Response of grape seedlings (*Vitisvinifera L.)*Halwani variety for adding ascorbic acid and glutathione compound on some vegetative traits

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Abstract

This experiment was conducted in the saran-covered plastic house of the College of Agriculture and Marshes, Thi-Qar University, on 20.02.2022, to study the effect of spraying with glutathione and ascorbic acid on the physical and chemical properties of the grape plant *Vitisvinifera* L at one year age.The effect of glutathione compound at four concentrations (0, 75, 150, 225) mg.l^{-1} and ascorbic acid at four concentrations also (0, 50, 100, 150) mg.l^{-1}, and the interaction between them was studied and implemented as a factorial experiment with two-factors(4×4×3) by following the randomized complete block design (RCBD) with three replications (48 seedlings per replicate). The averages were tested using the least significant difference L.S.D at the probability level of 5%.The most important results obtained indicate that the effect of spraying with glutathione significantly on the studied vegetative characteristics, where the spraying treatment (G3) with a concentration of (225 mg l^{-1}) was significantly superior to the control treatment (G0) in (plant height, leaves number, leaf area), where it gave the highest averages, respectively (99.637 cm, 34.427 leaves. plant^{-1}, 118.772 cm^2). As for the leaves carbohydrate content, it amounted to (18.483%), while the values of the leaves nutrients content (nitrogen, phosphorous) (0.283%, 1.275%) respectively.As for ascorbic acid, it had a significant effect on the studied vegetative characteristics, the spraying treatment (C3) with a concentration of (150 mg l^{-1}) was superior to (plant height (cm), leaves number (leaf plant^{-1}), leaf area (cm^2). Where it gave the highest averages, respectively (98.991, 32.647, 112.533 and carbohydrates (18.259%), while the percentage of nitrogen and phosphorous reached (0.274%, 1.192%).As for the binary interaction (compound glutathione + ascorbic acid) at a concentration (225 mg l^{-1} + 150 mg l^{-1}), the treatments showed apparent statistical differences, as the treatments excelled in vegetative and chemical characteristics (plant height, leaves number, leaf area, carbohydrates content of Leaves, nitrogen percentage, phosphorous percentage) had a significant effect on the comparison treatment. 8166

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1. INTRODUCTION

Grapes (*Vitisvinifera* L.)belong to the Vitaceae family, one of the trees of the temperate regions in Iraq or the world. Its cultivation has beenancient in Iraq since the emergence of the first civilizations to suit the environmental conditions. The cultivation of grapes was known to the Sumerians, and grape trees were found in the Hanging Gardens of Babylon.The grapes were mentioned in the Holy Qur'an eleven times. The area between the southern Black Sea and the Caspian Sea is in the middle of the area agreed upon by most botanists as the origin of the European grape *Vitisvinifera* L., from which all grapes originated before the discovery of the North American continent, and then spread Cultivation of it to the east and west (Xian, 2009).

Grapes occupy the first place among the various fruit trees in production and cultivated area. The cultivated area in the world is estimated at 8291220 hectares, and the total production is 74584600 tons. In Iraq, the total production is estimated at 896070 tons, the number of grape trees is 17.570000, and the production of one grape tree is 51 kg. Contains every 100 g of fresh grapes contains 81% water, 67 calories, 0.6 g of protein, 0.3 g of fat, 18 g of carbohydrates, 100 international units of vitamin A, 15 mg of vitamin B1, 20 mg of vitamin B2, vitamin B6, 50 mg vitamin, 50 mg. C, 170 mg potassium, 3 mg sodium, 18 mg iron, 12 mg phosphorous, 12 mg calcium (Al-Rubaie, 2009).

The grape is a deciduous tree with fast fruiting. The reason for human interest in this plant type is due to the importance of its fruits in terms of nutritional and therapeutic high. The fruits are eaten fresh or dried and used in various food manufacturing processes, such as molasses and vinegar. Grapes have many medical and therapeutic benefits as they prevent constipation and regulate glucose and cholesterol levels in the body. It protects against bowel cancer and is described as a stimulant for brain cells and heart muscles and a tonic for the liver and kidneys (Al-Abadi, 2019).There is great interest at present focused on the external addition of vitamins to improve plant growth and development, as they are natural products, including ascorbic acid (vitamin C), which is made biologically in high-end plants and affects plant growth and development, and plays an important role as a catalyst in enzymatic reactions in the transport system Electrons and metabolic processes (El - Kobisy*et al*.,, 2015).

Ascorbic acid is involved in many activities, including photosynthesis, cell wall growth, cell elongation, resistance to environmental stresses, and the biosynthesis of ethylene, gibberellins, anthocyanins and proline pigments (Galal*et al*.,, 2000; Smirnoff and Wheeler, 2000), Blokhina*et al.*, (2003) reported that ascorbic acid is one of the most abundant antioxidants that protect plant cells, and has been considered as a regulator of cell division and differentiation, adding that it is used in a wide range of functions. Function as a defensive antioxidant, photoprotection, and regulation of photosynthesis and growth.

Recently, it has been adopted to use modern agricultural methods that seek to improve plant growth, including biostimulants and antioxidants that have a role in curbing the oxidation and reduction processes in the plant cell. One of these antibiotics is the use of glutathione tripeptide, which is the most abundant in plant tissues, and has multiple functions in the development and growth of the plant cannot make or contribute other antioxidants, such as detoxification and stabilization or oxidation balance within the plant cell (Noctor*et al*.,, 2012).Its effect on plant growth is similar to that of growth regulators that encourage growth, as well as its role in reducing stress caused by temperature and toxins and stimulating respiration and cell division. It helps absorb nutrients, which is why it should be used with the glutathione compound to provide the plant's requirements that the roots are unable to provide.

This study was conducted with the aim of:-
1- Detection of the effect of some growth stimulants glutathione and ascorbic acid with different concentrations on grape seedlings' physical and chemical properties in Dhi-Qar Governorate.
2- Knowing the best concentrations for each of the factors of the study and the interaction between them in giving the trait of superiority to each of the studied features.

2. MATERIALS AND METHODS

2-1- The experiment location

The experiment was carried out in the saran-covered plastic house of the College of Agriculture and Marshes, University of Dhi-Qar, during the growing season 2021-2022. A number of one-year-old grape seedlings were selected to know the effect of spraying with glutathione and ascorbic acid on the physicochemical characteristics of grapes grown in Dhi-Qar governorate. An agricultural growth media consisting of mixed soil and peat moss was used in a ratio of 1:3. A random sample was taken from the river mixture and a sample of peat moss and analyzed in the laboratories of the Directorate of Agriculture of Dhi-Qar to identify some chemical properties of soil and peat moss.

Table (1) the chemical and physical properties of soil properties

Characteristic	Measuring unit	Value
pH		7.5
EC	$ds.m^{-1}$	2.51
Texture	Silty loam	loamy sand
Sand	$mg\ l^{-1}$	69.24
Silt		15.44
Clay		15.32
N		25.89
P		7.55
K		116.78

2-2 Study Factors:
The study included a factorial experiment with two factors:
Factor I: Glutathione complex in four concentrations (0, 75, 150, 225) $mg.l^{-1}$
Factor 2: ascorbic acid in four concentrations (0, 50, 100, 150) $mg.l^{-1}$

2-3 Dates and times of spraying
Grape seedlings, Halawani cultivar, were sprayed with glutathione at concentrations (0, 75,150, 225) $mg.l^{-1}$ and ascorbic acid at concentrations (0, 50,100,150) three times and according to the following dates:
First spray: On (25.03.2022) after the vegetative buds have opened
Second spray: 15 days after the first spray (10.04.2022)
Third spray: 15 days after the second spray (25.04.2022)
Measurements were taken a month after the third spray (25.05.2022).
A 5-liter capacity hand sprayer was used after adding the Tween-20 % to the spray solutions at a concentration of 0.1%to reduce the surface tension.

2-4 studied traits
1- Height plant (cm):-
The height of the plant was measured from its contact area in the soil to the end of the top of the main stem using metric tape.
2- Leaves number (leaf $plant^{-1}$):-
The number of leaves per plant was calculated by counting the number of leaves on the main stem, stalks and lateral branches for each seedling each repeat.
3- Leaves area (cm^2):
The plant's leaf area was measured by a leaf area measuring device (CI-202 LASER AREA METER) produced by the American CID company (C i D. BIO-Science Made in U-S-A).

Chemical properties
The total carbohydrate content was estimated by the method of Hedge and Hofreiter (1962), (0.2gm) of the sample to be measured is weighed, and (25 ml) of perchloric acid (1N) is added to it and placed in a test tube. The tubes are placed in a water bath at a temperature of (60) for 30 min. Then the sample is filtered using filter papers. A volume of (1 ml) is taken from the filtrate, and (9 ml) of distilled water is added to it to complete the volume to (10 ml) in a volumetric vial.Take from the last (1 ml) and add to it a concentration of phenol (5%) + (5 ml) concentrated sulfuric acid and leave it until it cools. It is measured at a wavelength (490 nm) with a spectrophotometer that prepares several concentrations of glucose (0.1, 0.2, 0.3, 0.4, 0.5), and the absorbance of the above readings are recorded (to make a calibration curve). Then the absorbance of the model is read and projected onto the calibration curve, and the concentration is extracted from it. Apply the following equation:

$$X = \frac{\text{Calibration curve concentration x (10 ml) x (25 ml)}}{\text{(0.2 gm x 1000)}} \times 100\%$$

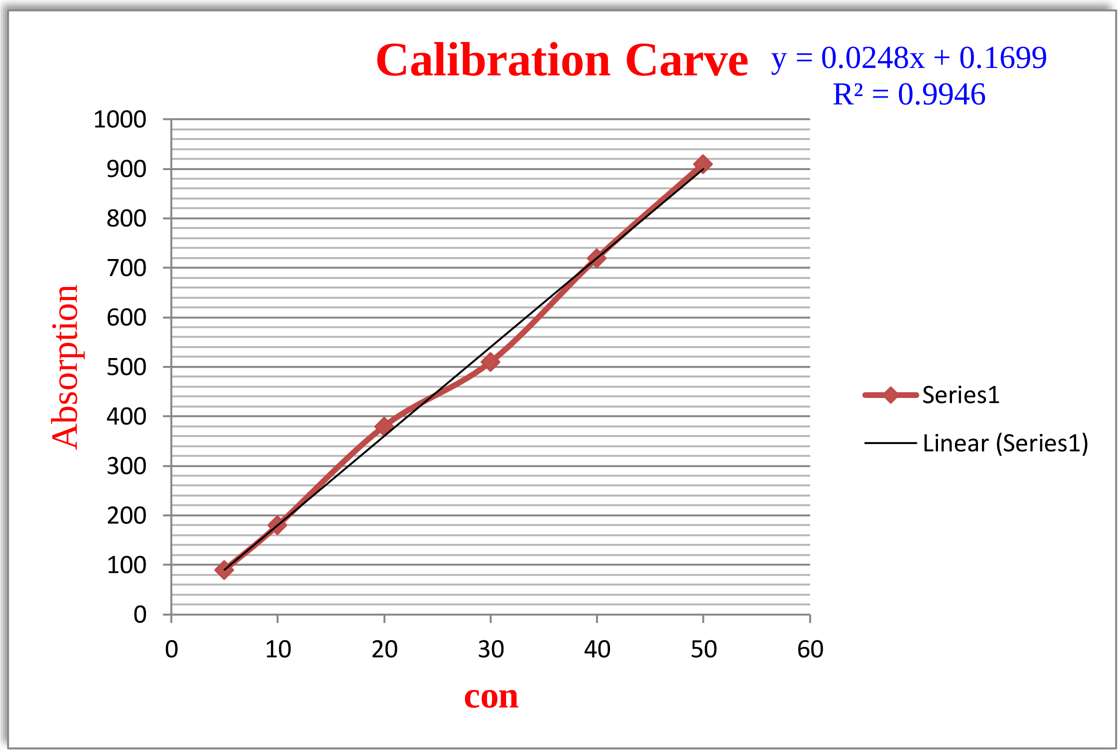
5-Total Nitrogen %:

The Kjeldahl method was used to estimate the percentage of nitrogen in the samples and based on the method mentioned by Van Dijk, D *et al.*, (2000) by taking a known weight of the model in the order of (0.5 g) placed in a beaker and adding to the sample (5 ml) of concentrated sulfuric acid. An appropriate amount of potassium sulfate and copper sulfate mixture was added. The digestion process was carried out by heating the contents, and after the digestion was completed, the mixture was turned into a clear, blue-colored liquid.The liquid was quantitatively transferred to the distillation flask of the Kieldahl apparatus, which contains a concentrated solution (40%) of sodium hydroxide connected to a condensed distillation flask that ends with a test tube immersed in a receiving flask containing a known volume of boric acid (20%) plus drops of red methyl guide. And dye (bromocresol blue). Then the determination flask is heated until the amount of the distilled liquid in the flask reaches about (25 ml). The collected liquid is flushed with hydrochloric acid (0.1) standard, and a control solution (plank) of chemicals in the above except for the sample is prepared. Nitrogen ratio according to the following equation:

Protein % = volume of HCl consumed x standard x 0.014 / sample weight x 100

6- Determination of phosphorous percentage (%):

The phosphorous content of the plant was estimated by taking a weight of (0.5 g) from the crushed and dried sample and dissolvingit with (5 ml) sulfuric acid and (2 ml) perchloric acid, and aluminiummolybdate and ascorbic acid were used (color method). Then it was measured using a spectrophotometer at a wavelength of (700 nm).



3. RESULTS AND DISCUSSION

1. The increase in seedling height (cm):

The results in Table (2) indicate that the addition of glutathione significantly affected the rate of increase in plant height. The addition treatment (G3) at a concentration of (225) mg l^{-1} significantly outperformed the control treatment (G0) by giving it the highest increase in plant height. It reached (99.637) cm. As for ascorbic, the results of the same Table indicate that the addition treatment (C3) at a concentration of (150) mg l^{-1} was significantly superior to the control treatment (C0), as it gave the highest increase in plant height of (98.991) cm.

The binary interaction (compound glutathione * ascorbic acid) exhibited a significant positive behavior, as the interaction (225 * 150) mg.l^{-1} was superior to the rest of the interactions. The increase in plant height was (110.554) cm, while the interaction treatment gave (0 * 0), and the lowest leaf content of the trait was (76,890) cm.

Table (2) the effect of glutathione, ascorbic, and their interaction on the increase in height plant (cm) for seedlings of grapes variety (Halwani)

Glutathione concentration	Ascorbic acid concentration				Glutathione average
	0	50	100	150	
0	76.890	77.221	83.264	92.331	82.427
75	79.211	90.180	88.110	97.112	88.654
150	90.550	88.662	95.392	95.963	92.642
225	89.662	91.662	106.671	110.554	99.637
Ascorbic acid average	84.078	86.931	93.359	98.991	
LSD 0.05					
G=2.517	C=2.517		G*C=5.035		

2. The increase in leaves number (leaf. plant^{-1}):

It is clear from the results shown in Table (3) that the treatment of adding glutathione to the plant at a concentration of (G3) at a concentration of (225) mg l^{-1} by giving it the highest increase in the number of leaves amounted to (34.427) leaves plant^{-1}. The lowest increase in the number of leaves reached (24.406) leaves plant^{-1}. As for adding ascorbic spray to the plant, the additional treatment with a concentration (C3) at a concentration of (150) mg.l^{-1} was superior to the control treatment (C0), in which the increase amounted to 32.647 leaves plant-1. The number of leaves in it increased (23.991) leaves plant^{-1}.

As for the interaction between the two factors of the study, the study treatment (225 * 150) mg l^{-1} showed a significant superiority over the rest of the interactions by giving it the highest value for increasing the number of leaves (38.657) leaves plant^{-1}. In contrast, the control treatment gave the lowest value for increasing the number of leaves reached (21.313) leaves plant^{-1}.

Table (3) the effect of glutathione, ascorbic, and their interaction on leaves number (leaf. plant^{-1}) for seedlings of grapes variety (Halwani)

Glutathione concentration	Ascorbic acid concentration				Glutathione average
	0	50	100	150	
0	21.313	22.317	22.653	31.340	24.406
75	16.627	32.653	20.040	37.290	26.653
150	27.687	30.640	26.093	23.300	26.931
225	30.337	33.087	35.627	38.657	34.427
Ascorbic acid average	23.991	29.674	26.103	32.647	
LSD 0.05					
G=0.373	C=0.373		G*C=0.747		

3. Leaves area (cm2):

The results in Table (4) indicate that the addition of glutathione significantly affected the average increase in leaf area. The addition treatment (G3) at a concentration of (225) mg l^{-1} significantly outperformed the control treatment (G0) by giving it the highest increase in leaf area rate. Where it reached 118.772 cm2. As for ascorbic, the results of the same Table indicate that the addition treatment (C3) at a concentration of (150) mg l^{-1} was significantly superior to the control treatment (C0), as it gave the highest increase in leaf area of (112.533) cm^2.

The binary interaction (compound glutathione * ascorbic acid) exhibited a significant positive behavior. The interaction (225 * 150) mg l^{-1} was superior to the rest of the interactions, and the increase in leaf area was (132.932) cm2, while the interaction treatment gave (0 * 0), the lowest leaf content of the trait was (44,551) cm^2.

Table (4) the effect of glutathione, ascorbic, and their interaction on Leaves area (cm2)for seedlings of grapes variety (Halwani)

Glutathione concentration	Ascorbic acid concentration				Glutathione average
	0	50	100	150	
0	44.551	55.301	80.652	100.872	70.344
75	51.342	72.550	78.620	103.113	76.406
150	90.880	96.230	103.703	113.224	101.009
225	101.861	116.362	123.931	132.932	118.772
Ascorbic acid average	72.159	85.111	96.727	112.533	
LSD 0.05					
G=1.996	C=1.996		G*C=3.992		

4. Carbohydrate content of leaves (%):

The results in Table (5) indicate that the addition of glutathione significantly affected the average increase in the carbohydrate content of the leaves. The addition treatment (G3) at a concentration of (225) mg l^{-1} significantly outperformed the control treatment (G0) by giving it the highest increase in the content of carbohydrates. Leaves of chlorophyll amounted to 18.483%. As for ascorbic, the results of the same Table indicate that the addition treatment (C3) at a concentration of (150) mg l^{-1} was significantly superior to the control treatment (C0), as it gave the highest increase in the carbohydrate content of leaves, which amounted to (18.259%).

The binary interaction (compound glutathione * ascorbic acid) exhibited a significant positive behavior, as the interaction (225 * 150) mg.l^{-1} was superior to the rest of the interactions. The increase in the carbohydrate content of the leaves was (19.170 %), while the interaction treatment gave (0 * 0), and the lowest carbohydrate content of the leaves was (16.270 %).

Table (5) the effect of glutathione, ascorbic, and their interaction on the carbohydrate content of leaves (%)for seedlings of grapes variety (Halwani)

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Glutathione concentration	Ascorbic acid concentration				Glutathione average
	0	50	100	150	
0	16.270	16.453	16.973	17.253	16.737
75	16.627	17.150	17.673	18.043	17.373
150	16.810	17.903	18.410	18.573	17.924
225	17.547	18.463	18.750	19.170	18.483
Ascorbic acid average	16.814	17.422	17.952	18.259	
LSD 0.05					
G=0.112	C=0.112		G*C=0.224		

5. Nitrogen content of leaves (%):

It is clear from the results shown in Table (6) that the addition of glutathione significantly affected the average increase in the concentration of nitrogen elements. The treatment (G3) at a concentration of (225) mg l^{-1} significantly outperformed the control treatment (G0) by giving it the highest increase in the concentration of nitrogen element, reaching (1.699%). As for ascorbic, the results of the same Table indicate that the addition treatment (C3) at a concentration of (150) mg l^{-1} was significantly superior to the control treatment (C0), as it gave the highest increase in nitrogen concentration, which amounted to (1.574%). As for the binary interaction (compound glutathione + ascorbic acid), it exhibited a significant positive behavior, as the interaction (225 * 150) mg l^{-1} was superior to the rest of the interactions, and the increase in nitrogen concentration was (2.115%). In contrast, the interaction treatment gave (0 * 0). The lowest nitrogen content in the leaves was (1.138%).

Table (6) the effect of glutathione, ascorbic, and their interaction on the nitrogen content of leaves (%)for seedlings of grapes variety (Halwani)

Glutathione concentration	Ascorbic acid concentration				Glutathione average
	0	50	100	150	
0	1.138	1.166	1.213	1.226	1.186

75	**1.177**	**1.216**	**1.244**	**1.263**	**1.225**
150	**1.194**	**1.254**	**1.441**	**1.692**	**1.395**
225	**1.342**	**1.338**	**2.003**	**2.115**	**1.699**
Ascorbic acid average	**1.213**	**1.243**	**1.475**	**1.574**	
LSD 0.05					
G=0.1045		**C=0.1045**		**G*C=0.2090**	

6. Phosphorous content of leaves (%):

Table (7) results indicate that adding glutathione significantly affected the average increase in phosphorous elements. The addition treatment (G3) at a concentration of (225) mg l^{-1} significantly outperformed the control treatment (G0) by giving it the highest increase in the concentration of elemental phosphorous, Where it reached (1.275)%. As for ascorbic, the results of the same Table indicate that the addition treatment (C3) at a concentration of (150) mg l^{-1} was significantly superior to the control treatment (C0), as it gave the highest increase in the concentration of the element, which amounted to (1.192%). As for the binary interaction (compound glutathione * ascorbic acid), it exhibited a significant positive behavior, as the interaction (225 * 150) mg l^{-1} was superior to the rest of the interactions, and the increase in plant height was (1.663%). In contrast, the interaction treatment gave (0 * 0); the lowest phosphorous content in leaves was (0.200%).

Table (7) the effect of glutathione, ascorbic, and their interaction on phosphorous content of leaves (%)for seedlings of grapes variety (Halwani)

Glutathione concentration	**Ascorbic acid concentration**				**Glutathione average**
	0	**50**	**100**	**150**	
0	**0.200**	**0.273**	**0.453**	**0.700**	**0.417**
75	**0.330**	**0.570**	**0.866**	**1.033**	**0.699**
150	**0.376**	**0.976**	**1.260**	**1.370**	**0.995**
225	**0.803**	**1.140**	**1.493**	**1.663**	**1.275**
Ascorbic acid average	**0.438**	**0.739**	**1.018**	**1.192**	
LSD 0.05					
G=0.019		**C=0.019**		**G*C=0.038**	

It is noticed from the tables (2, 3, 4) that the following studied vegetative traits (plant height, leaves number, leaf area) outperformed with a direct increase with the increase in the concentration of the glutathione compound. It gave a concentration of 225 mg l^{-1} to the highest averages (99.637 cm, 34.427 leaf plant^{-1}, 118.772 cm²) compared to the control treatment. This explains that the glutathione compound increases the efficiency of the plant in absorbing water and nutrients and that the glutathione compound has an active role in the various metabolic processes in the plant and then increases growth, which is positively reflected in the activity of the vegetative system, which leads to an increase in the characteristics of the vegetative system as a result of the expansion of cells by the action of growth hormones and this It leads to an increase in the number of branches and an increase in their length, and thus an increase in the number of leaves, and this, in turn, is reflected in other results. The reason for this is that the amino acids work to change the osmotic voltage and lead to its decrease, which leads to a reduction in the water effort of the cell and thus increases the ability of the cell to take water and dissolved nutrients and then leads to an increase in the vegetative growth of the plant (Amini and Ehsanpour 2005). This is consistent with the findings of Mahgoub*et al.*, (2006) in Egypt on the chrysanthemum plant.

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It was also shown that the significant increase that occurred in the above tables was given a concentration of 150 mg l^{-1} of ascorbic in the traits (plant height (cm), leaves number (leaf plant^{-1}), leaf area (cm^2) above the averages (98.991, 24.207, 112.533) compared to the comparison treatment. It may be attributed to the increase in growth characteristics due to spraying with ascorbic acid, mainly due to the stability of the structures of the cell membranes (Blokhinn*et al.*,, 2003). And the release of cell membrane fluidity like cholesterol and also the purity of the membrane to other ions and molecules and their participation in the regulation of cell division is that ascorbic acid participates in the regulation of cells and that ascorbic acid and the enzyme Ascorbate oxidase present in the cell wall participate in controlling growth. But the high activity of the ascorbate oxidase enzyme is associated with the rapid expansion of cells and thus increases the growth characteristics (Smirnof, 1996).

Regarding the moral superiority in the concentration of carbohydrates in the Table, this is because the glutathione compound increases the availability of nutrients ready to the plant in a more extended period and with a release compatible with the plant's growth. Which increases the formation of chlorophyll and the rate of photosynthesis and, as a result, increases the total growth. This, in turn, leads to an increase in the concentration of total carbohydrates and can the significant increase in carbohydrates is attributed to the compound that increases and regulates the action of growth hormones, cytokines and auxins, which have a key role in the course of the various metabolic processes of the plant, through the role of glutathione as an antioxidant, the rate of photosynthesis increases and the representation of CO2 increases, which leads to an increase in carbohydrates. This agrees with the study conducted by Eid*et al.,* (2011) on the Qadifa plant.

The significant superiority that occurred to carbohydrates in the Table is noted. The reason may be attributed to the role of ascorbic acid in stimulating it through its work as a co-enzyme for some enzymes responsible for the metabolism of carbohydrates and proteins and its regulation of the processes of division and expansion. It also affects the ratio between the processes of photosynthesis and respiration, thus increasing the content of carbohydrates in the leaves.

The results show that the addition of glutathione has had a positive effect. The increase in the mentioned characteristics is attributed to the fact that the glutathione compound has a large surface area and particle size less than the pores in the plant leaves, which can cause an increase in its penetration into plant tissues. Thus the absorption of nutrients and the compound is more efficient and streamlined. The direct absorption by the leaves of this compound significantly contributed to the increase in the leaves' content of it. It may also be attributed to the role of amino acids in regulating ions and modifying the movement of nutrients within plant tissues. These results agree with the mechanism Talaat&Aiziz (2005) on the chamomile plant.

When spraying the leaves with ascorbic acid, there is an increase in the content of nitrogen, phosphorous and potassium. It may be due to the increase in the organic acids secreted by the roots in the soil, thus increasing the solubility of nutrients released slowly in the root area of the rhizosphere where they can be used by the plant (Hanafy Ahmed 1996). These results agree with what was found by Ali (2000) by spraying the Flame seedless grape plants with ascorbic acid.

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