Plant Archives Volume 20 No. 1, 2020 pp. 2319-2326 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

THE EFFECT OF GLUTATHIONE AND ZINC OXIDE NANOPARTICLES APPLICATION AND THEIR INTERACTION ON SOME OF THE PHYSIOLOGICAL CHARACTERISTICS OF *VICIA FABA* L. EXPOSED TO SALINITY STRESS

Sahar F. Mahadi and Wifak A. Al-Kaisy*

Department of Biology, Collage of Education of Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

Abstract

The field experiment was conducted in the Botanical Garden of the Department of Biology at the College of Pure Sciences Education (Ibn Al-Haitham) and during the winter agricultural season 2019-2020 to study the effect of glutathione at the concentrations 0, 50,100 $mg.L^{-1}$, zinc oxide nanoparticles at the concentrations 0, 500, 1000 $mg.L^{-1}$ and their interactions on some physiological characteristics of the faba bean plant that exposed to different concentrations of NaCl 0,5,10,15 $dS.m^{-1}$.

The results showed a significant increase in the leaf content of vitamin C, proline and vitamin E when treated with NaCl, particularly the salinity concentration 15 $dS.m^{-1}$, concentration of glutathione 100 $mg.L^{-1}$ and 1000 $mg.L^{-1}$ of zinc oxide nanoparticles, while there was a significant decrease in the content of the leaves from chlorophyll at treated the plant with different concentrations of NaCl and particularly the salinity concentration 15 $dS.m^{-1}$, and there was a significant increase in the mean of this characteristic when treated with glutathione at concentration 50 $mg.L^{-1}$ and zinc oxide nanoparticles at concentration 100 $mg.L^{-1}$.

Key word s: *Vicia faba* L.; glutathione; zinc oxide nanoparticles; physiological characteristics.

Introduction

The faba bean plant is a winter legumes with nutritional value, represented by protein materials and essential elements nutrients to the body of the organism and abundant cultivation of faba bean in the countries of the Middle East and Ethiopia (Jensen *et al.*, 2010). The faba bean are nitrogen-fixative crops in the atmosphere but are very sensitive to certain diseases such as root rot or fungi, and the faba bean have a role in providing the body with the essential elements and energy necessary for the organism to carry its vitality (Clerc, 2013).

The stress to which the plant is exposed affects its biological and physiological functions, particularly salinity stress, which increases the accumulation of sodium and potassium ions in plant tissue cells, which negatively affects the vitality of the plant and its efficiency in the process of photosynthesis, gene expression and signal transmission (Ashraf *et al.*, 2015).

The use of leaf application for the faba bean by glutathione has a stimulating effect on plant cell growth and reduces the harmful effect of salinity in plant tissue cells because it is an antioxidant and consists of three amino acids, acting as a snip toe of the free roots resulting from stress, which provides protection for living cells, in particular, erythrocytes, the protection of cellular membranes and the assistance in the production of internal plant hormones that affect the process of cellular division and differentiation, the most important of which is auxin and kinetin hormones (Wu *et al.*, 2011).

Zinc oxide nanoparticles is an inorganic chemical compound with multiple functions of living organisms, as the small size of the zinc oxide nanoparticles molecule groups the rest of the atoms around the nanoparticle,

***Author for correspondence :** E-mail: estabraq_alqaissi@yahoo.com

increasing their effectiveness and physical properties (Zheng *et al.*, 2003). Zinc oxide nanoparticles has a role to play in increasing the rate of plant height and seed weight and increasing plant content from growth-regulating hormones (Jamali *et al.*, 2011).

Materials and Methods

The soil was prepared during the agricultural season 2019-2020 for cultivation in the Botanical Garden in Department of Biology, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, where the experiment was designed in the manner of Randomized Complete Block Design (R.C.B.D) and with three repeats, each of which contained 36 experimental units, each unit contained four lines of agriculture and each line included three holes the distance between the lines 25 cm, while the distance between hole and another was 20 cm and the seeds of the faba bean were cultivated on 15-10-2019 and the first cut was taken on 25-12-2019 and the characteristics of the following physiological growth were measured:

Chlorophyll Content (SPAD)

SPAD was used to measure the content of chlorophyll leaves by placing the broad leaf part under the arm of three random plants from each experimental unit and then calculated the mean.

Viamin C content in leaves (100 mg.gm^{-1} soft weight)

Take 1 gm soft vegetable leaves, placed in a glass counter, added 10 ml of oxalic acid solution and left for a whole day after the samples were filtered and the suspension took.

Used solutions

A: Ammonium molybdate, 5 gm of ammonium molybdate solution was weighted, then dissolved in 10 ml of distilled water.

B: Oxalic acid solution (0.05 M), the weight required for measurement was determined and in the same way the weight required for measurement was determined from EDTA (0.02 M), the two dissolved were mixed together and the volume was completed to 100 ml of distilled water.

C: Sulfuric acid solution, as 5 ml of this solution has been withdrawn and completed to 100 ml of distilled water.

D: A solution consisting of metaphosphoric acid+acetic acid, 5 gm of metaphosphoric acid solution and dissolved in 100 ml of distilled water and taken 30 ml of it and then mixed with 80 ml acetic acid solution and then completed to 50 ml of distilled water.

Methods

25 ml of leaker was withdrawn and mixed with 2.5 ml of B solution and added 0.5 ml of D solution, 1 ml of C solution and 2 ml of A solution, then completed the volume to 25 ml and read the absorption of samples by spectrophotometer at a wavelength of 670 nm, after that the measuring curve was prepared with 0.1 gm of ascorbic acid and dissolved in 100 ml of oxalic acid, then withdrawn 0.5, 1, 2, 3, 4, 4.5 ml of this curve to which the following concentrations were added 0.5, 1, 2, 3, 4, 4.5 ml of oxalic acid (0.05 M) and then placed in a conical glass 0.5 ml of solution D, 1 ml of solution C and 2 ml of solution A and completed volume to 25 ml with distilled water, after that read absorption at a wavelength of 760 nm, then the relationship between ascorbic acid concentration and the absorption values of samples was determined according to the Hussain *et al.* (2010) method:

1. **Proline content in leaves (mg.gm^{-1} soft weight):** 0.5 g of plant leaves were weighed then crushed and dissolved in 10 ml of salicylic acid, after that the samples were separated by centrifuge, 2 ml of leaker was taken and 2 ml of glacial acetic acid and 2 ml of ninhydrin solution, which preparer 1 gm of ninhydrin with 30 ml of glacial acetic acid and 20 ml of 6M phosphoric acid.
2. The tubes were placed in a hot water bath for 60 minutes until the appearance of red color then the tubes was cooled and put in each tube 4 ml of toluene solution, then pulled 3 ml of colored red layer from each tube and measured by absorption device spectrophotometer and at wavelength 520 nm by Bates *et al.* (1973) method.
3. **Vitamin E content (α-Tocophyrol) in plant leaves (mg.gm^{-1}):** A certain weight of plant leaves was crashed 1 gm and added 50 ml of sulphuric acid and left for 12 hours with a good mixture, then filtered mixture and put the leaker in test tubes and separated by centrifuge and then taken 0.2 2.2-dipyridyl and 0.6 ml of iron trichloride were added to the leaker, after optical density of the samples was measured at a wavelength of 460 nm and left the mixture until orange colored appeared, then the optical density of the leaker was read, but at a wavelength of 520 nm, the content of vitamin E in the samples was calculated according to the following equation (Rosenberg, 1992):

Vitamine E (α-Tocophyrol)= Sample (D520-D460)×0.29×0.15/D520(Standard)

Statistical analysis

The results were analyzed by SAS (SAS, 2012) to

compare the mean of all treatments with the lowest significant difference and a probability ratio of 0.05.

Results and Discussion

The results of table 1 indicated a significant decrease in the mean characteristic of chlorophyll content when treated with different concentrations of NaCl, particularly the salinity concentration 15 $dS.m^{-1}$, which gave the lowest mean of 46.89 SPAD and a decrease of 10.77% compared to the control treatment of 52.55 SPAD. This is due to the effect of salinity in the photosynthesis process through the accumulation of ions in the cytoplasm of plant cells, which hinders the process of the plant's absorption to other nutrient ions, including Mg^{+2}, which is involved in the synthesis of chlorophyll molecule, which affects the leaf content of chlorophyll (Zhani *et al.*, 2012).

This in agreement with Abdul Qados (2010) study on the faba bean plant. The table also indicated a significant increase in the mean of this characteristic when treated with glutathione 50 $mg.L^{-1}$, which gave the highest mean of 52.07 SPAD and an increase rate of 14.54% compared to the control treatment of 45.46 SPAD because of the glutathione is an antioxidant and it has a role in removing the harmful effect of free radicals and a co-enzyme factor in many interactions and works to remove many harmful substances to plant cells, which clearly contributes to raising the efficiency and rate of photosynthesis in the plant as well as increasing the amount of plant content of chlorophyll (Wang *et al.*, 2014). These results in agreement with Al-Hayani (2015) study on the mung bean plant. The results of the table showed a significant increase in the mean leaf content of chlorophyll in the treatment with zinc oxide nanoparticles, particularly concentration of 1000 $mg.L^{-1}$, which gave the highest mean of 50.38 SPAD and an increase rate of 5.99% compared to the control treatment of 47.53 SPAD.

This is due to the role of zinc in stimulating many interactions within the plant, including photoreactions, which increase the efficiency of the plant and its synthesis of chlorophyll molecule, in addition to its role in building plant growth regulators that stimulate plant growth and increase its efficiency, thereby increasing the content of green matter that represented by chlorophyll. The presence of zinc oxide nanoparticles stimulates the plant's absorption of other nutrients important for its growth, such as magnesium, which plays a role in the construction of the chlorophyll molecule (Yoon *et al.*, 2014). These results in agreement with Carcia-Lopez *et al.* (2014) study on the pepper plant. The table also showed a significant effect on the interaction between NaCl and glutathione, the highest concentration of this characteristic was 54.43 SPAD at the salinity concentration zero $dS.m^{-1}$ and the concentration 50 $mg.L^{-1}$ of glutathione compared to the lowest concentration of 41.11 SPAD at the salinity concentration 15 $dS.m^{-1}$ and the concentration zero $mg.L^{-1}$ of glutathione.

The table also indicated a significant

Table 1: The effect of glutathione, zinc oxide nanoparticles application and their interaction on the leaves content of chlorophyll (SPAD) in the faba bean plant exposed to salinity stress.

NaCl ($dS.m^{-1}$)	Glutathione ($mg.L^{-1}$)	ZnO nanoparticles ($mg.L^{-1}$)			Glutathione × NaCl
		0	500	1000	
0	0	47.3	54.93	56.33	52.86
	50	52.67	49.13	61.50	54.43
	100	60.31	48.20	42.77	50.37
5	0	40.50	46.43	47.87	44.93
	50	45.97	49.50	49.77	48.41
	100	52.10	50.20	50.23	50.84
10	0	33.30	46.27	49.20	42.92
	50	49.97	55.03	52.03	52.34
	100	48.80	45.87	52.67	49.11
15	0	35.63	42.50	45.20	41.11
	50	53.83	53.53	51.93	53.10
	100	50.10	44.23	45.07	46.47
Mean ZnO nanoparticles		47.53	48.82	50.38	3.31
L.S.D (0.05) ZnO nanoparticles		1.66			
L.S.D (0.05) Dual interaction		5.73			

ZnO nanoparticles×NaCl

NaCl	ZnO nanoparticles ($mg.L^{-1}$)			Mean of NaCl
	0	500	1000	
0	53.37	50.76	53.53	52.55
5	46.19	48.71	49.29	48.06
10	44.02	49.06	51.30	48.13
15	46.52	46.76	47.40	46.89
L.S.D (0.05)	3.31			1.91

Glutathione × NaCl

Glutathione	ZnO nanoparticles ($mg.L^{-1}$)			Mean of Glutathione
	0	500	1000	
0	39.18	47.53	49.65	45.46
50	50.61	51.80	53.81	52.07
100	52.78	47.13	47.68	49.20
L.S.D (0.05)	2.87			1.65

Table 2: The effect of glutathione, zinc oxide nanoparticles application and their interaction on the leaves content of vitamin C (mg.100gm^{-1} soft weight) in the faba bean plant exposed to salinity stress.

NaCl (dS.m^{-1})	Glutathione (mg.L^{-1})	ZnO nanoparticles (mg.L^{-1})			Glutathione × NaCl
		0	500	1000	
0	0	94.33	79.67	67.67	80.56
	50	96.00	87.00	76.00	86.33
	100	63.00	75.33	71.33	69.89
5	0	87.33	71.67	81.00	80.00
	50	87.00	74.00	91.67	84.22
	100	86.33	83.33	90.67	86.78
10	0	72.67	72.00	66.67	70.44
	50	66.67	71.67	77.33	71.89
	100	83.33	138.67	124.67	115.56
15	0	82.33	8.33	84.67	85.11
	50	96.33	76.00	88.33	86.89
	100	77.67	107.33	114.00	99.67
Mean ZnO nanoparticles		82.75	85.42	86.17	4.27
L.S.D (0.05) ZnO nanoparticles		2.14			
L.S.D (0.05) Dual interaction		7.41			

ZnO nanoparticles×NaCl				
NaCl	ZnO nanoparticles (mg.L^{-1})			Mean of NaCl
	0	500	1000	
0	84.44	80.67	71.67	78.93
5	86.89	76.33	87.78	83.67
10	74.22	94.11	89.56	85.96
15	85.44	90.56	95.67	90.56
L.S.D (0.05)	4.27			2.47

Glutathione × NaCl				
Glutathione	ZnO nanoparticles (mg.L^{-1})			Mean of Glutathione
	0	500	1000	
0	84.17	77.92	75.00	89.03
50	86.50	77.17	83.33	82.33
100	77.58	101.17	100.17	92.97
L.S.D (0.05)	3.70			2.14

effect of interaction between NaCl and ZnO nanoparticles and the highest interaction at the salinity concentration zero dS.m^{-1} and the concentration 1000 mg.L^{-1} from ZnO nanoparticles, compared to the lowest interaction of 44.02 SPAD at salinity concentration 10 dS.m-1 and the concentration zero mg.L-1 of ZnO nanoparticles. The table also showed a significant effect of the interaction between the glutathione and ZnO nanoparticles, as the concentration 50 mg.L^{-1} of glutathione and te concentration 1000 mg.L^{-1} of ZnO nanoparticles recorded the highest mean of 53.81 SPAD compared to the lowest mean of 39.18 SPAD at the concentration zero mg.L^{-1} of both glutathione and zinc oxide nanoparticles.

The results of the table also showed a significant effect of the interaction between the three experiment factors in the mean of this characteristic, as the salinity concentration zero dS.m^{-1} and the concentrations 50 mg.L^{-1} of glutathione and 1000 mg.L^{-1} of ZnO nanoparticles giving the highest mean of this characteristic was 61.50 SPAD compared to the lowest mean of 33.30 SPAD at salinity concentration 10 dS.m^{-1} and the concentration zero mg.L^{-1} of both glutathione and zinc oxide nanoparticles, this demonstrates the negative effect of salinity in the plant's chlorophyll content.

The results of table 2 also indicated a significant increase in the mean content of vitamin C in the leaves when treated with different concentrations of NaCl, particularly the saline concentration of 15 dS.m^{-1}, which gave the highest mean of 90.56 mg.100gm^{-1} soft weight and an increase rate of 14.73% compared to the control treatment of 78.93 mg.100gm^{-1} soft weight. The presence of salinity ions increases the plant's exposure to stress and exposure to reactive oxygen species, including OH^- and H_2O_2, which stimulates the efficiency of the plant's defensive mechanism to overcome the harmful effect of salinity on cellular components, increasing the plant's production of non-enzymatic antioxidants such as vitamin C (Salama and Al-Mutawa, 2009).

The results of the table also indicated a significant increase in the treatment of glutathione, particularly concentration of 50 mg.L^{-1}, which gave the highest value of 92.17 mg.100gm^{-1} soft weight and an increase rate of 17.63% compared to the control treatment of 79.03 mg.100gm^{-1} soft weight due to the role of glutathione being an antioxidant, sniping free radicals and antioxidant (Noctor *et al.*, 2012), and this increases the internal content of the plant from vitamin C. The results of the table also showed a significant increase in the level of vitamin C in the leaves when the plant was treated with zinc oxide nanoparticles, particularly concentration of 1000 mg.L^{-1}, which gave the highest mean of 86.17 mg.100 gm^{-1} soft weight and a percentage increase of 4.13% compared to the control treatment of 82.75 mg.100gm^{-1} soft weight.

The presence of zinc element increases the plant's absorption of other nutrients, including nitrogen and

Table 3: The effect of glutathione, zinc oxide nanoparticles application and their interaction on the leaves content of proline (ìg.gm^{-1} soft weight) in the faba bean plant exposed to salinity stress.

NaCl (dS.m^{-1})	Glutathione (mg.L^{-1})	ZnO nanoparticles (mg.L^{-1})			Glutathione × NaCl
		0	500	1000	
0	0	40.43	65.89	70.38	58.90
	50	46.45	81.46	81.80	69.90
	100	53.91	69.69	91.80	71.80
5	0	43.30	54.42	92.25	63.32
	50	35.05	67.87	92.52	65.15
	100	38.74	92.07	95.32	75.38
10	0	38.01	57.21	84.32	60.05
	50	51.51	73.92	93.68	73.03
	100	74.67	86.80	91.92	84.46
15	0	41.58	81.93	92.70	72.07
	50	43.46	63.89	93.90	67.08
	100	62.95	79.47	97.71	80.04
Mean ZnO nanoparticles		47.55	72.89	89.86	2.49
L.S.D (0.05) ZnO nanoparticles		1.24			
L.S.D (0.05) Dual interaction		4.31			

ZnO nanoparticles×NaCl

NaCl	ZnO nanoparticles (mg.L^{-1})			Mean of NaCl
	0	500	1000	
0	46.93	72.35	81.33	66.87
5	39.03	71.45	93.36	67.95
10	54.93	72.64	89.97	72.51
15	49.33	75.10	94.77	73.07
L.S.D (0.05)	2.49			1.43

Glutathione × NaCl

Glutathione	ZnO nanoparticles (mg.L^{-1})			Mean of Glutathione
	0	500	1000	
0	40.98	64.86	84.91	63.58
50	44.11	71.78	90.47	68.79
100	57.56	82.01	94.19	77.92
L.S.D (0.05)	2.15			1.24

phosphorus, which are essential elements involved in the synthesis of protein materials, and zinc increases the efficiency of photoreactions that occur within plant cells, which increases the rate of vegetative and root growth of plant. This in turn increases the absorption of nutrients elements hat inters in the synthesis of carbohydrates, which play a role in the synthesis of enzymatic antioxidants, including vitamin C (Dhookie *et al.,* 2013), and this in agreement with Gowayed and Kadasa (2016) study on the faba bean plant. The results of the table also indicated a significant effect of interaction in the mean of this characteristic at the salinity concentration 10 dS.m^{-1} and the concentration 100 mg.L^{-1} of the glutathione which gave the highest value of 115.56 mg.100 gm^{-1} soft weight compared to the lowest value of 69.89 mg.100 mg^{-1} soft weight at salinity concentration zero dS.m^{-1} and concentration 100 mg.L^{-1} of glutathione. The table also showed a significant effect of the interaction between NaCl and ZnO nanoparticles, the salinity concentration 15 dS.m^{-1} and the concentration 1000 mg.L^{-1} of ZnO nanoparticles gave the highest mean of this characteristic 95.67 mg.100gm^{-1} soft weight compared to the lowest value of 71.67 mg.100^{-1} soft weight at the salinity concentration zero dS.m^{-1} the concentration 1000 mg.L^{-1} of ZnO nanoparticles. The table also indicated a significant effect of the interaction between glutathione and zinc oxide nanoparticles, as the concentration 100 mg.L^{-1} of glutathione and the concentration ZnO nanoparticles 500 mg.L^{-1} recorded the highest mean of 101.17 mg.100gm^{-1} soft weight compared to the lowest interaction value of 75.00 mg.100gm^{-1} soft weight at the concentrations zero mg.L^{-1} of glutathione and 1000 mg.L^{-1} of ZnO nanoparticles.

The results of the table also showed a significant effect of the interaction between the three experiment factors in the mean of this characteristic, as the salinity concentration of 10 dS.m^{-1}, the concentrations 100 mg.L^{-1} of glutathione and 500 mg.L-1 of zinc oxide nanoparticles gave the highest mean of 138.67 mg.100gm^{-1} soft weight. The lowest mean was 63.00 mg.100gm^{-1} soft weight at salinity concentration zero dS.m^{-1} and the concentrations 100 mg.L^{-1} of glutathione and zero mg.L^{-1} of ZnO nanoparticles.

The results of table 3 indicated a significant increase when treating the plant with NaCl, particularly the salinity concentration 15 dS.m^{-1}, which gave the highest mean characterstic of leaves content in proline, which was 73.07 µg.gm^{-1} soft weight and an increase rate of 9.27% compared to the control treatment of 66.87 mg.gm^{-1} is a soft weight, due to the negative effect of salinity in increasing oxidative stress within the plant, which increases the efficiency of the plant's defensive mechanism to resist stress conditions of proline amino acid production (Boudjabi *et al.,* 2015), these results are consistent with Gumi *et al.* (2013) on potato plants. The results of the table also indicated a significant increase in the mean of this characteristic when treated with

Table 4: The effect of glutathione, zinc oxide nanoparticles application and their interaction on the leaves content of vitamin E (ìg.gm^{-1}) in the faba bean plant exposed to salinity stress.

NaCl (dS.m^{-1})	Glutathione (mg.L^{-1})	ZnO nanoparticles (mg.L^{-1})			Glutathione × NaCl
		0	500	1000	
0	0	0.0013	0.0050	0.0387	0.0150
	50	0.0373	0.0473	0.0247	0.0364
	100	0.0580	0.0773	0.0973	0.0776
5	0	0.0097	0.0407	0.0610	0.0371
	50	0.0717	0.1023	0.1127	0.0956
	100	0.0260	0.0467	0.0337	0.0354
10	0	0.0563	0.1030	0.1367	0.0987
	50	0.1067	0.1417	0.1513	0.1332
	100	0.1273	0.1790	0.3847	0.2303
15	0	0.0940	0.7377	0.7690	0.5336
	50	0.9283	1.0900	1.1233	1.0472
	100	1.0733	1.1400	1.2067	1.1400
Mean ZnO nanoparticles		0.2158	0.3092	0.3450	0.0568
L.S.D (0.05) ZnO nanoparticles		0.0284			
L.S.D (0.05) Dual interaction		0.0984			

ZnO nanoparticles×NaCl				
NaCl	ZnO nanoparticles (mg.L^{-1})			Mean of NaCl
	0	500	1000	
0	0.0322	0.0432	0.0536	0.0430
5	0.0358	0.0632	0.0691	0.0560
10	0.0968	0.1412	0.2242	0.1541
15	0.6986	0.9892	1.0330	0.9069
L.S.D (0.05)	0.0568			0.0328

Glutathione × NaCl				
Glutathione	ZnO nanoparticles (mg.L^{-1})			Mean of Glutathione
	0	500	1000	
0	0.0403	0.2216	0.2513	0.1711
50	0.2860	0.3453	0.3530	0.3281
100	0.3212	0.3607	0.4306	0.3708
L.S.D (0.05)	0.0492			0.0284

glutatione 100 mg.L^{-1}, which gave the highest mean of the characteristic was 77.92 µg.gm^{-1} soft weight and an increase of 22.55% compared to the control treatment of 63.58 µg.gm^{-1} soft weight. This is due to the role of glutathione as an antioxidant and works to protect the plant from oxidative damage and free radicals as well as to increase its efficiency in the production of dry matter (carbohydrates) thus increasing the internal content of the plant from proline (Pyngrope *et al.*, 2013).

The results of the table showed a significant increase in the mean characteristic of leaves content in proline when treated with ZnO nanoparticles, particularly concentration of 1000 mg.L^{-1}, which gave the highest mean of the characteristic was 89.86 µg.gm^{-1} soft weight and a percentage increase of 88.98% compared to the control treatment of 47.55 µg.gm^{-1} soft weight. This is due to the role of zinc oxide nanoparticles in maintaining the cellular structure as it is a micronutrient that covers the plant's basic needs due to its easy access to plant cells, which increases the rate of metabolism of the plant and the amount of carbohydrates production, which contribute to increase proline concentration (Prasad *et al.*, 2012). Also, there is a significant effect of the interaction between NaCl and glutathione, with the highest interaction value being 84.46 µg.gm^{-1} soft weight at the salinity concentration 10 dS.m^{-1} and the concentrations 100 mg.L^{-1} of glutathione, while the concentration zero mg.L^{-1} and salinity concentration zero dS.m^{-1} recorded the lowest interaction value 58.90 µg.gm^{-1} soft weight.

The table also showed a significant effect of interaction between NaCl and ZnO nanoparticles, as the highest value of 94.77 µg.gm^{-1} soft weight at salinity concentration 15 dS.m^{-1} and the concentration 1000 mg.L^{-1} of ZnO nanoparticles, while the lowest value for interaction was 39.03 µg.gm^{-1} soft weight at salinity concentration 5 dS.m^{-1} and the concentration zero mg.L^{-1} of ZnO nanoparticles. The table also indicated a significant effect of the interaction between the glutathione and ZnO nanoparticles, as the concentration 100 mg.L^{-1} of glutathione and the concentration 1000 mg.L^{-1} of ZnO nanoparticles gave the highest value of 94.19 µg.gm^{-1} soft weight, and the lowest interaction value of 40.99 µg.gm^{-1} soft weight at zero mg.L^{-1} of both glutathione and ZnO nanoparticles nanoparticles.

The results of the table also indicated a sigificant effect of the interaction between the three experiment factors in the mean of this characteristic, as the highest mean of 97.71 µg.gm^{-1} soft weight at salinity concentration 15 dS.m^{-1} and the concentrations of 100 mg.L^{-1} of glutathione and 1000 mg.L^{-1} of ZnO nanoparticles, while the lowest interaction value amounted to 35.05 µg.gm^{-1} soft weight at salinity concentration 5 dS.m^{-1} and the concentrations 50 mg.L^{-1} of glutathione and 0 mg.L^{-1} of ZnO nanoparticles.

The results of table 4 indicated a significant increase in the mean content of vitamin E when treated with NaCl

solution, particularly the salinity concentration of 15 $dS.m^{-1}$, which gave the highest mean of 0.9069 $\mu g.gm^{-1}$ and an increase rate of 2.00% compared to the control treatment of 0.0430 $\mu g.gm^{-1}$. The presence of Na^+ and Cl^- elements increases the plant's exposure to oxidative conditions and its exposure to free radicals, thus increasing the plant's defensive mechanism against these radicals and the defensive mechanism is the presence of vitamin E (Parida *et al*., 2002).

The results of the table also indicated a significant increase in the mean of this characteristic when treated with glutathione, particularly the concentration 100 $mg.L^{-1}$, which gave the highest mean characteristic of 0.3708 $\mu g.gm^{-1}$ and an increase rate of 116.71% compared to the control treatment of 0.1711$\mu g.gm^{-1}$, due to the role of glutathione as an antioxidant and a reduced agent has a role in removing the toxic effect of certain substances and removing the effect of reactive oxygen species, which increases the presence of enzymatic antioxidants, including vitamin E (Noctor *et al*., 2012).

The results of the table showed a significant increase in the mean of vitamin E content in the leaves when treated with zinc oxide nanoparticles, particularly the concentration 1000 $mg.L^{-1}$, which gave the highest mean of 0.3450 $\mu g.gm^{-1}$ and an increase rate of 59.87% compared to the control treatment of 0.2158 $\mu g.gm^{-1}$, this is due to the role of zinc oxide nanoparticles in increasing the efficiency of photosynthesis and increasing the rate of vegetative and root growth of the plant and thus its absorption of nutrients necessary to raise the plant's defensive mechanism against oxidative conditions resulting from environmental stresses, including vitamin E (Bouis, 2003).

The results of the table also indicated a significant effect of interaction between NaCl and glutathione, with the highest interaction value being 1.1400 $\mu g.gm^{-1}$ at salinity concentration 15 $dS.m^{-1}$ and the concentration 100 $mg.L^{-1}$ of glutathione, while the lowest interaction value was 0.0150 $\mu g.gm^{-1}$ at salinity concentration zero $dS.m^{-1}$ and zero $mg.L^{-1}$ of glutathione. The table also indicated a significant effect of interaction between NaCl and ZnO nanoparticles, with the highest interaction value being 1.0330 $\mu g.gm^{-1}$ at the salinity concentration of 15 $dS.m^{-1}$ and the concentration 1000 $mg.L^{-1}$ of ZnO nanoparticles, while the lowest mean was 0.0322 $\mu g.gm^{-1}$ at the control treatment.

The table also indicated a significant effect of the interaction between the glutathione and ZnO nanoparticles, as the concentrations 100 $mg.L^{-1}$ of glutathione and 1000 $mg.L^{-1}$ of ZnO nanoparticles, the highest value of 0.4306 $\mu g.gm^{-1}$ compared to the lowest value of 0.0403 $\mu g.gm^{-1}$ at zero $mg.L^{-1}$ of both glutathione and ZnO nanoparticles.

The results of the table also indicated a significant effect of the interaction between the three experiment factors in the mean of this characteristic, particularly the salinity concentration 15 $dS.m^{-1}$ and the concentrations 100 $mg.L^{-1}$ of glutathione and 1000 $mg.L^{-1}$ of ZnO nanoparticles, which gave the highest mean of the characteristic 1.2067 $\mu g.gm^{-1}$, compared to the lowest mean of 0.0013 $\mu g.gm^{-1}$at salinity concentration zero $dS.m^{-1}$ and zero $mg.L^{-1}$ for both glutathione and zinc oxide nanoparticles.

Conclusions

The plant's exposure to salinity stress with increased concentrations has raised the plant's internal content of non-enzymatic antioxidants, including vitamin C, proline and vitamin E by reducing plant content from chlorophyll, especially at concentration 15 $dS.m^{-1}$, which gave the highest mean of anti-oxidants non-enzymatic oxidation to increase the reactive oxygen species that formation by salinity stress, which increases plant resistance to free radicals, as well as the use of foliar application of the plant with glutathione and zinc oxide nanoparticles, has increased the plant's content from non-enzymatic antioxidants and chlorophyll. It also has a positive role for glutathione and ZnO nanoparticles in reducing the damage caused by salinity stress.

References

Abdul Qados, A.M.S. (2010). Effect of salt stress on plant growth hand metabolism of bean plant *Vicia faba* (L). *J. Saudi. Soci. Agric. Sci*., **10:** 7-15.

Al-Hayani, I.H.H. (2015). Effect of glutathione and hydrogen peroxide and their interaction on some qualitative and quantitative characteristics of *Vigna radiata* L. plant. PhD thesis, College of Education for Pure Sciences, Ibn Al-Haitham, University of Baghdad, 208.

Ashraf, M.X., M. Roohi, Z. Iqbal, M. Ashraf, M. Ozturk and S. Gucel (2015). Cadmium (cd) and lead (pb) induced inhibition in grouth and alteration in some biochemical attributes and mineral accumulation in mung bean (*Vigna radiata* L. Wilcek). Communication in soil.Sci. Plant analysis. *Taylor and Francis*, 1532-2416.

Bates, L.S., R.P. Waldes and I.D. Teare (1973). Rapid determination free proline for water stress studies. *Plant Soil*, **37:** 205-207.

Boudjabi, S., M. Kribaa and H. Chenchouni (2015). Growth physiology and yield of durum wheat (*Triticum durum*) treated with sewage studge under water stress conditions. *Excl. J*., **14:** 230-334.

Bouis, H.E. (2003). Micronutrient fortification and phytoremediation. *Curr. Opin. Plant Biol*., **12:** 373-380.

Carcia-Lopez, J.I., G. Nino-Medina, E. Olivares-Saenz, R.H. Lira-Saldivar, E.D. Barriga-Castro, R. Vazquer-Alvarado, P.A. Rodnguez-Salinas and F. Zavala-Garcia (2019). Folar application of Zinc oxide nano particles and Zinc sulfate boosts the content of the bioative compounds in Habanero peppers. *Plant Articles*, **8(254):** 1-20.

Clerc, M. (2013). Fourniture deazote par larotation cultural. FIBIC Inst. Res. Agric. Bio. Lausanne. Switzerland, 1001-1008.

Dhookie, M.S.S., M.A. Al-Obeidi and A.O. Ismail (2013). The effect of irrigation water quality in the growth and yield maize (*Zea mays* L.) in calcareous soils in Erbil-Kurdistan region of Iraq. *J. Kirkuk Univ. Agric. Sci.*, **4(2):** 6-18.

Gowayed, S.M.H. and N.M. Kadasa (2016). Effect of Zinc oxide nono particles on antioxidative system of faba bean (*Vicia faba* L.) seeding exposed to cadmium. *Life. Sci. J.*, **13(3):** 18-27.

Gumi, A.M., A.A. Aliero, K. Shehu and A. Danbaba (2013). Salinity stress: effect on growth, biochemical parameters and ion homeostasis in *Solanum lycospersicum* L. (N. Daneka). *Central Euro. J. Exper.*, **2(3):** 20-25.

Hussain, I., M.A. Khan, F.V. Khan, S. Ayaz and F.U. Khan (2010). UV spectrophotometric analysis profile of ascorbic acid in medical plants of Pakistan. *World Appl. Sci. J.*, **9(7):** 800-805.

Jamali, G., S.H. Enteshani and S.M. Hosseini (2011). Study effect adjustement drought stress application potassium and zinc in corn. *Iranian. J. Crop. Ecophysiol.*, **3(3):** 216-222.

Jensen, E.S., M.B. Peoples and H. Hauggaard-Nielsen (2010). Faba bean in cropping system. *Field Crops Res.*, **115(3):** 203-216.

Noctor, G., A. Ahmadi, S. Chaouch, Y. Han, J. NeuKermans, B. Marquez-Garcia, G. Queval and C.H. Feoyer (2012). Glutathine in plants: An integrated overview. *Plant Cell Environ.*, **35(2):** 454-484.

Parida, A., A.B. Das and P. Das (2002). Nacl stress causes changes in photosynthetic pigments protiens, and other metabotic component in the leaves of true mangrove. Bruguiera parvioflora in hydroponic culures. *J. Plant Biol.*, , **45:** 28-36.

Prasad, T.N.V.K.V., P. Sudhakar, Y. Sreenivasulu, P. Latha, V. Munaswamy, K. Raja Reedoly, T.S. Sreeprasad, P.R. Sajanlal and T. Pradeep (2012). Effect of nano scale zinc oxide particles on the germination growth and yield of peanut. *J. Plant Nutr.*, **35:** 905-927.

Pyngrope, S., K. Bhoomika and R.S. Dubey (2013). Reactive oxygen species ascorbate glutatyhine pool, and enzymes of their metabolism in drought-sensitive and tolerant indica rice (*Oryza sativa* L.) seedling subjected to progressing levels of water deficit protoplasma., **250:** 585-600.

Rosenberg, H.R. (1992). Chemistry and physiology of vitamins-inter science publishers. Inc.New York.

Salama, K.H.A. and M.M. Mutawa (2009). Glutahione-triggered mitigation in salt induced alterations in plasma lemma of onion epidermal cell. *Int. J. Agric. Biol.*, **11:** 639-642.

SAS. (2012). Statistical analysis system uers Guide. Statistical version 9. 1th ed. SAS. Inst. Jnc. Cary. N. C.. USA.

Wang, R., S. Liu, F. Zhou and C. Hua (2014). Exogenous ascorbic acid and glutathione alleviated *Oryza sativa* L., Zeitschrift fur Nature Forschung, **69(5-6):** 226-236.

Wu, J.C., S.H. Sun, Y.T. Ke, C.P. Xie and F.X. Cgen (2011). Effect of glutathione on chloro plast membane fludity and the glutathione circulation system in young loguat fruits under low temberature stress. *Acta. Hortic.*, **887:** 221-225.

Yoon, S.J., J.J. Kwak, W.M. Lee, P.A. Holden and Y.J. An (2014). Zinc oxide nanoparticles delay soy bean development: A stanard soil microcosm study. *Ecotoxical. Environ. Safety*, **100:** 131-137.

Zhani, K.B., F. Mariem, M. Fardaous and H. Cherif (2012). Impact of salt stress (NaCl) on growth, chlorophyll content and fluoresence of Tunisian cultivars chili peper (*Capsicum frutescens* L.). *J. Stress. Physiol. Bio. Chem.*, **8(4):** 236-252.

Zheng, M., F. Davidson and X. Hung (2003). ZnO nano particles: Growth, properties and applications. *J. Am. Chem. Soc.*, **125:** 7790.