

# Effect Of Adding Sulfur And Glutathione On Some Biochemical Characteristics Of Leaves Of The Tissue Date Palm (*Phoenix Dactylifera L.*) Berhi Cultivar Under Heat Stress.

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## Abstract

A study was conducted on the effect of heat stress on some biochemical characteristics of the leaves of the tissue date palm variety Berhi and the role of adding sulfur (S) and spraying with glutathione (GSH) in alleviating the damage from heat stress. Five-year-old palm trees of approximately equal sizes were selected and planted in a private orchard south of Basrah Governorate, Iraq. And conducted the same service operations of fertilization and watering.

The following treatments were applied to date palm trees, as agricultural sulfur was added at a concentration of (0, 1, and 2) kg/tree, and glutathione at a concentration of (0, 100, and 200) mg/liter as well as their interactions. The results showed that the highest values with a significant effect for the traits (glutathione and chlorophyll) and the lowest value for a significant decrease in the traits (proline, carbohydrates, hydrogen peroxide and MDA) was for treatment (2 kg S x 200 mg/L GSH) recorded (45.92  $\mu\text{mol g}^{-1}$  and 6.38 mg 100 g<sup>-1</sup>). and (4.51  $\mu\text{mol g/g}$ , 10.66 mg/gm, 0.62  $\mu\text{mol g}$ , and 1.94 nmol/gm), respectively, compared with the control treatment that recorded (40.10  $\mu\text{mol g/g}$  and 3.36 mg/gm), and (11.19  $\mu\text{mol g/g}$ , 14.02 mg/gm and 1.06  $\mu\text{mol/g. g}$  and 4.27 nmol/g) respectively for the previous treatments. The results of the current study showed that the addition of sulfur and glutathione helped reduce the damage caused by heat stress and improve biological processes and growth.

**Keywords:** - heat stress, glutathione, sulfur, date palm.

## INTRODUCTION:

Environmental stress represents a major challenge in our quest for sustainable food production because it reduces yield by up to 70% in crop plants. (Ahmad and Prasad.2012) Heat stress is the most dangerous type of environmental stress that causes a defect in some plant functions and leads to oxidative stress and thus increases Levels of reactive oxygen species that in turn influence cytotoxicity and cause degradation of lipid membranes of cell walls affecting the regulation of intercellular exchange and plant growth, photosynthesis, pollen development, reproduction, protein denaturation and alteration in many enzyme activities (Hussain et al. 2019 ). However, an increase in temperature to such levels causes deterioration in plant growth and development, resulting in severe and irreparable damage often defined as heat stress. Usually, an increase in temperature, (10-15) degrees Celsius above the required rate is considered heat stress (Sharma, et al, 2017).

The date palm, (*Phoenix dactylifera L.*), is an evergreen fruit tree belonging to the family Arecaceae. It is a monocotyledonous plant and its cultivation is spread in tropical and semi-tropical areas between latitudes 15-30 north of the equator. The palm tree bears high temperatures, drought and salinity and is considered one of the most adaptable plants to the environment. Therefore, it is considered a symbol of the desert environment and the Arabian Gulf region is home to the palm tree (El-Shibli and Korelainen, 2009; Govarets and Dransfield, 2005). Palm shoots resulting from tissue culture grown in an environment with a temperature of 53-58 were affected by photosynthesis processes, leaf characteristics and the production of reactive oxygen species, as well as plant pigments and high levels of antioxidants (Khair & Karim, 2015; Al-busaidi, 2002). Textile palm trees are affected by high or low temperatures as they are caused by laboratory conditions and their leaves are thin and the amounts of protective wax on their leaves differ from what it is in the seedlings produced by their mothers in the field (Al-busaidi, 2002). Among (EL Hadrami et al., 2011) tissue palm offshoots were affected by abiotic stresses such as drought, salinity and temperature. Palms try to regulate their growth

and development processes to reduce the amount of damage caused by the environmental burden (Shareef.2019,). Moreover, the growth of date palm offshoots decreases from May to September as a result of the abnormal condition of salinity, water scarcity and high temperatures (Jasim et al. 2016). Palm shoots resulting from tissue culture grown in an environment with a temperature of 53-58 were affected by photosynthesis processes, leaf characteristics and the production of reactive oxygen species, as well as plant pigments and high levels of antioxidants (Khair & Karim, 2015; Al-busaidi, 2002). . Palm trees resulting from tissue culture are affected by high or low temperatures as they are the result of laboratory conditions and their leaves are thin and the amounts of protective wax on their leaves differ from what it is in the seedlings produced by their mothers in the field (Al-busaidi, 2002 ).E A study (Hadrami et al., 2011) showed that tissue palm offshoots were affected by abiotic stresses such as drought, salinity and temperature.. Palms try to regulate their growth and development processes to reduce the amount of damage caused by the environmental burden (Shareef.2019,). Moreover, the growth of date palm offshoots decreases from May to September as a result of the abnormal condition of salinity, water scarcity and high temperatures (Jasim et al. 2016). In order to reduce the damages of heat stress and improve crop production, various techniques have been introduced, including mineral feeding in the agricultural system to prepare various nutrients for plants. The use of agricultural sulfur (S) and glutathione (GSH) is one of the effective ways to make plants tolerant of heat stress (Ihsan et al. 2019).

Sulfur is an important nutrient required by plants under stress conditions. Sulfur compounds play an important role as structural components of important cellular molecules within the cell. Sulfur is of great importance in plant growth, meaning that through the formation of many amino acids and compounds responsible for the environmental response, such as cysteine (Cys), methionine (Met) and glutathione (GSH) it enters into the formation of protein. Plants need these sulfur biomolecules to develop effective defense mechanisms against various stresses (Rennenberg & Herschbach, 2012; Khan et al, 2014).

(Abbas et al., 2015) found that sulfur is one of the factors that reduce stress on plants, as it was used to reduce the damages of salt stress on date palm shoots. (Ihsan et al., 2019) showed that sulfur is one of the available methods as a heat stress reliever. He also showed the important role of sulfur as it is part of the components of vitamins and some amino and fatty acids and is included in the composition of some antioxidants. Glutathione (GSH) is a tripeptide in (c-glutamyl-cys teinyl-glycine; c-Glu-cys-Gly). The structure of GSH includes a bond between the carboxyl group of the glutamate side chain (Glu) and the amine group of cysteine (Cys) that is linked to glycine by a peptide bond. However, the thiol group is the main group for the chemical reaction with regard to its biological and biochemical functions (Zagorchev et al. 2013). The treatment of the palm trees of the Halawi cultivar with plant hormones led to an increase in the content of leaves and fruits of hormonal antioxidants, which is one of the mechanisms of plant resistance. To oxidative stress from high temperatures (Al Khalifa and. Al-Meer.2018).

Glutathione is the most important part of the plant's resistance system to stress, which is highly dependent on the supply of sulfur to plants. In sulfur deficiency, the GSH level in the cell drops rapidly and GSH returns to its original level when sulfur fertilizers are applied (Nazar et al., 2011).

Glutathione has multiple functions in plant growth and development that other antioxidants cannot perform, as it protects membranes by maintaining a reduced state of both a-tocopherol and zeaxanthin, preventing oxidative denaturation of proteins under stress conditions by protecting special thiol groups. out. It is also considered as a chelating material for toxic metals such as metalloids, then transported, and isolated in the gap. In addition to detoxifying ROS, GSH is involved in methylglyoxal (MG) detoxification.

Glutathione stabilizes or balance oxidative stress within the stress-affected plant cell. Glutathione enhances plant tolerance to various abiotic stresses including high temperature, low temperature (HT), salinity, drought, and toxic metal stress. Glutathione regulates cell proliferation, apoptosis, and fibrosis. Growth, development, cell cycle, gene expression, protein activity, and immune function (Noctor et al. 2012; Nahar et al. 2015a; Hasanuzzaman et al. 2017). The addition of sulfur and glutathione improved many of the biochemical and anatomical characteristics of date palm trees under heat stress (AL-Arab et al. 2022).

## **METHODS AND MATERIALS:**

The study was conducted in the season 2021 in one of the private orchards south of Basra Governorate in Iraq. Twenty-seven seedlings of date palm cultivar Al-Barhi cultivar, aged 5 years, were selected. To study the biochemical characteristics of leaves under heat stress conditions and the effect of treatment with agricultural sulfur and glutathione in mitigating the harmful effects.

Agricultural sulfur was added as a fertilizer for palm shoots at a concentration of (0-1-2) kg/palm and treated with glutathione sprayed on the leaves at a concentration (0-100-200) mg/l as well as the interactions. leaves samples were taken for the following tests::

### **1- PROLINE.**

The proline content of leaf samples was estimated according to the method (Bates et al, 1973).

115.5 molecular weight of proline.

## 2- GSH-GLUTATHIONE:

The glutathione content in the leaves was estimated according to the method of Moron et al. (1979). Glutathione reacts with dithiobis-2-nitrobenzoic acid (DTNB-5,5D TNB) to produce a yellow colour.

## 3- TOTAL CHLOROPHYLL:

The total chlorophyll pigment in leaves taken from the middle fronds was estimated by the method described by (1976, Goodwin).

## 4- TOTAL CARBOHYDRATES:

The method described by him (Watanabe et al., 2000) was used to estimate total carbohydrates,

## 5- HYDROGEN PEROXIDE (H<sub>2</sub>O<sub>2</sub>):

The H<sub>2</sub>O<sub>2</sub> content in the leaves was measured by applying the method described by Sergiev et al. 1997.)

## 6- MALONDIALDEHYDE (MDA) CONTENT:

MDA tissue content was estimated using the (Packer and Heath, 1968) method.

## EXPERIMENT DESIGN AND STATISTICAL ANALYSIS.

The experiment was designed using complete randomized blocks (CRBD) and the results were analyzed using Anatomical Structure Variance Analysis, SPSS. The averages were also analyzed and significance was tested by the least significant difference (RLSD) test at the level of (0.05) (Snedecor and Cochran, 1986). The study aims to know the effect of adding sulfur and glutathione in reducing the damage caused by heat stress, which may be one of the ways to counteract heat stress and improve the growth and production of date palm.

## RESULTS AND DISCUSSION

### Proline:

The results of Table (1) showed the effect of adding sulfur and glutathione on the proline content of leaves, as it was noted that all treatments were significantly superior to the control treatment in reducing the level of proline in leaves, and that the treatment (2 kg S x 200 mg/L GSH) recorded the lowest proline content of (10.73 Micromole GM-1) Significantly superior to all treatments. As for the highest level of proline, it was recorded in the comparison treatment and it was (11.29 micromol gm-1).

(Table (1) Effect of adding (S) and (GSH) on the content of date palm leaves of proline ( $\mu\text{mol g}^{-1}$ )

Sulfur kg/tree	Glutathione mg/L			sulfur average
	0	100	200	
0	11.29	11.23	11.21	11.24
1	11.23	10.99	10.91	11.04
2	11.19	10.88	10.73	10.93
Glutathione average	11.23	11.03	11.01	R.L.S.D=0.059

### GLUTATHIONE:

Table (2) shows the effect caused by the addition of sulfur and glutathione on the glutathione content of leaves under heat stress, as it was shown from the results of the table that the treatment (2 kg S x 200 mg/L) was significantly superior to all treatments by recording the highest level of glutathione in the leaves, which is (43.25 micromole g). As for the other treatments, there was a significant difference between them and the control treatment in the glutathione content of the leaves, which recorded the lowest value was (40.10 micromole g-1).

Find (2) the effect of adding (S) and (GSH) on the glutathione (GSH) content of date palm leaves ( $\mu\text{mol g}^{-1}$ )

Sulfur kg/tree	Glutathione mg/L			sulfur average
	0	100	200	
0	40.10	40.53	40.38	40.39
1	40.38	41.47	41.76	41.28
2	40.44	42.33	43.25	43.08
Glutathione average	40.56	41.40	42.78	R.L.S.D=2.685

## TOTAL CHLOROPHYLL:

The results in Table (3) indicate that there is a significant effect of adding sulfur and glutathione to tissue palm shoots under heat stress. The treatment (2 kg S x 200 mg/L GSH) achieved the highest rate of chlorophyll (3.50 mg.100 g) significantly superior to all The other treatments, including the control treatment, which recorded the lowest content of chlorophyll was (3.14 mg.100 g), with a significant difference from all treatments.

**Table (3)** Effect of adding (S) and (GSH) on the total chlorophyll content of date palm leaves (mg.100gm)

Sulfur kg/tree	Glutathione mg/L			sulfur average
	0	100	200	
0	3.14	3.19	3.19	3.17
1	3.21	3.33	3.37	3.30
2	3.27	3.39	3.50	3.38
Glutathione average	3.21	3.30	3.35	R.L.S.D=0.145

## CARBOHYDRATES:

Table (4) shows that the addition of sulfur and glutathione had a significant effect on the carbohydrate content of the leaves of the date palm cultivar Barhi under heat stress, as the carbohydrate rate decreased significantly in all treatments compared to the comparison treatment that recorded the highest carbohydrate rate of (14.02 mg / g) and that the treatment ( 2 kg S x 200 mg/L GSH) recorded the lowest rate of carbohydrates amounting to (12.25 mg/g), achieving a significant decrease in the rate of carbohydrates than all other treatments.

**Table (4)** Effect of adding (S) and (GSH) on the carbohydrate content of date palm leaves (mg/g)

Sulfur kg/tree	Glutathione mg/L			sulfur average
	0	100	200	
0	14.02	13.73	13.69	13.81
1	13.71	13.24	12.91	13.28
2	13.61	12.73	12.25	12.86
Glutathione average	13.78	12.23	12.95	R.L.S.D=0.025

## HYDROGEN PEROXIDE (H2O2):

The results of Table (5) clarified the effect of adding sulfur and glutathione on the hydrogen peroxide content of leaves, as it was noted that all treatments recorded a significant difference over the comparison treatment in reducing hydrogen peroxide level in leaves, and that the treatment (2 kg S x 200 mg/L GSH) recorded less Average hydrogen peroxide amounted to (0.65) micromole g with a significant difference from all treatments, while the highest level of proline was recorded by the control treatment and it was (1.07) micromole g.

**Table (5)** Effect of adding (S) and (GSH) on the content of date palm leaves from (H2O2 (micromol g)

Sulfur kg/tree	Glutathione mg/L			sulfur average
	0	100	200	
0	1.07	0.92	0.86	0.95
1	0.90	0.79	0.74	0.81
2	0.83	0.73	0.65	0.74
Glutathione average	0.93	0.81	0.75	R.L.S.D=0.033

## MALONDIALDEHYDE (MDA):

The results of table (6) showed the moral effect of adding sulfur and glutathione to the heat-stressed Burhi date palm trees, as all treatments achieved a significant decrease of the MDA rate in leaves from the highest rate recorded by the comparison treatment which was (4.27 nmol/gm), while the treatment recorded (2 kg S x 200 mg/L GSH) had the lowest rate of MDA (3.908 nmol/g) with a significant difference from all other treatments.

**Table (6)** Effect of adding (S) and (GSH) on the content of date palm leaves from (MDA) (nmol/g)

Sulfur kg/tree	Glutathione mg/L			sulfur average
	0	100	200	
0	4.27	3.99	3.92	4.06
1	3.98	3.82	3.78	3.86
2	3.92	3.26	3.08	3.42
Glutathione average	4.05	3.69	3.59	R.L.S.D=0.014

## DISCUSSION:

Environmental stress affects a decrease in the biological characteristics of the plant and its degradation. The degree of damage depends on the type of stress, the duration of the stress period, and the ability to adapt to it (Watkins et al. 2017). The decrease in chlorophyll content may be a direct result of heat stress, which causes a deterioration in the biological composition of the various plant pigment parts and damage to the membranes, and may cause a decrease in the moisture content of leaves and thus affect photosynthesis (Hnilickova et al. 2017; Shah et al. 2017).

Perhaps the reason for the increase in the content of glutathione is because it works in removing stress by binding to toxic molecules and then enzymes bind to glutathione, or it may be due to the fact that some enzymes use glutathione as an aid in their work because glutathione is small reducing and oxidative molecules and it also has a role in the formation of salicylic acid and plant defense signals (Rouhier et al. 2008). It works to increase the efficiency of the roots to absorb nutrients, especially NPK. (Abdi et al., 2011)

Proline may have a direct role as an antioxidant agent in removing or controlling ROS in plants under stress, or an indirect role in balancing the oxidative state in plant tissues through the activation of antioxidant enzymes. It may be explained that heat stress induces ROS formation, either directly via the Fenton and Weiss-Harber reactions, or indirectly by inhibiting plant antioxidant mechanisms (Remero-Puertas et al. 2007). Hydrogen peroxide is one type of ROS that plays several roles. Roles in the plant, as well as its accumulation in plant tissues in response to stress (Abass and Morris. 2013). This study agreed with some previous studies, as it was (Awad et al. 2019) found that the exposure of date palm to poisoning resulting from stress of the aluminum element at a concentration (200 mg/L) caused a reduction in total proteins and free amino acids, as well as the accumulation of malondialdehyde, H<sub>2</sub>O<sub>2</sub> and peroxidase and the generation of toxic reactive oxygen species. found (Faisal and Abdullah.2021) that adding sulfur at a concentration of 500 g/palm tree increased the percentage of total sugars, reducing sugars in fruit, chlorophyll, and total carbohydrates, in the leaves compared to the control, and this treatment also led to A significant decrease in the percentage of the concentration of proline and sodium in the leaves compared to the control treatment. The use of sulfur with silicon, boron and zinc on oil palm increased the yield and amount of chlorophyll in the leaves (Nur et al.2021). When adding sulfur and humic acid, it led to an increase in the content of the fruits of nitrogen, phosphorous, yield and total sugars (Tuaema.2021).

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