Tikrit Journal for Agricultural Sciences
ISSN:1813-1646 (Print); 2664-0597 (Online)
Journal Homepage: http://www.tjas.org
E-mail: tjas@tu.edu.iq

TJAS
Tikrit Journal for Agricultural Sciences

DOI: https://doi.org/10.25130/tjas.25.1.4

The effect of Fulvic Acid on the Activity of Some Antioxidant Enzymes in the leaves of Date Palm Offshoots, Nabayti Cultivar Resulting from Tissue Culture under Conditions of Salt Stress

Ahmed D. K. Al-Asadi, and Batool H. F. Al-Zubaidy

Department of Horticulture and Land Escaping, College of Agriculture and Marshes, University of Thi – Qar, Thi – Qar, Iraq.

*Correspondence email: **ahmed-d@utq.edu.iq**

KEY WORDS:
Sodium Chloride, Peroxidase, Glutathione Peroxidase, Glutathione Reductase

Received: 11/06/2024
Revision: 23/11/2024
Proofreading: 26/12/2024
Accepted: 18/01/2025
Available online: 31/03/2025

ABSTRACT

This study was conducted at the College of Agriculture and Marshlands, Thi Qar University during the growing season of 2021 to study the effect of sodium chloride and fulvic acid and their interactions on some antioxidant enzymes of the leaves of date palm offshoots, a Nabayti cultivar, resulting from tissue culture. The study was implemented as a factorial experiment according to a Completely Randomized Block Design (CRBD) with two factors, the first is sodium chloride at four concentrations (0, 50, 100 and 150) mM, and the second is fulvic acid at three concentrations (0, 2.5 and 5) g L^{-1} with three replicates for each treatment. The results showed that the fulvic acid treatment at a concentration of (5 g L^{-1}) achieved the highest averages of activity of peroxidase, glutathione peroxidase and glutathione reductase enzymes (10.738, 26.357 and 25.681) units g^{-1} min^{-1} respectively, compared to the control treatment. The sodium chloride treatment at a concentration of (150 mM) achieved the highest averages of activity of peroxidase and glutathione reductase enzymes (13,429 and 26,556) units g^{-1} min^{-1} respectively, while the concentration (10 mM) achieved the highest average of activity of glutathione peroxidase enzyme (26,277 units g^{-1} min^{-1}) compared to the control treatment. The interaction treatment between (sodium chloride at a concentration of (150 mM) and fulvic acid at a concentration of (5 g L^{-1}) achieved the highest averages of activity of peroxidase, glutathione peroxidase and glutathione reductase enzymes (14,580 and 28,267 and 27,623) units g^{-1} min^{-1} respectively, compared to the control treatment.

تأثير حامض الفولفيك في فعالية بعض الإنزيمات المضادة للأكسدة لأوراق فسائل نخيل التمر صنف نبايتي الناتجة من زراعة الأنسجة تحت ظروف الإجهاد الملحي

احمد دينار خلف الاسدي وبتول حنون فالح الزبيدي
قسم البستنة وهندسة الحدائق، كلية الزراعة والاهوار، جامعة ذي قار، العراق.

الخلاصة

أجريت هذه الدراسة في كلية الزراعة والأهوار جامعة ذي قار خلال موسم النمو 2021 لدراسة تأثير كلوريد الصوديوم وحامض الفولفيك وتداخلاتهما في بعض الانزيمات المضادة للأكسدة لأوراق فسائل نخيل التمر صنف نبايتي الناتج من زراعة الأنسجة. نفذت الدراسة كتجربة عامليه وفق تصميم القطاعات العشوائية الكاملة (CRBD) وبعاملين، الأول هو كلوريد الصوديوم بأربعة تراكيز (0 و50 و100 و150) مليمولر، والثاني هو حامض الفولفيك بثلاثة تراكيز (0 و2.5 و5) غرام لتر$^{-1}$ وبواقع ثلاثة مكررات لكل معاملة. وأظهرت النتائج ان معاملة حامض الفولفيك بتركيز (5 غم لتر$^{-1}$) حققت اعلى المعدلات لفعالية انزيمات البيروكسيديز والجلوتاثيون بيروكسيديز والجلوتاثيون ريدوكتيز (10.738 و26.357 و25.681) وحدة غم$^{-1}$ دقيقة $^{-1}$ على التوالي، بالمقارنة مع معاملة السيطرة. أما معاملة كلوريد الصوديوم بتركيز (150 مليمولر) فقد حققت أعلى المعدلات لفعالية انزيمات البيروكسيديز والجلوتاثيون ريدوكتيز (13.429 و26.556) وحدة غم$^{-1}$ دقيقة$^{-1}$ على التوالي، في حين حقق التركيز (10 مليمولر) أعلى معدل لفعالية انزيم الجلوتاثيون بيروكسيديز (26.277 وحدة غم$^{-1}$ دقيقة$^{-1}$) بالمقارنة مع معاملة السيطرة. اما معاملة التداخل بين (كلوريد الصوديوم بتركيز (150 مليمولر) وحامض الفولفيك بتركيز (5 غم لتر$^{-1}$) فقد حققت أعلى المعدلات لفعالية أنزيمات البيروكسيديز والجلوتاثيون بيروكسيديز والجلوتاثيون ريدوكتيز (14.580 و28.267 و27.623) وحدة غم$^{-1}$ دقيقة$^{-1}$ على التوالي وبالمقارنة مع معاملة السيطرة.

الكلمات الافتتاحية: كلوريد الصوديوم، بيروكسيديز، كلوتاثيون بيروكسيديز، كلوتاثيون ريدوكتيز.

INTRODUCTION:

The date palm *Phoenix dactylifera* L. is the first tree in Iraq. It is an evergreen fruit tree belonging to the palm family Arecaceae and the order Arecales. It is a monocotyledonous tree, the Arabian Gulf region is one of the largest palm cultivation areas in the world, and from there its cultivation spread to all regions of the world with a suitable climate (Al-Jabouri, 2002). Iraq has many cultivars, amounting to about (627 cultivar), divided into commercial, local, and rare scales, the Nabayti cultivar is considered one of the good and rare cultivars, and Basra Governorate, southern Iraq, is considered its original home, it is present in small numbers, and its fruits during the khalal stage are yellow in color, sweet in taste, oval in shape, medium in size, and their ripening time is also medium. (Al-Bakr, 1972).

Iraq has the largest land cultivated with date palms in the world, as the cultivation of the finest cultivars is widespread , characterized by their commercial and nutritional importance and abundant production , in addition to the great role that palm orchards play in improving the environment and providing the main cover under which many fruit trees are grown , especially in the central and southern regions from Iraq, and until recently, Iraq occupied the first position in the global date trade for a number of cultivars such as Al-Zahdi, Al-Halawi, Al-Sayer, Al-Khadrawi, Al-Barim, Al-Jabjab, and others (Ismail, 2010).

Despite these indications, the process of cultivation, production and manufacturing of dates has witnessed a significant decline in recent decades , as this national wealth was not invested optimally, and a number of reasons contributed to this decline, including the neglect and damage to which the palm groves were exposed as a result of the wars that Iraq entered , in addition to the process of smuggling good cultivars out of the country, such as the Barhi , the migration of a large

number of palm farmers and producers , and the neglect of orchard service , control and care, in addition to the scarcity of water and the salinity of soil and water in recent decades as a result of climate change , which Iraq is considered one of the countries most affected by , were among the reasons , the main factor that led to this decline in the number of palm trees and the quantities of dates produced, especially in the southern region of Iraq , especially since the problem of salinity (salinity of soil and irrigation water) is one of the most important problems facing agriculture globally, especially in arid and semi-arid areas (Munns and Tester 2008; Alturki, 2018), and the use of salt water for irrigation affects approximately one-third of the world's irrigated lands in wet areas as well as dry and semi-arid areas (Yaish and Kumar, 2015).

Salinity is one of the most widespread abiotic stresses that leads to significant losses in agricultural crop production, especially in arid and semi-arid regions (Hernandez, 2019). Recently, the amount of land affected by salinity was estimated at 1,125 million hectares worldwide (Hossain, 2019). This area is expected to increase with increasing climate change, human activities, and freshwater scarcity (Arguelles, 2020; Tomaz *et al*., 2020). Salinity can induce different molecular, physiological, and biochemical malformations (Elkelish,2019; Ramadan *et al*.,2022; Ola *et al*.,2012). It inhibits plant growth and development by affecting plant water relations, cell turgor pressure, and disturbing plant growth regulators, restricting cell division and enlargement (Qi and Zhang,2020). Additionally, it can negatively affect photosynthesis, transpiration, and stomatal conductance (Alnusairi *et al*.,2021; Lotfi *et al*.,2020). Furthermore, salinity stress disintegrates the cell membrane and disturbs ion homeostasis, leading to variations in cell ultrastructure and imbalances in nutrient uptake (Ola *et al*.,2012; Manaa *et al*.,2019). Several studies have indicated that salinity stress induced oxidative damage at the cellular level due to the excessive release of reactive oxygen species (ROS) in different plant species (Ramadan *et al*.,2022; Ola *et al*.,2012; Alnusairi *et al*.,2021; Akyol *et al*.,2020).

It has become necessary to use some possible alternative techniques at the present time with the aim of improving the plant's tolerance to salt stress conditions (Jasim *et al*., 2010) , recent studies have shown that there are many chemical compounds, nutrients, and growth stimulants that can be used to reduce the effects of salinity on the growth and production of plants , among these compounds is fulvic acid, which is one of the plant organic acids that is naturally produced from the humic substance resulting from the decomposition of organic matter, which causes its addition to the soil or plant increases the absorption of nutrients, especially when exposed to salt stress (Cimrin *et al.* ,2010), and it works to improve the physical, chemical and biological properties of the soil and reduces the problems and damages of excessive salinity and alkalinity and thus increases the strength of the root system and its ability to absorb (Shaaban *et al.*, 2009). One of the most important fulvic acid components is the aromatic and aliphatic compounds consisting of carbohydrates and amino acids. It is characterized by its low partial weight, ranging from 1000 to 10,000 Daltons (Al-Shater and Al-Balkhi, 2010).

Because the plant has two defense systems against the effects of environmental stresses: the enzyme antioxidant system, which includes antioxidant enzymes, and the non-enzyme antioxidant system, which includes osmotic solutes such as total soluble carbohydrates, the amino

acid proline, and others. This study came with the aim of knowing the effects of sodium chloride salt and fulvic acid and their interactions in the activities of some antioxidant enzymes (peroxidase, glutathione peroxidase, and glutathione reductase) and their role in improving the tolerance of date palm offshoots, Nabayti cultivar, resulting from tissue culture to salt stress conditions, thus increasing their susceptibility to acclimatization to environmental conditions in the southern region of Iraq.

MATERIALS AND METHODS:

This study was conducted at Thi - Qar University, College of Agriculture and Marshlands, during the 2021 growing season on 36 offshoots resulting from tissue culture of the Nabayti cultivar, similar in size and three years old. It was designed as a factorial experiment according to the Randomized Completely Blocks Design (RCBD) and with two factors , the first of which includes : using four concentrations of sodium chloride salt (0, 50, 100, and 150) mM , the offshoots were watered with low salt concentrations, gradually increasing (25 mM) in each watering until the required study concentrations were reached and maintained for the purpose of avoiding exposure of the plants to shock due to salt stress , then the offshoots were watered with the above study concentrations (10) watering , 10 days between one watering and the next until the study was completed.

As for the second factor, it was fulvic acid, at three concentrations (0, 2.5, and 5) g L^{-1} and it was given as an addition to the soil and for (10) watering between one and other (10 days) until the completion of the study , the soil was prepared by mixing a ratio of (1:1:2) of the soil and peat moss and vermiculite, respectively, and treated with the fungicide HIMEX and the insecticide (Rivadan) as a preventive measure to avoid insect and fungal infections, according to the recommendation mentioned on the boxes by the producing company , this soil was placed in anvils of diameter (40 cm) and then the offshoots were transferred to it , the electrical conductivity (EC) of the soil and irrigation water used in the study was (2.73 and 1.17) ds m^{-1}, respectively, and the pH of the soil and irrigation water was (6.93 and 7.10) , respectively. The offshoots were treated with a fertilization program throughout the study period, consisting of N.P.K complex fertilizer and amino acids at a concentration of (2 gm L^{-1}), watered once every 10 days for each, while the microelements at a concentration of (1 ml L^{-1}) were sprayed once every 10 days.

Experimental measurements:

Estimation of peroxidase enzyme activity (POD):

The activity of POD enzyme was estimated according to the method described by (Nezih, 1985). The materials and solutions used were: Guaicaol solution, which was prepared by mixing (1.36 ml) of guaicaol in a volumetric flask, then the volume was completed to (250 ml) using distilled water, and hydrogen peroxide solution H_2O_2 with a concentration of (0.1%), which was prepared by taking a volume of (0.4 ml) of H_2O_2 (30%) and completing the volume to (120 ml) using distilled water. The method of work included mixing (1 ml) of H_2O_2 with (1 ml) of guaiacol, and then the enzyme activity was estimated by adding (2 ml) of the reaction mixture in the cell of the Japanese spectrophotometer (Spectromlab22), then (0.1 ml) of the sample was added and the

change in light absorption was monitored every 30 seconds for 3 minutes at a wavelength of 420 nm. The activity of the POD enzyme was calculated according to the following equation:

$$\text{POD enzyme activity (unit } g^{-1}min^{-1}) = \frac{\Delta \text{ Device reading} / \Delta \text{ Time}}{0.1 \times 0.01}$$

0.1: Sample size

0.01: One unit of enzyme (the amount of enzyme that causes an increase in light absorption of 0.01 unit per minute at a wavelength of 420 nm).

Estimation of glutathione peroxidase enzyme activity (GPX):

The activity of GPX enzyme was estimated according to the method of (Flohe and Gunzler, 1984). The following solutions were used: phosphate buffer solution (0.1 M) and pH 7.4. This solution contains a certain volume of solution B added to 200 ml of solution A until the pH value reaches 7.4, reduced glutathione solution (0.002 M), sodium azide solution (0.010 M), hydrogen peroxide solution (0.03 M), 5% Trichloroacetic Acid (TCA) solution and Dithiobs-2-nitrobenzoic acid (DTNB) solution. The method included two stages: the first was to put (0.3 ml) of the extract in test tubes and add (0.3 ml) of phosphate buffer (0.1 M) (pH 7.4), (0.2 ml) of reduced glutathione (0.002 M), (0.1 ml) of sodium azide solution (0.01 M) with (0.1 ml) of hydrogen peroxide (0.03). And then put the tubes in a water bath at 37 °C for 15 minutes after which (0.3 ml) of (TCA) solution was added to the tubes and then cooled in an ice bath and then put in a centrifuge at 1500 rpm for 5 minutes. The filtrate was taken and read in a spectrophotometer at a wavelength of 420 nm compared to Blank which contains (0.1 ml) of distilled water. The second stage included placing (0.1 ml) of the extract in test tubes and adding (0.3 ml) of phosphate buffer and (0.7 ml) of (DTNB), then the mixture was taken and read in a spectrophotometer at a wavelength of 420 nm compared to a blank containing (0.1 ml) of distilled water. The enzyme activity was calculated according to the following equation:

$$\text{GPX enzyme activity (unit } g^{-1}min^{-1}) = \frac{1000000 \times (AB - AT)}{1 \times 622}$$

AT: Absorption of samples in the first treatment at a wavelength of 420 nm.

AB: Absorbance of samples in the second treatment at a wavelength of 420 nm.

622: Extinction coefficient.

1: Cuvette length (1 cm).

Estimation of glutathione reductase activity (GR):

The activity of the GR enzyme was estimated according to the method of (Teisseire and Guy, 2000) by measuring the increase in absorbance at a wavelength of 412 nm resulting from the reduction of 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) to 2-nitro-5- thiobenzoic acid (TNB). The enzyme activity was calculated according to the following equation:

$$\text{GR enzyme activity (unit } g^{-1}min^{-1}) = \frac{\Delta A / t \times Vt \times 1000}{E \times Vs \times d}$$

ΔA: The difference between the two absorptions.
t: Time
Vt: Total solution volume.
Vs: Volume of solution for sample.
E: Absorption coefficient (6.22).
d: Dilution factor.

Statistical analysis:

The results of the experiment were analyzed statistically as a factorial experiment according to the Complete Randomized Block Design, and the differences between the means were tested using the least significant difference (L.S. D) under the level of significance of 0.05 (Al - Rawi and Abdul Aziz, 1980).

RESSULTS AND DISCUSSION:

The results in Table (1) showed that fulvic acid had a significant effect on increasing the activity of the peroxidase enzyme with an increase in its concentration in the growth medium, as the concentration (5 ml L^{-1}) achieved the highest average of (10.738 unit $g^{-1}min^{-1}$) compared to the control treatment, which gave the lowest average of (8.845 unit $g^{-1}min^{-1}$).

As for the effect of sodium chloride, it caused a significant increase in enzyme activity averages with an increase in the concentration of sodium chloride in the growth medium, as the concentration (150 mM) gave the highest average of (13.429 unit $g^{-1}min^{-1}$) compared to the control treatment, which recorded the lowest average of (5.427 unit g^{-1} min^{-1}).

Some of the interaction treatments had a significant effect by increasing the activity of the peroxidase enzyme, and the interaction treatment between (fulvic acid at a concentration of (5 g L^{-1}) and sodium chloride at a concentration of (150 mM) achieved the highest average of (14.580 unit $g^{-1}min^{-1}$), while the control treatment recorded the lowest average of (4.643 unit g^{-1} min^{-1}).

Table (1) the effect of fulvic acid, sodium chloride and their interactions on the peroxidase enzyme activity (unit g^{-1} min^{-1}).

NaCl con. (mM)	**fulvic acid con.(g L^{-1})**			**NaCl averages**
	0	**2.5**	**5**	
0	6.250 h	5.387 i	4.643 j	5.427 d
50	9.750 f	9.673 f	7.650 g	9.024 c
100	12.373 c	11.600 d	10.310 e	11.428 b
150	14.580 a	12.930 b	12.777 bc	13.429 a
Fulvic acid averages	10.738 a	9.897 b	8.845 c	
L. S. D. (0.05)	**Interactions**		**NaCl**	**Fulvic acid**
	0.4965		**0.2867**	**0.2483**

Values with similar letters are not significantly different according to the least significant difference (L.S. D) testing with 5% probability

It is noted from the results in Table (2) that fulvic acid had a significant effect on increasing the activity of the glutathione peroxidase enzyme with an increase in its concentration in the growth medium, the concentration treatment (5 g L^{-1}) achieved the highest average of (26.356 unit g^{-1} min^{-1}), followed by the concentration treatment (2.5 g L^{-1}) at an average of (25.710 unit g^{-1} min^{-1}), compared to the control treatment, which recorded the lowest average of (24.227 unit g^{-1} min^{-1}).

As for the effect of sodium chloride, it was also significant by increasing the activity of the glutathione peroxidase enzyme with increasing its concentration, the concentration treatment (100 mM) achieved the highest average of (26.277unit g^{-1} min^{-1}), which did not differ significantly with the concentration treatment (150 mM), which recorded an average of (26.161unit g^{-1} min^{-1}), compared to the control treatment, which recorded the lowest average of (23.903unit g^{-1} min^{-1}).

The interaction treatment between (sodium chloride at a concentration of (150 mM) and fulvic acid at a concentration of (5 g L^{-1}) had a significant effect and achieved the highest average of activity of the glutathione peroxidase enzyme, amounting to (28.267 unit g^{-1} min^{-1}), while the control treatment recorded the lowest average amounting to (23.273 unit g^{-1} min^{-1}).

Table (2) the effect of fulvic acid, sodium chloride and their interactions on the glutathione peroxidase enzyme activity (unit g^{-1} min^{-1}).

NaCl con. (mM)	**fulvic acid con.(g L^{-1})**			**NaCl averages**
	0	**2.5**	**5**	
0	24.630 d	23.807 e	23.273 f	23.903 c
50	25.797 c	25.687 c	24.663 d	25.382 b
100	26.730 b	26.537 b	25.563 c	26.277 a
150	28.267 a	26.807 b	23.410 ef	26.161 a
Fulvic acid averages	26.356 a	25.710 b	24.227 c	
L. S. D. (0.05)	**Interactions**		**NaCl**	**Fulvic acid**
	0.5164		**0.2981**	**0.2582**

Values with similar letters are not significantly different according to the least significant difference (L.S. D) testing with 5% probability

The results in table (3) showed that fulvic acid caused a significant increase in the activity of the enzyme glutathione reductase with an increase in its concentration in the growth medium, and the concentration treatment (5 g L^{-1}) achieved the highest average of (25.681 unit g^{-1} min^{-1}), compared to the control treatment that was recorded the lowest average (21.786 unit g^{-1} min^{-1}).

As for the sodium chloride treatments, they also caused a significant increase in the activity of the enzyme with an increase in the concentration of sodium chloride in the growth medium, the concentration treatment (150 mM) achieved the highest average of (26.556 unit g^{-1} min^{-1}) compared to the control treatment, which recorded the lowest average of (20.755 unit g^{-1} min^{-1}).

The interaction treatment between sodium chloride at a concentration of (150 mM) and fulvic acid at a concentration of (5 g L^{-1}) was significantly superior and achieved the highest average of enzyme activity amounting to (27.623 unit g^{-1} min^{-1}), while the control treatment recorded the lowest average amounting to (16.693 unit g^{-1} min^{-1}).

Table (3) the effect of fulvic acid, sodium chloride and their interactions on the glutathione reductase enzyme activity (unit g^{-1} min^{-1}).

NaCl con. (mM)	fulvic acid con.(g L^{-1})			NaCl averages
	0	2.5	5	
0	24.293 g	21.280 h	16.693 j	20.755 d
50	25.693 c	24.747 ef	20.703 i	23.714 c
100	25.113 de	24.923 def	24.530 fg	24.855 b
150	27.623 a	26.827 b	25.217 d	26.556 a
Fulvic acid averages	25.681 a	24.444 b	21.786 c	
L. S. D. (0.05)	**Interactions**		**NaCl**	**Fulvic acid**
	0.4033		**0.2329**	**0.2017**

Values with similar letters are not significantly different according to the least significant difference (L.S. D) testing with 5% probability

The results in tables (1, 2, and 3) showed a significant increase in the activity averages of the enzymes peroxidase, glutathione peroxidase, and glutathione reductase due to the effect of sodium chloride salt and fulvic acid and their interactions , the reason for this increase may be due to the salt stress resulting from the influence of sodium chloride can induce a state of oxidative stress (Seis, 2015), which results in active forms of reaction oxygen species (ROS) such as : Super Oxide (O_2^-) , Hydroxyl radicals (OH^-), Hydrogen Peroxide (H_2O_2) and others in quantities larger than what is produced under normal conditions , which causes the occurrence of there are many damages when the plant remains under conditions of stress , and the most important of these damages is the damage to the plant's metabolic processes due to the oxidative stress that occurs to membrane lipids, proteins, and nucleic acids (Hassanein, 2004; Parida and Das,2005) . Generally, salt stress can restrict plant growth by affecting cell division (Ferjani *et al*.,2003), photosynthesis (Alnusairi *et al*.,2021), plant water relations (Stepien and Klbus, 2006), homeostasis of plant hormones (Yu *et al*.,2020), and uptake of essential nutrients (Nahhas *et al*.,2021; Youssef *et al*.,2021). As a result, the plant's defense systems are induced and activated to remove or reduce their harmful effects (Chookhampaeng, 2011; Elsahookie, 2013), the most important of which is the enzymatic defense system that has the ability to remove or neutralize free radicals (Ashraf and Foold, 2007; Ashraf *et al*., 2008 and Ashraf, 2009). The first action that the plant takes to limit the effects of free radicals is to direct the enzyme superoxide dismutase to convert the superoxide radical into a water molecule and hydrogen peroxide (H_2O_2) , then the enzymes peroxidase, ascorbate peroxidase, glutathione peroxidase, and glutathione reductase convert hydrogen peroxidase into water and oxygen , so antioxidant enzymes are the organic key that plays an effective role in the plant's protective system and increases its tolerance to salt stress or other environmental stresses, Increasing the activity of antioxidants reduces oxidative stress, increases osmotic pressure, increases the selective absorption of beneficial ions, and prevents accumulation of excess toxic ions and thus helps the plant tolerance conditions of salt stress (Ashraf, 2009), especially for date palm offshoots produced by tissue culture, which greatly improves their ability to adapt to salt stress conditions as shown by the results of this study.

The reason for the increase in the activity of these enzymes with the increase in the concentration of fulvic acid used may be due to the roles played by fulvic acid in improving the ability of plants to withstand environmental stress conditions, including salt stress, as it is considered a regulator of plant growth, has multiple functions in increasing the permeability of the cell membrane, photosynthesis, and controlling hormone levels (Cimirin *et al.*,2010). Fulvic acid is an organic acid that has many benefits for soil and plants. It is a natural chelating substance that contributes to chelating and facilitating the nutrients present in the soil. It also contributes to reducing soil salinity by chelating the calcium element present in the soil, making it free, active and available in it. In addition, it plays a major role in improving the physical properties of the soil, as it makes its color dark, which helps it absorb sunlight significantly, which leads to raising its temperature and thus helping to warm the roots and increase their ability to absorb water and nutrients, in addition to its role in encouraging root formation, as it contributes to raising the level of natural auxins (Aminifard, 2012). As a result of the above-mentioned roles of fulvic acid and its ability to chelate and facilitate nutrients in the soil such as iron, zinc, manganese, copper, selenium and calcium, and the association of some of them with some antioxidant enzymes such as superoxide dismutase, peroxidase, glutathione peroxidase and glutathione reductase, which causes an increase in the effect of antioxidant enzymes to reduce or protect the plant from the effects of free radicals resulting from oxidative stress resulting from salinity and thus increase its tolerance to stress conditions as shown by the results of this study.

The peroxidase enzyme is one of the enzymes of the plant's defense system against free radicals, which works to remove free oxygen radicals and protect membrane lipids from oxidation, its main function is to reduce hydrogen peroxide to water and oxygen molecules, in addition to other physiological functions such as contributing to the process of photosynthesis, respiration, auxin metabolism, and resistance to virus infection (Lin and Kao, 2001).

As for the enzyme glutathione peroxidase, which is from the peroxidase family, which is involved in the synthesis of glutathione, it has a role in protecting cells and plastids. Its activity lies in reducing toxic fatty hydro peroxidase compounds to alcohols and reducing toxic hydrogen peroxide to water, thus protecting cells from oxidative stress (Roy *et al*, 2005), and the element selenium is included instead of sulfur in glutathione, thus this enzyme becomes more efficient in protecting the plant, and studies have proven that it has a role in prolonging the life of cells and preserving them from the effects of stress. As for the enzyme glutathione reductase, it is also one of the types of glutathione. This enzyme catalyzes the reaction of (GSSH) to (GSH), as each mole of oxidized glutathione requires a mole of NADPH to reduce it to GSH, and this enzyme works to stimulate the plant to an alternative energy pathway (Locato *et al*., 2009).

The results of this study were consistent with what a number of researchers found through their studies on date palms, as they found a significant increase in the activity of some antioxidant enzymes under the salt stress and some nutrients, including (Darwash, 2013; Ati, 2016; Shareef, 2016; Al-Jabri, 2017, Faisal, 2019).

CONCLUSION:

The concentrations of fulvic acid used in this study caused a significant increase in the activity of some antioxidant enzymes, namely peroxidase, glutathione peroxidase, and glutathione reductase, which contributed to reducing the oxidative effects of sodium chloride salt, and this was clearly reflected in improving the tolerance of date palm offshoots, a Nabayti cultivar resulting from tissue culture, for salt stress conditions.

REFERENCES:

Akyol, T.Y.; Yilmaz, O.; Uzilday, B.; Uzilday, R.O.; Turkan, İ. (2020). Plant response to salinity: An analysis of ROS formation, signaling, and antioxidant defense. *Turk. J. Bot.*, 44 (1): 1–13. doi 10.3906/bot-1911-15

Al-Bakr, A. J. (1972). The date palm, its past, present, and what is new in its cultivation, industry, and trade, Al-Ani Press, Baghdad, Iraq: 1085.

Al-Jabouri, H. J. (2002). The importance of date palm trees *Phoenix dactylifera* L. in the State of Qatar. Proceedings and activities of the training course on plant tissue culture applications in improving agricultural production. Publications of the Arab Organization for Agricultural Development (21-27) January, Doha - Qatar: 1-25.

Al-Jabri, K. M. A. (2017). Seasonal variation of heavy metal pollution and the effect of cadmium and lead treatment on some biochemical, anatomical and genetic characteristics of date palm *Phoenix dactylifera* L., Barhi cultivar. (Doctoral dissertation, College of Science, Basra University - Iraq: 206.

Alnusairi, G.S.; Mazrou, Y.S.; Qari, S.H.; Elkelish, A.A.; Soliman, M.H.; Eweis, M.; Abdelaal, K.; El-Samad, G.A.; Ibrahim, M.F.; ElNahhas, N. (2021). Exogenous nitric oxide reinforces photosynthetic efficiency, osmolyte, mineral uptake, antioxidant, expression of stress-responsive genes and ameliorates the effects of salinity stress in wheat. *Plants (Basel)*, 10 (8): 1693. doi: 10.3390/plants10081693.

Al-Rawi, K. M. and Abdul Aziz, M. K. (1980). Design and analysis of agricultural experiments. Dar Al-Kutub Foundation for Printing and Publishing - University of Mosul - Iraq.

Al-Shater, M. S., and Akram, M. B. (2010). Soil fertility and fertilization. *Kindergarten Press*, Damascus University Publications. Faculty of Agriculture. The Syrian Arab Republic:290.

Alturki, S. (2018). Effect of NaCl on Growth and Development of in vitro Plants of Date Palm *Phoenix dactylifera* L. 'Khainazi 'Cultivar. *Asian Journal of Plant Sciences*, 17 (3): 120-128. doi:10.3923/ajps.2018.120.128

Aminifard, M. H; Arolee, H.; Nemati, H.; Azizi, M. and Hawa, Z. E. J. (2012). Fulvic acid affects pepper antioxidant activity and fruit quality, *Afr. J. Biotechnol.*, 11 (68):13179-13185. doi: 10.5897/AJB12.1507

Arguelles, M.C. (2020). A review of recent advances and future challenges in freshwater salinization. *Limnetica,* 39(1): 185–211. doi: 10.23818/limn.39.13

Ashraf, M.; Athar, H. R.; Harris, P. J. C. and Kwon, T. R. (2008). Some prospective strategies for improving crop salt tolerance. *Adv. Agron*., 97:46-110. doi:10.1016/S0065-2113(07)00002-8

Ashraf, M. and Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and experimental botany*, 59 (2): 206 – 216. https://doi.org/10.1016/j.envexpbot.2005.12.006

Ashraf, M. (2009). Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotechnol Advances*, 27 (1): 84-93. doi: 10.1016/j.biotechadv.2008.09.003.

Ati, M. A. Z. (2016). The effect of spraying with some anti-environmental stress agents on some physiological, anatomical and productive characteristics of date palm *Phoenix dactylifera* L. Halawi cultivar. Doctoral dissertation, College of Agriculture, University of Basra: 225.

Chookhampaeng, S. (2011). The effect of salt stress on growth, chlorophyll content, proline content and antioxidative enzymes of pepper *Capsicum annuum* L. seedling. *European Journal Science Research*, 49 (1): 103 – 109.

Cimrin, K. M.; Onder, T.; Metin, T. and Burcu, T. (2010). Phosphorus and humic acid Application alleviate salinity stress of Pepper seedling. *Afr. J. Biotechnol.*, 9(36): 5845-5851. http://www.academicjournals.org/AJB

Darwesh, R.S.S., (2013). Improving growth of date palm plantlets grown under salt stress with yeast and amino acids applications. *Annals of Agricultural Sciences*, 58(2): 247-256. https://doi.org/10.1016/j.aoas.2013.07.014

Elkelish, A.A.; Soliman, M.H.; Alhaithloul, H.A.; El-Esawi, M.A. (2019). Selenium protects wheat seedlings against salt stress-mediated oxidative damage by up-regulating antioxidants and osmolytes metabolism. *Plant Physiol. Biochem*., 137: 144–153. doi: 10.1016/j.plaphy.2019.02.004.

Elsahookie, M. M. (2013). Breeding Crops for Abiotic Stress: A Molecular Approach and Epigenetic. College of Agriculture, University of Baghdad:244.

Faisal, H. A. I. (2019). The effect of spraying with ascorbic acid, tocopherol, and silicon on some growth characteristics of date palm seedlings *Phoenix dactylifera* L. (cv. Barhi) growing in a saline environment. Doctoral dissertation, College of Agriculture, University of Basra, Iraq: 210.

Ferjani, A.; Mustardy, L.; Sulpice, R.; Marin, K.; Suzuki, I.; Hagemann, M.; Murata, N. (2003). Glucosylglycerol, a compatible solute, sustains cell division under salt stress. *Plant Physiol*., 131(4):1628–1637. doi: 10.1104/pp.102.017277

Flohe, L. and Gunzler, W.A. (1984). Assay of glutathione peroxidase. *Elsevier* , Volume 105: 114-120 . https://doi.org/10.1016/S0076-6879(84)05015-1

Hassanein, A. M. (2004). Effect of relatively high concentration of mannitol and sodium chloride on regeneration and gene expression of stress tolerant (Alhagi graecorum) and stress

sensitive (*Lycopersicon esculentum* L.) plant species. *Bulg, J. Plant Physiol*., 30 (3-4): 19-36. http://www.bio21.bas.bg/ipp/gapbfiles/v-30/04_3-4_19-36.pdf

Hernandez, J.A. (2019). Salinity tolerance in plants: Trends and perspectives. *Int. J. Mol. Sci*., 20 (10): 2408. https://doi.org/10.3390/ijms20102408

Hossain, M.S. (2019). Present scenario of global salt affected soils, its management and importance of salinity research. *Int. Res. J. Biol. Sci.*, 1(1): 1–3. https://www.researchgate.net/publication/334773002

Ismail, R. S. (2010). Palm trees and dates in Iraq and ways of development (agriculture, production, marketing and manufacturing). A study presented to the Fourth International Date Palm Conference. Abu Dhabi, United Arab Emirates, 15-17 March 2010. Republic of Iraq - Ministry of Agriculture - General Authority for Palm Trees: 70.

Jasim, A. M.; Abbas, M. F. and AL-zubiady, B. H. (2010). Effect of salt stress and proline on chemical content of embryo genic callus and somatic embryos of date palm *Phoenix dactylifera* L. cv. Ashkar. *Acta Horticulturae* , 882(882): 219 – 224. doi:10.17660/ActaHortic.2010.882.24

Lin, C.C. and Kao, C.H. (2001). Cell wall peroxidase activity, hydrogen peroxide level and NaCl-inhibited root growth of rice seedlings. *Plant and Soil*, 230(1): 135-143. doi:10.1023/A:1004876712476

Locato, V.; Depint, M. C. and De Gara, L. (2009). Different involvement of Mitochondrial plastidial and cytosolic ascorbate – glutathione redox enzymes in heat shock responses. *Physiol. Plant*, 135 (3) :296 -306.

Lotfi, R.; Ghassemi-Golezani, K.; Pessarakli, M. (2020). Salicylic acid regulates photosynthetic electron transfer and stomatal conductance of mung bean (*Vigna radiata* L.) under salinity stress. *Biocatal. and Agric. Biotechnol.*, 26:1 - 8. doi: 10.1016/j.bcab.2020.101635

Manaa, A.; Goussi, R.; Derbali, W. and Cantamessa, S (2019). Salinity tolerance of quinoa (Chenopodium quinoa Willd) as assessed by chloroplast ultrastructure and photosynthetic performance. *Environ. Experi. Bot*., 162 (54): 103–114. doi: 10.1016/j.envexpbot.2019.02.012

Munns, R. and Tester, M. (2008). Mechanism of salinity tolerance. *Annual Review of Plant Biology*, 59 (1): 651- 681. doi: 10.1146/annurev.arplant.59.032607.092911

Nahhas, N.E.; AlKahtani, M.D; Abdelaal, K.A.; Al Husnain, L.; AlGwaiz, H.I.; Hafez, Y.M.; Attia, K.A.; El-Esawi, M.A.; Ibrahim, M.F. and Elkelish, A. (2021). Biochar and jasmonic acid application attenuates antioxidative systems and improves growth, physiology, nutrient uptake and productivity of faba bean (*Vicia faba* L.) irrigated with saline water. *Plant Physiol. Biochem*.,166: 807–817. doi: 10.1016/j.plaphy.2021.06.033. Epub 2021 Jun 20.

Nezih, M. (1985). The peroxidase enzyme activity of some vegetables and its resistance to heat. *Journal of the Science of Food and Agriculture*, 36 (9): 877 – 880. https://doi.org/10.1002/jsfa.2740360918

Ola, H.A.E.; Reham, E.F.; Eisa, S. and Habib, S. (2012). Morpho-anatomical changes in salt stressed kallar grass (*Leptochloa fusca* L. Kunth). *Res. J. Agric. & Biol. Sci*., 8(2): 158-166. https://www.aensiweb.net/AENSIWEB/rjabs/rjabs/2012/158-166.pdf

Parida, A. K. and Dass, A. B. (2005). Salt tolerance and salinity effects on plants. A review. *Ecotoxicol. Environ. Saf*., 60 (3): 324-349. doi: 10.1016/j.ecoenv.2004.06.010.

Qi, F.; Zhang, F. (2020). Cell cycle regulation in the plant response to stress. *Front. Plant Sci*., 10: 1- 10. doi: 10.3389/fpls.2019.01765

Ramadan, K.M.A.; Alharbi, M.M.; El-Beltagi, H.S. and Darwish, D. (2022). Alpha Lipoic Acid as a Protective Mediator for Regulating the Defensive Responses of Wheat Plants against Sodic Alkaline Stress: Physiological, Biochemical and Molecular Aspects. *Plants,* 11 (787): 1- 16. doi:10.3390/plants11060787

Roy, G.; Sarma, B.K.; phadnis, P.P. and Mugesh, G. (2005). Selenium – containing enzymes in mammals; chemical perspectives. *Journal of Chemical Sciences,* 117(4):287-303. doi:10.1007/BF02708441

Seis, H. (2015). Oxidative stress: A concept in redox biology and Medicine. *Redox Biology*., 4:180-183. doi: 10.1016/j.redox.2015.01.002

Shaaban, S.H.A.; Manal, F. M. and Afifi, M.H.M. (2009). Humic acid foliar application to Minimize soil applied fertilization on surface irrigated wheat. *World Journal of Agriculture sciences*, 5 (2): 207 – 210. https://doi.org/10.5555/20093185745

Shareef, H. J. (2016). Improving Salt Tolerance in Date palm offshoots *Phoenix dactylifera* L. Berhi and Sayer cultivars using some Anti-Salinity Compounds. Doctoral dissertation, College of Agriculture, University of Basrah, Iraq.

Stepien, P.; Klbus, G. (2006). Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. *Biol. Plant*, 50 (4): 610–616. doi:10.1007/s10535-006-0096-z

Teisseire, H. and Guy, V. (2000). Copper – induced changes in antioxidant enzymes activities in fronds of duck weed (Lemna minor). *Plant Science*, 153 (1): 65 – 72. doi:10.1016/S0168-9452(99)00257-5

Tomaz, A.; Palma, P.; Alvarenga, P. and Gonçalves, M.C. (2020). Soil salinity risk in a climate change scenario and its effect on crop yield. In Climate Change and Soil Interactions; *Elsevier*, Amsterdam, Netherlands: 351–396. doi:10.1016/B978-0-12-818032-7.00013-8

Yaish, M. W., and Kumar; P. (2015). Salt tolerance research in date palm tree *Phoenix dactylifera* L past, present and future perspectives. *Front. Plant Sci.,* 6: 1 – 5. https://doi.org/10.3389/fpls.2015.00348

Youssef, M.H.; Raafat, A.; El-Yazied, A.A.; Selim, S.; Azab, E.; Khojah, E.; El Nahhas, N. and Ibrahim, M.F. (2021). Exogenous Application of Alpha-Lipoic Acid Mitigates Salt-Induced Oxidative Damage in Sorghum Plants through Regulation Growth, Leaf Pigments, Ionic Homeostasis, Antioxidant Enzymes, and Expression of Salt Stress Responsive Genes. *Plants (Basel),* 10 (11): 2519. doi: 10.3390/plants10112519

Yu, Z.; Duan, X.; Luo, L.; Dai, S.; Ding, Z. and Xia, G. (2020). How plant hormones mediate salt stress responses. *Trends Plant Sci*., 25 (11): 1117–1130. doi: 10.1016/j.tplants.2020.06.008