



Effect of sulfur and glutathione treatment on some anatomical characteristics of the tissue date palm leaves Phoenix dactylifera L. Barhi cultivar under heat stress

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Abstract:

The study was conducted in one of the orchards located in the south of Basrah Governorate / Iraq. In the growing season of 2021 to show the effect of adding sulfur at a concentration of (0, 1, 2) kg / seedling and glutathione at a concentration of (0, 100 and 200) mg / liter and their interactions in reducing the damage caused by heat stress on the anatomical characteristics of the leaves of the palm tree cuttings of the cultivar Barhi, and the results showed the superiority of the treatment (2 kg S × (200 mg/L GSH) in the average length and width of the vascular bundle and thickness of wood and bark and on the comparison treatment which recorded (252.1, 177.7, 84.8 and 65.6) μm , respectively, while the comparison treatment recorded the lowest values for the mentioned traits and it was (216.0, 140.8 and 68) .4 and 54.4 μm , respectively, and no treatment recorded a significant superiority for the characteristics of upper and lower epidermal layer thickness and cuticle thickness for both skins, and the widest spread of tannin cells in the control treatment, while the treatment (2 kS x 200 mg/L GSH) recorded the least spread of tannin cells.

Key words: sulfur, glutathione, anatomical characteristics, heat stress.

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Introduction:

Abiotic stress is one of the major constraints to crop production and food security worldwide. Crop growth is adversely affected by abnormal temperatures due to physical damage, physiological disturbances and biochemical changes reducing crop yield by an estimated 50% annually. (Hasanuzzaman et al. 2012a; Fahad, et al. 2017).

Heat stress is often defined as a rise in temperature above the levels necessary for plant growth and development for a period of time sufficient to cause irreparable damage. In general, an increase in temperature, (10-15) degrees Celsius above the required rate is considered heat stress (Sharma, et al, 2017). Heat stress causes a defect in some plant functions and leads to oxidative stress, and thus increases the levels of reactive oxygen species, which in

turn affects the events of cellular toxicity and causes the deterioration of the fatty membranes of cell walls, which affects the regulation of exchange between cells and plant growth, photosynthesis, pollen development, and reproduction. Protein denaturation and alteration of several enzyme activities (Hussain et al. 2019).

The date palm, *Phoenix dactylifera* L., is an evergreen fruit tree belonging to the Arecaceae family. It is a monocotyledonous plant. Its cultivation is spread in tropical and subtropical areas between latitudes 15-30 north of the equator. The Arab Gulf region is home to the palm tree and considers it a symbol of the desert environment, as it is one of the most adapted plants to the desert environment because it bears high temperatures, drought and salinity



El-Shibli and Korelainen, 2009) ; Dransfield, Govarets, 2005. Temperature plays an important role in plant growth. Although date palms can withstand harsh climatic conditions, excessive and widespread levels of environmental stresses, including sudden rise in temperature and drought, may cause severe damage, significant reduction in yield and quality. Fruits and reduce physiological and metabolic processes in plants (Patankar et al. 2019).

Palm trees resulting from plant tissue cultivation 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). Between (Hadrami et al., 2011) tissue palm offshoots were affected by abiotic stresses such as drought , salinity and heat. studying the effect of these stresses is one of the challenges facing plant growth and development. The palm shoots resulting from tissue culture exposed to the effect of high temperatures between 53-58 were affected by photosynthesis and leaf characteristics, oxidation complications occurred and the production of reactive oxygen species, as well as the effect of plant pigments and high levels of antioxidants (Khair & Karim, 2015; Al-busaidi, 2002).

To improve the performance of crops under heat stress, various techniques such as mineral feeding have been introduced into the agricultural system to process various nutrients for plants. The use of agricultural sulfur and glutathione (GSH) is one of the effective ways to enhance plant tolerance against high temperature stress (Ihsan et al. 2019).

Treating al-Halawi palm trees with plant hormones led to an increase in the leaves and fruits content of hormonal antioxidants, which are one of the mechanisms of plant resistance to oxidative stress resulting from the high temperature (Al Khalifa and. Al-Meer.2018).

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. Moreover, it has an important role as a signaling factor for intercellular communication, and the importance of sulfur for plant growth lies in the fact that it enters into

protein formation through the formation of many amino acids and compounds responsible for environmental response such as cysteine (Cys), methionine (Met) and glutathione (GSH). 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 chemical reaction group with respect to its biological and biochemical functions (Zagorchev et al. 2013).

Glutathione is the most important part of a plant's stress system that is largely dependent on sulfur supply to plants. In the case of sulfur deficiency, the level of GSH in the cell drops rapidly while the use of sulfur fertilizers has returned to its original level (Nazar et al., 2011)..

Glutathione has multiple functions in plant development and growth that other antioxidants cannot perform. For example, it protects membranes by maintaining a reduced state of both a-tocopherol and zeaxanthin, preventing oxidative denaturation of proteins under stress conditions by protecting their thiol groups. Glutathione also helps chelate toxic metals such as metalloids and then transport and isolate them in the vacuole. Apart from detoxifying ROS, GSH is involved in methylglyoxal (MG) detoxification.

Glutathione detoxifies, biosynthesis and stabilizes oxidative stress within the stress-affected plant cell. Glutathione enhances plant tolerance to various abiotic stresses including salinity, drought, high temperature (HT), low temperature and toxic mineral stress. Glutathione regulates cell proliferation, and

death. Apoptosis, fibrosis, growth, development, cell cycle, gene expression, protein activity, and immune function (Noctor et al. 2012; Nahar et al. 2015a; Hasanuzzaman et al. 2017)

Materials and methods:

The study was conducted in the season 2021 in one of the private orchards in Siba district, Basra Governorate, southern Iraq. 27 seedlings of date palm cultivar Al-Barhi cultivar, aged 5 years, were selected. To study the changes in the anatomical structure of leaves under heat stress conditions and the effect of treatment with agricultural sulfur and glutathione in mitigating the harmful effects.

The treatments of the experiment were carried out as agricultural sulfur was added at a concentration (0-1-2 kg/vessel) and the amino acid glutathione was sprinkled on the leaves at a concentration (0-100 mg/L-200 mg/L) as well as the interaction treatments.

Paper samples were taken for anatomical examinations, as leaf samples were collected from the middle row and five sheets were taken from each duplicate and the fixation process was performed on them in A.A.F solution for 48 hours, then the samples were passed with ascending concentrations of ethyl alcohol. Samples were immersed in paraffin wax at a temperature of 58-60°C, and leaf models were cut by Microtome Rotary with a thickness of 10 µm, and loaded onto glass slides. To stain the samples, they were placed in Safranin dye for 30-60 minutes and then placed in Fast green dye for 15 seconds, after which the slide cover was placed with drops of DPX to prepare the slide for microscopy (Tomlinson, 1990; Thomas and Franceschi, 2013). Then the slides were studied and the measurements were taken in µm meter micro by a micrometer ocular lens in an Olympus type optical microscope equipped with a camera connected to a computer

Experiment design and statistical analysis.

The experiment was designed using randomized complete blocks (CRBD) and the results were analyzed using analysis of variance for anatomical structure, the SPSS statistical program. Also, the averages were analyzed and significance was tested according to the least significant difference test (RLSD) at the probability level (0.05) (Snedecor and Cochran, 1986).

Results :

The results of Table (1) showed the effect of treatment with sulfur and glutathione on the length of the vascular bundles of the leaves of the tissue date palm variety Barhi. Which recorded the largest length of the bundle, which amounted to (252.1 µm), with a significant superiority over the comparison treatment, which recorded the lowest length of the vascular bundle, which amounted to (216.0 µm).

Table (2) shows the extent to which the width of the vascular bundle was positively affected by the addition of sulfur and glutathione under high temperature stress, as the bandwidth in the interaction treatment reached ((2 kg S x 200 mg/L GSH (177.7 µm), recorded the highest values with significant superiority than the comparison treatment Which recorded lows value of (140.8 µm).

The results in Table (3) showed the effect of adding sulfur and glutathione on the average thickness of wood, leaves, palm trees, cultivar Barhi under high temperature stress, as it was the interference treatment.

((2 kg S x 200 mg/L GSH had the most effect on the amount of wood thickness as it reached) 84.87 (µm) with a significant superiority than the comparison treatment which recorded the lowest value of wood thickness which was (78.40 µm).

As for the thickness of the bark, the results of Table (4) showed the effect of adding sulfur and glutathione on the average thickness of the bark leaves of the palm tree tissue variety Barhi under high temperature stress. (65.60 (µm) with a significant difference from the comparison treatment which recorded the lowest thickness of the bark was (78.40 µm).

Regarding the thickness of the epidermis and the upper and lower cuticle layers, the results of the table (5, 6, 7 and 8) showed the effect of treatment with sulfur and glutathione on the thickness of the upper and lower epidermis and the cuticle layers for both skins. . The pictures in panel (2 and 3) showed the upper and lower epidermal layers and the two cuticle layers in the study parameters.

Plate (1) showed the proliferation of tannin compounds in tissue cells of date palm leaves of Barhi variety treated with sulfur and glutathione, as histological sections showed the proliferation

of dense tannin in the comparison treatment, and it was noted that the proliferation of tannin cells varied among other treatments, and that the treatment (2kgS×200mg\ l GSH) had the lowest prevalence of tannin in comparison with other treatments including the control treatment. The proliferation of tannin cells was observed around the large and small vascular bundles.

treatment	0	100mg/l GSH	200mg/lGSH	average
0	216.0	212.6	235.7	221.4
1kgS	219.4	242.6	246.4	236.1
2kgS	237.8	248.2	252.1	246.0
average	224.4	234.4	244.7	LSD=18.59

Table (1) Effect of sulfur and glutathione on average length of vascular bundles (µm) of leaves of the tissue date palm cultivar Barhi.

treatment	0	100mg/l GSH	200mg/lGSH	average
0	140.08	146.27	149.33	145.46
1kgS	149.47	159.93	166.80	158.73
2kgS	155.87	169.33	177.77	167.65
average	148.71	158.51	164.63	LSD=8.519

Table (2) Effect of sulfur and glutathione on the average width of the vascular bundles (µm) of the leaves of the tissue date palm cultivar Barhi

treatment	0	100mg /l GSH	200mg/lG SH	average
0	78.40	80.93	81.53	80.60
1kgS	81.30	82.47	82.63	81.70
2kgS	83.80	82.80	84.87	83.38
average	81.16	82.12	82.95	LSD=1.864

Table (3) Effect of sulfur and glutathione on average wood thickness (µm) of leaves of the tissue date palm cultivar Barhi

treatment	0	100mg /l GSH	200mg/lG SH	average
0	54.40	55.47	58.00	57.88
1kgS	56.80	59.47	63.77	58.09
2kgS	62.83	63.67	65.60	64.03
average	58.01	58.77	62.63	LSD=1.864

Table (4) Effect of sulfur and glutathione on average phloem thickness (µm) of leaves of the tissue date palm cultivar Barhi.

treatment	0	100mg /l GSH	200mg/lGS H	average
0	4.83	4.93	5.10	4.95
1kgS	5.12	5.13	5.23	5.16
2kgS	5.21	5.21	5.36	5.99
average	5.05	5.09	5.70	LSD=0.726

Table (5) Effect of sulfur and glutathione on the average thickness of the upper epidermis (µm) of leaves of the tissue date palm cultivar Barhi.

treatment	0	100mg /l GSH	200mg/lGS H	average
0	2.80	2.82	2.94	2.85
1kgS	2.86	2.91	2.97	2.91
2kgS	2.96	2.93	3.08	2.99
average	2.87	2.88	2.99	LSD=0.714

Table (6) Effect of sulfur and glutathione on the average thickness of the lower epidermis (µm) of leaves of the tissue date palm cultivar Barhi

treatme nt	0	100mg /l GSH	200mg/lGS H	average
0	2.00	2.17	2.46	2.21
1kgS	2.30	2.43	2.66	2.46
2kgS	2.46	2.59	2.63	2.48
average	2.25	2.43	2.65	LSD=0.67

Table (7) Effect of sulfur and glutathione on the average thickness of the lower epidermal cuticle (µm) of leaves of the tissue date palm cultivar Barhi

treatme nt	0	100mg /l GSH	200mg/lGS H	average
0	2.40	2.46	2.53	2.46
1kgS	2.48	2.53	2.55	2.52
2kgS	2.55	2.57	2.57	2.57
average	2.47	2.52	2.55	LSD=0.205

Table (8) Effect of sulfur and glutathione on average thickness of the upper cuticle (µm) of leaves of the tissue date palm cultivar Barhi

Discussion:

The reduction in the size of the vascular bundles may be attributed to their effect on abiotic stress, which caused a reduction in the number and size of the transport vessels (wood and phloem), or the deterioration of the transporting elements. Sandalio et al., 2001; Vollenweider et al., 2006) and this was confirmed by the current study and it agreed with the study (Neama et al., 2017). The decrease in the diameter of the vascular bundles is directly related to the reduction in the area of the woody vessels, which is responsible for nutrient delivery (Ortega et al. 2006). The effect of abiotic stress on reducing the thickness of the vascular bundles may be due to the plant's adaptation to environmental stress by the mechanism of uptake and transport of water and nutrients from the roots, which is associated

with increased stress (Ceccoli et al. 2011.) Abiotic stress stimulates the plant to make some tissue modifications in order to Maintaining the most important water content within the tissues and reducing the evaporation process by increasing the thickness of the epidermis and cuticles (Hameed et al. 2013).The increase in the number of epidermal cells or the decrease in the volume of the median tissue is due to the influence of the tissue inside the leaf by stress, which reduces the length and diameter of the bundles and reduces Photosynthesis (Atabayeva et al. 2013) The results of the study (Alnajjar, et al. 2020) indicated that the use of antioxidants led to the thickness of the epidermal layer, the upper and lower epidermis and the cuticle layers of the epidermis, the length and width of the vascular bundles, and The diameter of the primary and secondary xylem, and the thickness of the bark of the leaf. The upper two properties of the epidermis play an important role in isolating reactive oxygen species (ROS) away from mesenchymal cells important in photosynthesis processes (2011. (Gomes et al.. A study (Shareef and Sweed.2020) showed that heat stress is the most harmful factor for the anatomical characteristics of the plant than other stress factors, as abiotic stress led to an increase in the thickness of the upper epidermis of leaves and an increase in the thickness of the acuticle in July compared to other treatments. The reason is due to some changes in the anatomical structure of the date palm leaves as a result of their exposure to heat stress, as it is considered as a defense method to reduce water loss by increasing the thickness of the cuticle layer (Challa and Van Beusichem. 2004).The presence of environmental stress led to a significant decrease in the thickness of the epidermis with a decrease The number of epidermal cells from the upper and lower epidermis thus reduces leaf thickness (Duarte et al. 2013).

Tannin compounds are naturally present in plant tissues in small quantities, and the plant's production of these compounds increases when exposed to oxidative stress. (Abass et al. 2016; Dalcoros et al. 2013) Stresses, including oxidative stress, lead to an increase in the build-up and accumulation of phenolic compounds, including tannin, in plant tissues as a non-enzymatic defense mechanism to mitigate the harmful or toxic effects of free radicals and the active oxygen species ROS formed by stress. This



is in view of the fact that these compounds have antioxidant properties (Agati et al. 2012; Zhang et al. 2015) and the result of this study is similar to the results of many studies that showed that the accumulation of reactive oxygen species ROS is a common feature of all stresses to which plants are exposed, and this is what Explains the increased production of tannins that are an adaptive response as a non-enzymatic defense mechanism to counteract the harmful and toxic

effects of oxidative stress.(Constabel and Lindroth 2010).

The results of the current study showed that the increase in temperatures to extreme levels led to changes in the anatomical structure of the leaves of date palm trees, which may be part of the plant's defense mechanisms to reduce the harmful effect of heat stress, or to its effect in increasing the concentrations of the active oxygen species ROS with toxic effects on plant cells.

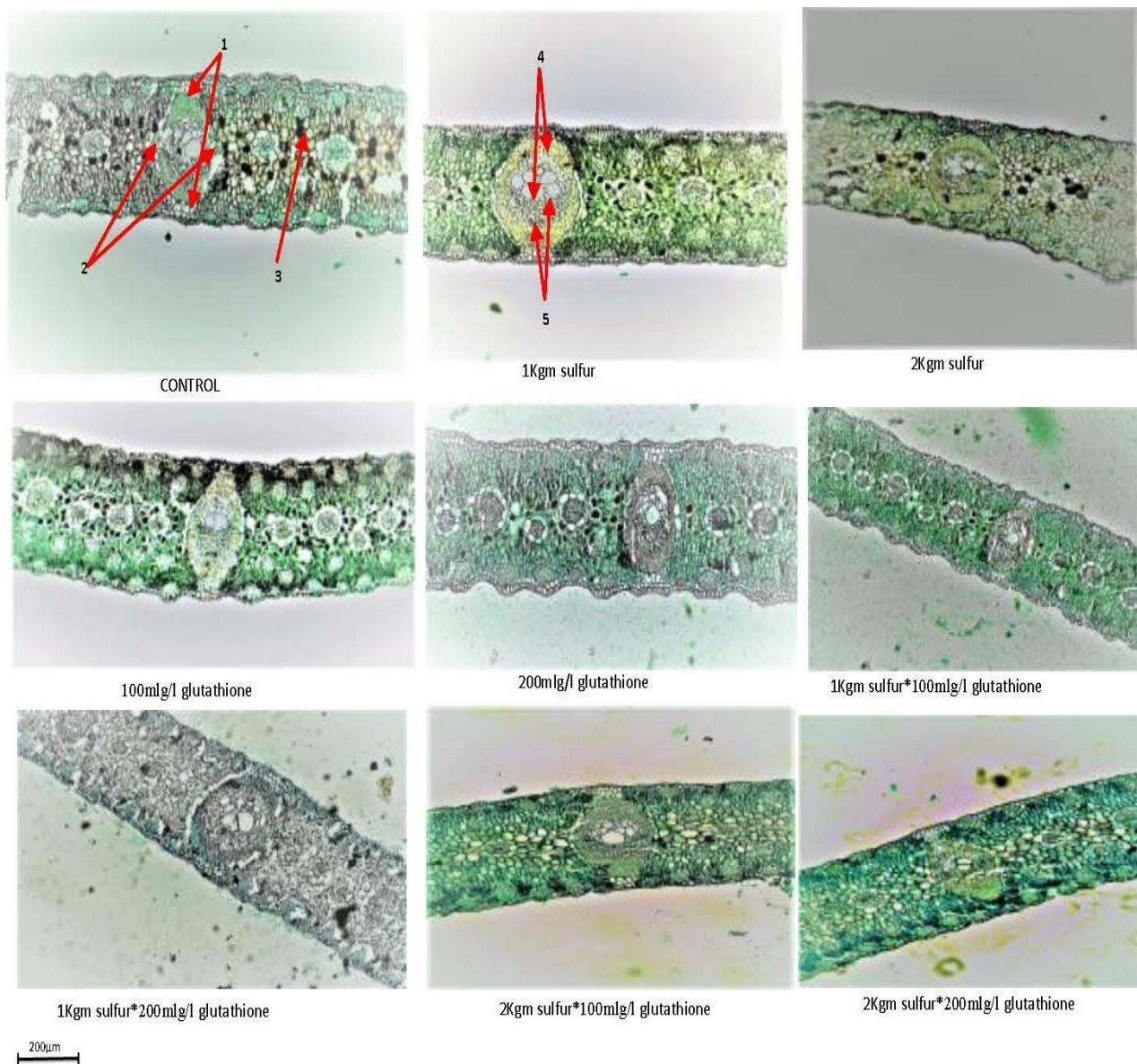


Plate (1): Effect of sulfur and glutathione supplementation on the length and width of the vascular bundle, the thickness of wood and phloem, and the proliferation of tannin cells.

1- length vascular 2- width vascular 3- tannin cells 4- thickness of wood 5- thickness of phloem

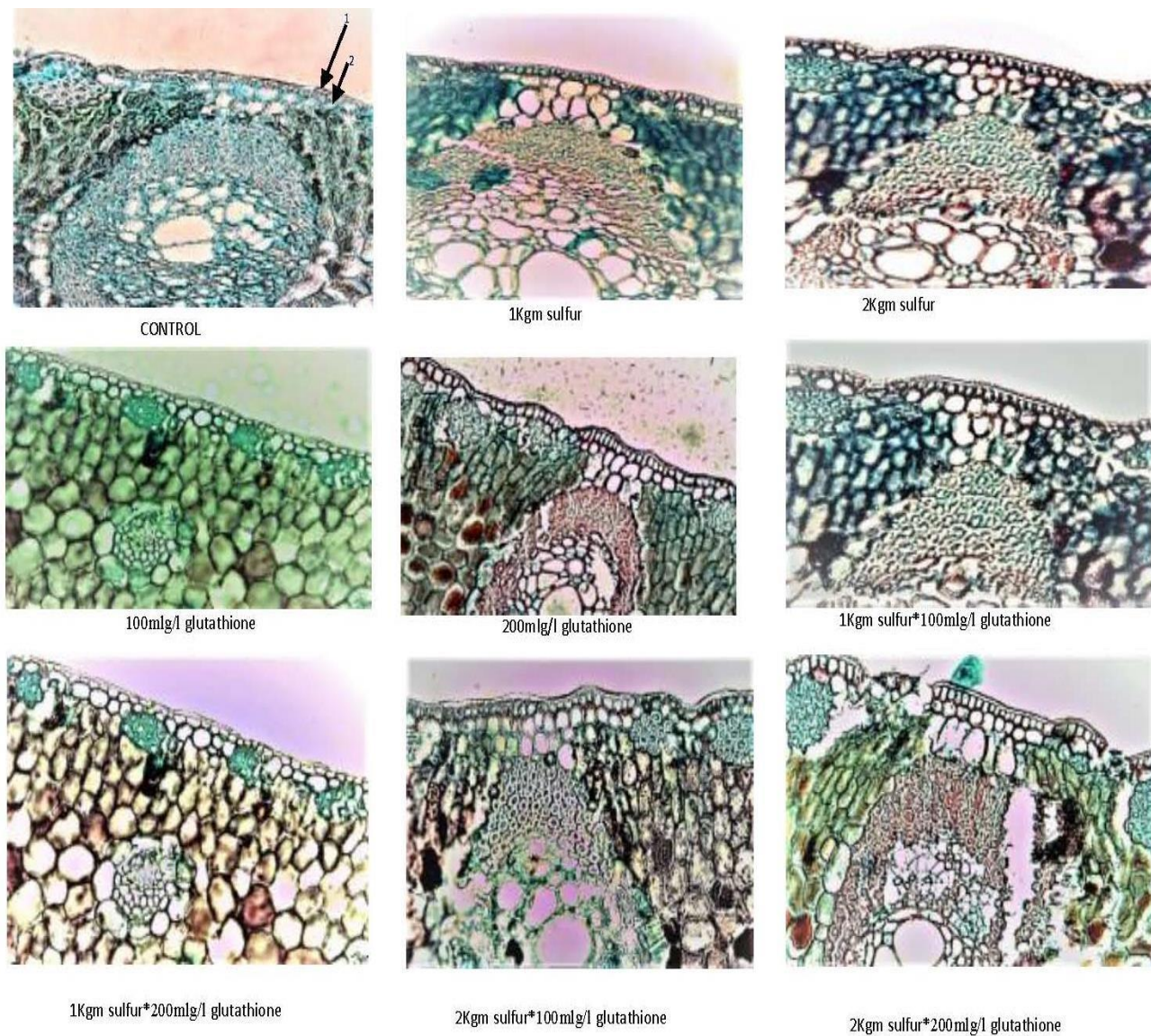


Plate (2) Effect addition of sulfur and glutathione in the thickness of the upper epidermis and the upper cuticle

1-cuticle 2-epidermis

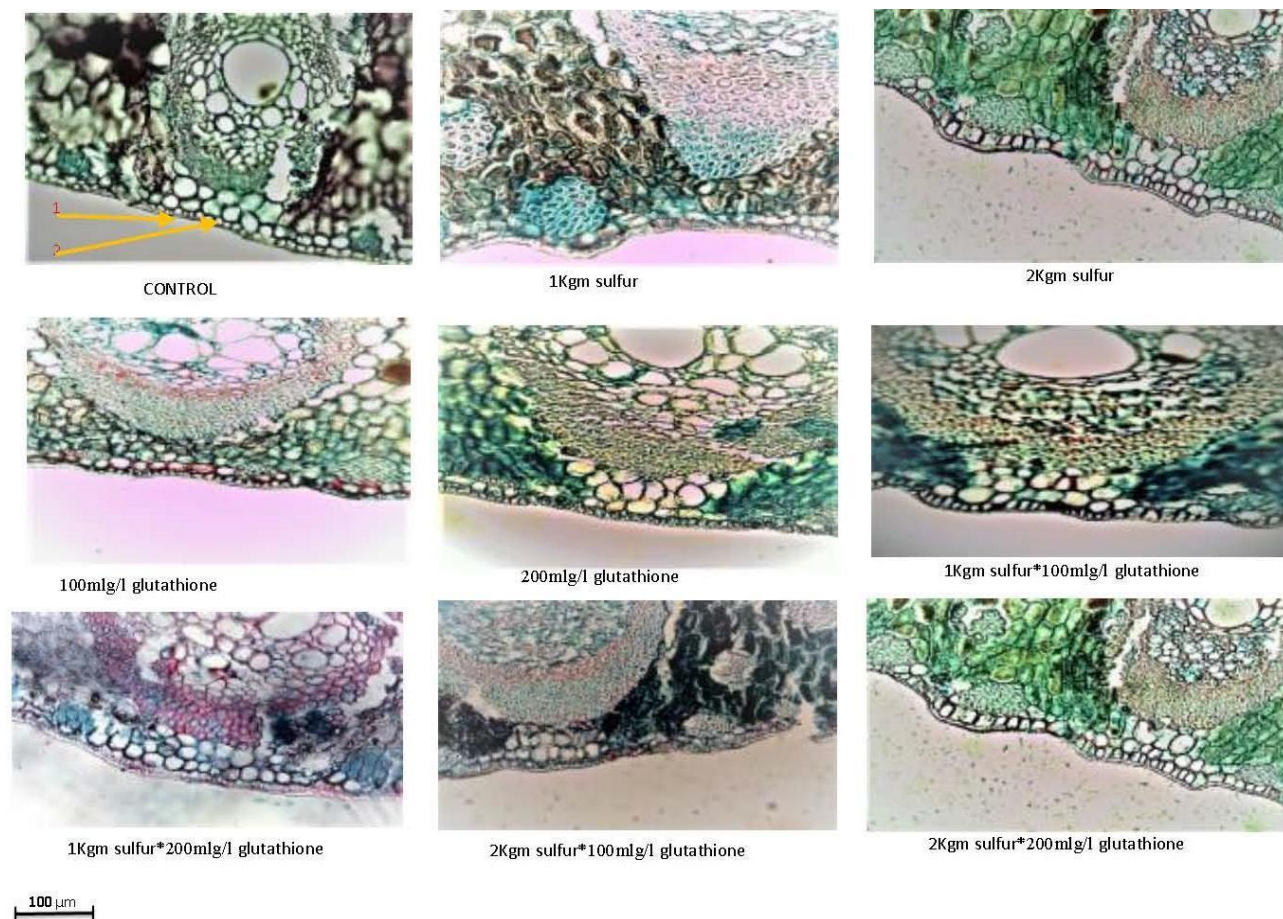


Plate (3) Effect of adding sulfur and glutathione on the thickness of the lower epidermis and the lower cuticle under heat stress

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