in suitable quantities to the plant increases the plant's adaptation to drought conditions and lack of water.

Glutathione concentration in plant leaves

The soil moisture content significantly influenced the concentration of Glutathione in plant leaves, which was significantly higher in the third level of irrigation (W3) compared to W1 and W2 by 30.3% and 10.8% respectively (table 4). the increased concentration of Glutathione, which is from non-enzymatic antioxidants with low moisture content of soil, due to increased production and accumulation of Reactive Oxygen Species. Glutathione is one of the methods used by the plant to form types of short-chain proteins antioxidant, this process is called Glutathionylation of Proteins (Gupta *et al*., 2015). The results in table 4 show a significant increase in the average concentration of glutathione as the application rate of zinc increases. The concentration of Glutathione increased from 61.5μM.gm^{-1} to 87.1 μM.gm^{-1} when the application rate of zinc increased from zero to 40 mg.L^{-1}. Zinc is a non-enzymatic antioxidant which is an adjunct factor to the enzyme Superoxide Dismutase, which exists in the cytoplasm (Cu / Zn SOD), and plays an important role in the removal of the effective Reactive Oxygen Species (Gupta *et al*., 2015). Spraying with Selenium resulted in a significant effect on the concentration of Glutathione in plant leaves (table 4). The application rate Se4 was significantly higher than Se0, Se2 and Se6 by 54.20, 24.90% and 39.73%, respectively. Selenium is classified as one of the most powerful non-enzymatic antioxidants and its entry into the structure of glutathione leads to inhibition of oxidative enzymes such as NADPH oxidase, FAD Protein oxidase and Iron oxidase and loses its destructive ability of cells (Drahoòovský *et al*., 2016).

The interaction between selenium and zinc significantly affected the concentration of Glutathione in plant leaves. The application rate (Zn 40 + Se 4) was significantly higher than other interactions, recording an average of 111.4 $\mu M.gm^{-1}$ of Glutathione, while the lowest was 52.2 $\mu M.gm^{-1}$ at application rate (Zn0 + Se 0). Gupta *et al*. (2015), Cakmak *et al*. (2010) noted that the selenium and zinc are important non-enzymatic antioxidants, these elements enter the structural structure of many important antioxidant enzymes to remove the harmful effect of stress in cells. Interaction between zinc spraying levels and moisture levels significantly affected the concentration of Glutathione in plant leaves. The (W3 + Zn 40) treatment recorded the highest mean (97.9$\mu M.gm^{-1}$) of this interference, while the lowest average was 52.9$\mu M.gm^{-1}$ at interference (W1 + Zn0). When comparing the levels of zinc application rate with the low levels of moisture (W3), it is noticed that the interference level (W3 + Zn 40) exceeded the level of (W3+Zn0) and (W3+Zn20) with a significant increase by 41.0% 12, 7 respectively. For the interference between the application rate of soil moisture content and selenium concentration, the highest mean of Glutathione in plant leaves was observed at (W3 + Se 4) treatment, recording 112.8 $\mu M.gm^{-1}$. While the lowest mean was observed at interference (W1 +Se0), recording 55.0 $\mu M.gm^{-1}$. By comparing the levels of selenium spraying with the low level of moisture (W3), the interference (W3 + Se 4) was significantly higher than (W3 + Se 0), (W3+Se2) and (W3 +Se 6) by 73.53%, 29.80% and 52.02% respectively. Selenium is highly capable of controlling the magnetic force of the Reactive Oxygen Species generated during stresses, leading to the removal and disposal of these groups (Pal *et al*., 2013).

In the study, the highest concentration of Glutathione in plant leaves was observed at (W3+ Se4 +Zn40) with an average of 136.2 $\mu M.gm^{-1}$, while the lowest concentration was observed at (W1 +Se0+ Zn0) with an average 43.6 $\mu M.gm^{-1}$. Glutathione is a source of amino acids when necessary, especially when stress occurs, resulting in the breakdown of important amino acids such as Cysteine and Glycine (Mansouri and Radhouane, 2015). Dawood (2016) noted that the increased concentration of polyamines such as Glutathione in the plant is a contribution to osmotic modification to preserve cells from the effect of loss of the water of the plasm Plasmolysis by increasing its osmotic in favor of water entering it.

Total efficacy of Glutathione peroxidase

There was a significant increase in the overall efficacy of Glutathione peroxidase with lower moisture content of the soil (table 5). The irrigation rate (W3) was significantly higher than W1 and W2 by 45.5% and 15.5%. The effectiveness increase of this enzyme resulted from Reactive Oxygen Species activity under the influence of water stress. The results of table 5 showed that the overall efficacy of Glutathione peroxidase at Zn40 treatment was significantly higher than Zn0 and Zn 20 treatments 59.1% and 19.0% respectively. Zinc plays an important role in many physiological and biochemical interactions, particularly enzyme activity and action (Kim *et al*., 2006). The overall efficacy of Glutathione peroxidase increased significantly by increasing plant treatment with selenium. When the concentration of selenium increased from 0 $mg.L^{-1}$ to 4 $mg.L^{-1}$ the total efficacy of Glutathione peroxidase increased from 44.1 $U.mL^{-1}$ to 92.8 $U.mL^{-1}$. However, the mean effectiveness of this enzyme was observed to decrease as the application rate of selenium increased. The reduction in this characteristic started at Se6 treatment, recording 61.2 $U.mL^{-1}$ as an average. Selenium is used the synthesis of the Glutathione in this enzyme instead of the sulfur therefor, as a result, this enzyme becomes more efficient in protecting the plant and prolonging the age of the cells and maintaining its aging under the influence of water stress (Al-Helaly, 2013).

There overlap between the concentration of zinc and selenium significantly increased of the total activity of the enzyme Glutathione peroxidase. The application rate (Se 4 + Zn 40) was the highest, reaching 117.0 $U.mL^{-1}$, and the lowest was 31.9 $U.mL^{-1}$ for the level of non-spraying of these two elements. This result is in corresponding to what is found in table 4 of the increased concentration of Glutathione, which enters the structure of the enzyme at the same level of addition (Se4 + Zn40). Glutathione works to remove stress through its association with oxidizing molecules and then binds to the outer surface enzymes of Glutathione and its role in the stimulation of defense signals of the plant (Rouhier *et al*., 2008). For zinc and water interface, results indicated that the highest mean of this the total efficacy of this

Table 4 : Effect of zinc, selenium and moisture levels on Glutathione content in leaves (ìM.g^{-1} fresh weight) (The average two seasons).

Zn*Se	Water Stress			Selenium	Zinc
	W3	W2	W1		
52.2	59.2	54.0	43.6	Se 0	Zn 0
60.0	67.5	60.1	52.5	Se 2	
77.4	89.5	77.0	65.7	Se 4	
56.5	61.5	58.0	50.1	Se 6	
62.9	65.4	66.9	56.5	Se 0	Zn 20
78.5	89.7	79.0	67.0	Se 2	
97.2	112.8	98.6	80.2	Se 4	
71.5	79.6	71.6	63.3	Se 6	
70.3	70.6	75.6	64.9	Se 0	Zn 40
90.4	103.4	90.0	78.0	Se 2	
111.4	136.2	109.5	88.5	Se 4	
76.6	81.5	78.0	70.4	Se 6	
4.0	4.8			LSD 0.05	
Average Zn					
61.5	69.4	62.2	52.9	Zn 0	Zn * W
77.5	86.8	79.0	66.7	Zn 20	
87.1	97.9	88.2	75.4	Zn 40	
3.5	3.1			LSD 0.05	
Average Se					
61.8	65.0	65.5	55.0	Se 0	Se * W
76.3	86.9	76.3	65.8	Se 2	
95.3	112.8	95.0	78.1	Se 4	
68.2	74.2	69.2	61.3	Se 6	
3.5	0.15			LSD 0.05	
	84.7	76.4	65.0	W Average	
	3.5			LSD 0.05	

Table 5 : Effect of zinc, selenium and moisture levels on Glutathione peroxidase enzyme (U.mL^{-1}) (The average two seasons).

Zn*Se	Water Stress			Selenium	Zinc
	W3	W2	W1		
31.9	36.5	33.7	25.5	Se 0	Zn 0
55.7	65.0	57.5	44.5	Se 2	
67.6	85.5	68.3	49.0	Se 4	
47.6	61.5	46.5	35.0	Se 6	
45.1	52.1	46.0	37.2	Se 0	Zn 20
68.8	77.3	70.1	59.0	Se 2	
94.1	112.0	92.0	78.4	Se 4	
63.5	73.5	61.2	55.9	Se 6	
55.4	64.2	60.5	41.5	Se 0	Zn 40
78.0	88.5	82.4	63.3	Se 2	
117.0	136.9	119.5	94.6	Se 4	
72.7	84.2	73.4	60.5	Se 6	
5.0	7.5			LSD 0.05	
Average Zn					
50.7	62.1	51.5	38.5	Zn 0	Zn * W
67.8	78.7	67.3	57.6	Zn 20	
80.7	93.4	83.9	64.9	Zn 40	
4.0	4.5			LSD 0.05	
Average Se					
44.1	50.9	46.7	34.7	Se 0	Se * W
67.5	76.9	70.0	55.6	Se 2	
92.8	111.4	93.2	74.0	Se 4	
61.2	73.0	60.3	50.4	Se 6	
4.0	5.1			LSD 0.05	
	78.0	67.5	53.6	W Average	
	4.0			LSD 0.05	

enzyme was observed at (W3 + Zn40), recording 93.4 U.mL^{-1} and the lowest mean was 38.5 U.mL^{-1} at (W1 + Zn0). The negative effect of water tension decreased due to the increase in zinc concentration. the application rate of these two factors (W3 + Zn40) was significantly higher than (W3 + Zn0) and (W3 + Zn20) by 50.4% and 18.6%, respectively. This element is necessary in plant nutrition, increasing plant's cell ability to resist oxidation and increasing the susceptibility of the roots to water stress, and its contribution to raising the efficiency of Plasma lemma membrane functions in root cells (Gupta *et al*., 2015). For the effect of the interaction between moisture and selenium, the treatment (W3 + Se 4) gave the highest average (111.4 U.mL^{-1}), while the treatment (W1 + Se0) gave the lowest average (34.7 U.mL^{-1}). The application rate (W3 + Se4) was significantly higher than other rates (W3 + Se0), (W3 + Se2) and (W3 + Se6) by 118.8%, 44.9% and 52.6% respectively. Pal *et al*. (2013) state that the presence of the diamagnetism phenomenon, which is opposite to the selenium act, this phenomenon reduces the magnetic determination of the Reactive Oxygen Species. This phenomenon arise when the plants expose to stresses, then become stabile and loss of the destructive magnetic effect of the cells, which helps the system of antioxidant enzymatic to reduce the damage impact of Reactive Oxygen Species. In the concentration of the enzyme.

The interference between three factors significantly affected the Glutathione peroxidase value. The concentration of (Se4 + Zn40) at the level of the third moisture (W3) was the highest, recording 136.9 $U.mL^{-1}$ while the level of non-spraying of these two elements at the level of the first moisture (W1) was the lowest average (25.5 $U.mL^{-1}$). By comparing the spraying of the two factors with low levels of moisture, the interference of (W3 + Se4 + Zn40) was significantly higher than (W3 + Se0 + Zn0) with an average of 136.9 $U.mL^{-1}$ and 36.5 $U.mL^{-1}$, respectively. In summary, the total activity of the Glutathione peroxidase enzyme increased with zinc and selenium application. This enzyme is one of the most important antioxidant enzymes that acts as an antioxidant for unsaturated fats, this enzyme is often found in the mitochondria (Dias *et al*., 2016).

Concentration of proline in plant leaves

Table 6 shows a significant increase in the concentration of proline in the plant leaves of the sun flower plant, with the low moisture of soil, W3 exceeds the level of the first moisture W1 and the second W2. The quantitative accumulation of amino acids, especially proline, is a means of defense used by the plant to face water shortage. This positive role of proline in regulating the plant's osmotic potential and increasing the ability of the cells to withdraw water and nutrients from the growth environment results in maintaining elongation of cells and open the gaps and the process of photosynthesis and thereby maintaining plant growth under water shortage (Sinay *et al*., 2015). The concentration of proline in the leave significantly differed among zinc treatment, in which the highest concentration was achieved by Zn40 treatment 8.80 $mg.g^{-1}$ while the lowest concentration was achieved by Zn0 (4.37 $mg.g^{-1}$). Zinc has a significant role in metabolic reactions of proteins and its involvement in the synthesis and activation of more than 300 enzymes, especially those related to the production of nucleic acids in the cell and the metabolism of protein (Kim *et al*., 2016). The concentration of proline in the plant leaves was significantly increased by the addition of selenium. The fourth application rate of selenium had the highest concentration 8.74 $mg.g^{-1}$, while the first, second and third application rates had an average of 4.17, 5.80 and 7.01 $mg.g^{-1}$, respectively. The properties of selenium photovoltaic properties associated with water stress have a significant role in the electron transport system and increase the efficiency of Ribulose-1,5-bisphosphate carboxylase enzyme (Jalal and Jing Quan, 2016). Selenium also plays a role in inhibition of photorespiration, resulting in producing Reactive Oxygen Species and the accumulation of glycolyte.

Table 6 : Effect of zinc, selenium and moisture levels on proline acid concentration in leaves ($mg.g^{-1}$ fresh weight) (The average two seasons).

Zn*Se	Water Stress			Selenium	Zinc
	W3	W2	W1		
3.19	4.33	3.09	2.16	Se 0	Zn 0
2.87	5.04	4.27	2.30	Se 2	
4.69	5.90	4.78	3.41	Se 4	
5.75	7.50	5.85	3.90	Se 6	
4.22	5.50	4.95	2.22	Se 0	Zn 20
5.67	7.18	5.80	4.05	Se 2	
6.59	8.40	6.62	4.75	Se 4	
7.84	9.55	8.90	5.08	Se 6	
5.12	6.89	5.44	3.05	Se 0	Zn 40
7.88	10.95	7.17	5.52	Se 2	
9.75	12.05	11.32	5.90	Se 4	
12.65	16.85	4.551	6.55	Se 6	
1.9	2.5			LSD 0.05	
Average Zn					
4.37	5.69	4.49	2.94	Zn 0	Zn * W
6.07	7.65	6.56	4.02	Zn 20	
8.80	11.68	9.62	5.25	Zn 40	
1.3	1.5			LSD 0.05	
Average Se					
4.17	5.57	4.49	2.47	Se 0	Se * W
5.80	7.72	5.74	3.95	Se 2	
7.01	8.78	7.57	4.68	Se 4	
8.47	11.3	9.76	5.17	Se 6	
1.3	1.5			LSD 0.05	
	8.34	6.89	4.07	W Average	
	1.3			LSD 0.05	

There was a significant overlap between zinc concentration and selenium concentration. The highest concentration of proline in the plant leaves was observed at treatment (Se6 + Zn40) with an average 12.65 $mg.g^{-1}$, while the lowest value was 3.19 $mg.g^{-1}$ of non-spraying of these two elements. The same table showed a significant overlap between the levels of moisture and levels of zinc, in which the highest mean was 11.68 mg.

g^{-1} at treatment (W3 + Zn40) and the lowest mean was 2.94 mg.g^{-1} at treatment (W1 + Zn0). The value of interference (W3 + Zn40) was significantly higher than (W3 + Zn0) and (W3 + Zn20) by 105.27% and 52.67%, respectively. Zinc was reported to enter the structure of cellular membranes and participate in a number of its functions, and contribute to protecting the cell from the harmful effects of reactive oxygen species (Broadly *et al*., 2006). The combination of two factors (soil moisture content and selenium) had different values of proline according to the level of application. The treatment (W3+ Se6) had the highest value compared to the rest of the treatments with an average of 11.30 mg.g^{-1}, while the treatment (W1 + Se0) had the lowest value with an average 2.94 mg.g^{-1}. One of the important functions of selenium is that it protects the enzymatic system Nitrogen fixation (Nitrogenase and Nitrate reductase) as an enzyme adjunct factor, leading to increases the amino acids in the plant (Hernández-Castro *et al*., 2015). The level of interference (W3 + Se6) was significantly higher than the level of interference (W3 + Se0) 102.87%. This indicates that the amount of proline in the plant leaves increases as the selenium application rate increases at a low soil moisture content, Ashihara *et al*. (2011) notes that selenium enters as enzymatic facilities to represent proline when stressed by the pyrroline-5-Carboxylase enzyme.

The tripe overlap between zinc plus selenium and W3 (Se6 + Zn40 + W3) was observed to have the highest value of proline 16.85 mg.g^{-1} while the lowest value of this property 2.16 mg.gm^{-1} was observed at zero concentration of these two elements and W1 (Se0 + Zn0 + W1). This correlation indicates that zinc and selenium play a significant role in increasing proline when the sunflower plant exposes to water tension due to the accumulation of amino acid Glutamic acid, which is the main source of proline in the plant (Manaf, 2016).

What can be concluded from this research is reconsidering the role of selenium as it is no longer included in plant nutrition elements in all existing local references and working to highlight its importance, especially with the stress situations. It had a clear role in the system of Enzymatic Antioxidants and Non-Enzymatic Antioxidant. Researchers should work on the development of Long-term experiments to determine the suitable concentrations of this element because the boundary between the increase and the deficiency of this element is very small. It is very important to add zinc element to the plant because the Iraqi soil is very poor of this element, which is included in the structure of many enzymes, especially antioxidant enzymes. In addition, to the lack of zinc has a direct impact on the general human health.

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