



ORIGINAL ARTICLE

STUDY OF THE PHYSIOLOGICAL INTERACTIONS OF TREATMENT WITH THE ANTIOXIDANTS CITRIC ACID AND GLUTATHIONE TO REDUCE OIL POLLUTANTS ON DATE PALM TREES *PHOENIX DACTYLIFERA L.*

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Abstract: The study was conducted in one of the orchards in the Al-Dair District, Nahran Omar District, and Basra Governorate to determine the effectiveness of ground treatment with citric acid and spraying with the antioxidant glutathione to reduce the harmful effects of oil pollutants on Al-Sayer date palm trees. Citric acid was added at two concentrations of 200 and 400 gm. palm⁻¹ and glutathione was sprayed at a concentration of 100, 200, and 300 mg. L⁻¹. The results showed that the treatment T7 (add citric acid at a concentration of 400 g. Palm⁻¹, glutathione spray with a concentration of 300 mg. L⁻¹) significantly reduced the concentration of heavy metals such as lead, nickel, cadmium, cobalt, and chromium as well as oil pollutants, hydrocarbons, alkanes, amino acids proline, and phenolic compounds in the leaves of the date palm of the Sayer variety compared to the control treatment, which recorded the highest values of the above qualities. T7 treatment was considerably superior in terms of chlorophyll a, b pigment concentration, total chlorophyll in the plant, carotene and anthocyanin concentration, peroxidase enzyme activity, and glutathione concentration in the leaves. while the comparison treatment is based on the lowest values for the plant pigments and the aforementioned attributes, respectively. The usage of antioxidants such as citric acid and glutathione has played an important role in buffering the detrimental effects of oil pollutants on Sayer palm trees, which has resulted in improved tree development and increased production under severe environmental conditions.

Key words: Citric, Glutathione, Al-sayer, Pollution, Date palm.

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1. Introduction

The date palm belongs to the family Aracaceae, and it is believed that the date palm is found in the Arabian Gulf and southern Iraq, and is considered an important fruit tree in many countries of the world. Wet which is a monocots, a dicotyledonous plant characterized by its cultivation for its fruits of global nutritional value and for aesthetic aspects. It is of cultural, economic and social importance as well as being of agricultural importance all over the world, and in the Middle East in particular [Jones *et al.* (2011)]. The

problem of environmental pollution is one of the most important problems facing the world today, and this problem is related to the health of the environment and living organisms.

Pollution is defined as an undesirable change in the physical, chemical and biological properties of air, water and soil, or those that harm life or may cause a potential danger to the health of any living organism, and any substance that causes pollution by pollutants, which can be defined as any substance solid, gaseous, or liquid, and they exist in certain concentrations that

may cause harm to the environment and living organisms [Al-Hasany *et al.* (2020)]. Oils contain toxic organic and inorganic compounds (toxic metal ions, dissolved and suspended substances, phenolic compounds, hydrocarbons, sulfides, carbon oxides, cyanide, *etc.*), this makes it one of the most dangerous sources of environmental pollution for water, air and soil. One of the most sensitive organisms to pollution is plants because they are immobile, as well as the susceptibility of their cellular tissues to the accumulation of pollutants that enter the plant either through the soil or the deposition of air pollutants [Zurayk *et al.* (2001), Al-Hasany *et al.* (2021)]. Citric acid is one of the non-enzymatic antioxidants, as it acts as a scavenger to get rid of free radicals resulting from abiotic stresses to which the plant is exposed, which affect disturbed food transformations, affect the electron transport chain, and increase the destruction of plasma membranes, and increase hydrogen peroxide [Saqr (2012)]. Among those stresses are salt and alkaline stress, pollutants and others. As for glutathione, it is a tripeptide consisting of three amino acids: glutamic, cysteine, and glycine. Glutathione is found in plant cells and is low in molecular weight, works to remove reactive oxygen species (ROS) and relieve stress by linking glutathione with molecules and then enzymes bind to the outer surface of glutathione [Roubier *et al.* (2008), Noaema *et al.* (2020)]. The mechanism of action of glutathione as an antioxidant is that it interacts with atomic oxygen, superoxide and hydroxide and thus acts indirectly on the capture of free radicals, and it works to increase the stability of the composition of plant membranes by removing the Acycleperoxide formed by the reaction of Lipid peroxidation, and a reducing agent that converts the oxidized form of the ascorbic acid cycle back into the shorthand form [Saqr (2006)]. A study conducted at the site of the Najibiyah power station in Basra showed that heavy elements (lead, cadmium and chromium) recorded the highest concentrations in palm leaves in the dry season (summer), it reached (43.34, 5.67, 15.13) mg. kg⁻¹, respectively [Al-Jabri (2017), Aljaberi *et al.* (2020)]. While Abi Al-Khasib site recorded the highest concentration of cobalt at 24.87 mg. kg⁻¹. Kazem (2019) explained in a study evaluating the oil pollution of West Qurna field that the highest concentration of hydrocarbons, alkanes and heavy elements lead, nickel, and cadmium found in the leaves plant of *Suaeda vermiculata* in the summer was (10.63, 12.47, 28.75,

106.77, 15.03) µg.g⁻¹ respectively. Faisal *et al.* (2020) indicated that when studying the seasonal variations of oil pollutants, the highest concentrations of hydrocarbons, alkanes and lead in the leaves of the jabjab palms trees was in summer in orchards near West Qurna Oil Field No. 2, while the lowest concentrations were in the winter season and in Orchards far from the oil field. Therefore, this study aims to use the antioxidants citric acid and glutathione to mitigate the negative effects of oil pollutants on Al-Sayer date palm trees, which in turn is reflected in improving the growth of trees and increasing their productivity and quality.

2. Materials and Methods

This research was carried out in one of the personal orchards in the Dair district, Nahran Omar region-Basra governorate, with the aim of knowing the effect of ground treatment with citric acid and spraying with the antioxidant glutathione to mitigate the negative effects of oil pollutants represented by hydrocarbons, alkanes and heavy elements in the leaves of date palm trees of Al-Sayer cultivar 27. This was accomplished by selecting 27 date palm trees of the Al-Sayer variety at the age of 13 years that were as close to identical in age, height, and vegetative growth as feasible, and free of pathological infection. The process of adding citric acid to the soil by making a circular trench around the trunk of a palm, 30 cm deep and 40 cm wide, one meter away from the trunk of the palm, and scattering citric acid in the trench in an amount of 200 and 400 g. palm⁻¹, in two stages, the first stage is in the middle of January and the second is in the middle of February, and then agricultural soil was added to the trench. The solutions used have been prepared, where glutathione was prepared at a concentration of (100, 200, 300) mg. L⁻¹ in addition to control treatment (without adding and spraying with distilled water). The first spray was conducted at the beginning of the third month (March), and a month after the first spray, the second spray took place. A diffuser (Tween 20) concentrate (0.01%) was added to the solutions during the spraying process, which was conducted in the morning hours on the shoots until complete wetness. Leaf traits measurements were taken in the month of September by taking the tufts (leaflets) of the fronds located in third line after growth of the apex leaves [Al-Ani (1998)]. An analysis of the orchard soil was conducted before conducting the study (Table 1).

Table 1: The chemical properties of soil.

Properties	Values
Electrical conductivity (E. C) d / m	9.38
Soil Reactivity (PH)	7.69
Ready nitrogen (mg. Kg ⁻¹)	201.65
Ready-made phosphorous (mg.kg ⁻¹)	29.80
Ready-made potassium (mg. Kg ⁻¹)	86.63
Total lead mg. kg ⁻¹	136.35
Total Nickel mg. kg ⁻¹	70.04
Total Cadmium mg. kg ⁻¹	12.65
Total Cobalt mg. kg ⁻¹	42.89
Total Chromium mg. kg ⁻¹	135.79

1. Extraction of the total concentration of heavy metals in the soil: The total content of heavy metals was extracted from the soil according to the method described in Davidsonk (2013).
2. Extraction of heavy metals from palm leaves: heavy metals were extracted from the leaves of date palm trees according to the method described in Jones (1984).
3. Plant pigments: The plant pigments were extracted from palm leaves according to method of Arnon (1949), and the plant pigments were estimated based on the following equations according to Asare- Boamah *et al.* (1986) and it was expressed in unit (mg. g⁻¹).
 Total chlorophyll (mg. L⁻¹) = 20.2 (O.D. 645) + 8.02 (O.D. 663)
 Chloropyll a (mg.L⁻¹) = 12.7 (O.D. 663) – 2.69 (O.D. 645)
 Chloropyll b (mg. L⁻¹) = 22.9 (O.D. 645) – 4.68 (O.D. 663)
 Anthocynine (mg.L⁻¹) = 0.0821 (O.D 534) – 0.0439 (O.D 645) – 0.002423 – (O.D 661).
 Carotine (mg. L⁻¹) = (O.D 740) – 17.1) (chl a + chl b) – 9.47 (anthocynine) / 119.26.
 where O.D = absorbance at each wavelength.
4. Estimation of total phenols: The method of Singleton and Rossi (1965) was followed to estimate total phenols in palm leaf tissues.

Transactions and Statistical Design

The experiment included seven proxy transactions:

- (T₁) - Control treatment (without adding and spraying with distilled water
 (T₂) - Citric acid at a rate of 200 g. palm⁻¹ and

Table 2: Effect of treatment with the antioxidants citric acid and glutathione on the concentration of heavy metals in the leaves date palm trees Al-Sayer cultivar µg.g⁻¹.

Treatment	Elements				
	Lead	Nickel	Cadmium	Cobalt	Chromium
T ₁	25.52	73.48	9.35	21.30	25.50
T ₂	23.49	62.88	9.03	20.75	21.97
T ₃	22.74	62.07	8.76	16.35	20.82
T ₄	22.53	61.58	8.29	14.42	18.69
T ₅	21.81	60.96	7.92	13.00	17.00
T ₆	20.81	59.83	7.39	12.14	14.48
T ₇	19.44	58.61	6.97	10.37	12.51
L.S.D	1.07	5.36	1.24	5.15	2.03

spray treatment with glutathione concentration of 100 mg.g⁻¹.

(T₃) - Add citric acid at a rate of 200 g. palm⁻¹ and spray treatment with glutathione concentration of 200 mg.g⁻¹.

(T₄) - Add citric acid at a rate of 200 g. palm⁻¹ and spray treatment with glutathione concentration of 300 mg.g⁻¹.

(T₅) - Add citric acid at a rate of 400 g. palm⁻¹ and spray treatment with glutathione concentration of 100 mg. g⁻¹.

(T₆) - Add citric acid at a rate of 400 g. palm⁻¹ and spray treatment with glutathione concentration of 200 mg. g⁻¹.

(T₇) - Adding citric acid at a rate of 400 g. palm⁻¹ and spray treatment with glutathione concentration of 300 mg. g⁻¹.

The experiment was carried out by Randomized complete block design (RCBD) which is a simple experiment with seven coefficients and three replicates. Results were statistically analyzed using the statistical program Genstat version 7 and the means were compared using the least significant difference (L.S.D) test at the 0.05 probability level [Al-Rawi and Khalaf Allah (2000)].

We note from Table 2 that the treatments with the antioxidants citric acid and glutathione significantly affected the leaves content of heavy metals, as these treatments decreased significantly by recording the lowest values compared to the comparison treatment (without adding and spraying with distilled water), so that the treatment T₇ recorded (adding citric acid at a

Table 3: Effect of treatment with the antioxidants citric acid and glutathione on the concentration of plant pigments in the leaves date palm trees Al-Sayer cultivar mg.g^{-1} .

Plant pigments					
Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotene	Anthocynins
T ₁	1.91	0.63	2.55	0.266	0.046
T ₂	2.23	0.74	2.97	0.423	0.057
T ₃	2.34	0.78	3.13	0.563	0.066
T ₄	2.53	0.84	3.38	0.69	0.073
T ₅	2.68	0.89	3.58	0.753	0.081
T ₆	3.47	1.15	4.64	0.853	0.088
T ₇	3.97	1.32	5.30	0.903	0.101
L.S.D	0.32	0.105	0.47	0.241	0.013

Table 4: Effect of treatment with the antioxidants citric acid and glutathione on some physiological characteristics in the leaves date palm trees Al-Sayer cultivar.

Characteristics						
Treatment	Activity of the peroxidase enzyme units. minute .g ⁻¹	Glutathione concentration $\mu\text{mol.g}^{-1}$	Proline concentration $\mu\text{g.g}^{-1}$	Hydrocarbon concentration $\mu\text{g.g}^{-1}$	Alkanes concentration $\mu\text{g.g}^{-1}$	Phenolic concentration $\mu\text{g.g}^{-1}$
T ₁	53.22	20.28	43.26	12.29	12.99	10.33
T ₂	61.33	29.87	33.63	11.39	11.54	9.46
T ₃	81.44	34.99	27.43	10.59	10.37	9.11
T ₄	92.52	48.00	21.76	9.57	9.57	8.48
T ₅	95.51	53.46	19.59	9.36	8.42	7.74
T ₆	98.78	56.80	18.75	9.08	7.21	6.60
T ₇	121.29	61.33	17.43	8.70	6.05	5.48
L.S.D	6.52	6.64	3.02	0.29	0.76	0.681

rate of 400 g. palm⁻¹ and spraying with glutathione at a concentration of 300 mg. L⁻¹) the lowest values of 19.44, 58.61, 6.97, 10.37, 12. $\mu\text{g.g}^{-1}$ compared to the control treatment that recorded the highest values, amounted to 25.52, 73.48, 9.35, 21.30 and 25.50 $\mu\text{g.g}^{-1}$ for the elements lead, nickel, cadmium, cobalt, chromium, respectively.

We notice from Table 3 that treatment with the antioxidants citric acid and glutathione had a significant effect on the plant pigments in the leaves, where the treatment with antioxidants led to an increase in the concentration of plant pigments in the leaves chlorophyll a, b, total, carotene and anthocyanin, where the treatment T₇ recorded the highest values of 3.97, 1.32, 5.30, 0.903, 0.101 mg. g⁻¹, compared to the control treatment, which recorded low values, which were 1.91, 0.63, 2.55, 0.266 and 0.046 mg. g⁻¹.

We note from Table 4 that treatment with the antioxidants citric acid and glutathione had a significant

effect on the physiological characteristics under study, as the increase in the concentration of citric acid and glutathione led to an increase in the activity of the peroxide enzyme in the leaves, whereby treatment T₇ gave the highest activity of the peroxidase enzyme amounting to 121.29 units. minute. g⁻¹ compared to the comparison treatment, which recorded the lowest activity of peroxidase enzyme, which was 53.22 units. minute.g⁻¹. The same treatment also led to an augmentation in concentration of glutathione in the leaves, where the highest values were recorded, reaching 61.33 $\mu\text{mol.g}^{-1}$, while the control treatment showed the lowest values of 20.28 $\mu\text{mol.g}^{-1}$. The table also shows that treatment with the antioxidants citric acid and glutathione resulted in a significant decrease in the concentration of the amino acid proline, hydrocarbons, alkanes and phenolic compounds in the leaves, where the treatment T₇ recorded the lowest values of 17.43, 8.70, 6.05 $\mu\text{g.g}^{-1}$, 5.48 mg.g⁻¹ compared with the control treatment that recorded highest values

of 43.26, 12.29, 12.99 $\mu\text{g}\cdot\text{g}^{-1}$ and 10.33 $\text{mg}\cdot\text{g}^{-1}$ for proline, hydrocarbons, alkanes and phenolic compounds, respectively.

Tables 2, 3 and 4 show that treatment with the antioxidants citric acid and glutathione led to a reduction in the concentration of heavy metals, hydrocarbons and alkanes, an increase in the activity of peroxidase enzyme and glutathione concentration and an increase in the concentration of plant pigments in the leaves. This may be because citric acid is a non-enzymatic antioxidant as it acts as a sweeper to get rid of free radicals resulting from the stresses to which the plant is exposed, which affect turbulent food transformations, and affect the electron transport chain, increase the demolition of plasma membranes and increase hydrogen peroxide [Saqr (2012)], or perhaps due to the role of citric acid, which works to catch free radicals and protect. The cell is protected from the effects resulting from it and prevents the accumulation of toxic ions and thus leads to an improvement in the nutritional status of the plant. Or it may be due to the role of glutathione, which is one of the antioxidants, which works to protect cells from destruction and helps cells to remain shape active and that glutathione consists of three amino acids, glycine, glutamic and cysteine, and that amino acids cause an increase in the levels of the element Mg that enters construction of chlorophyll [Amin *et al.* (2011)], and that glutathione works to accumulating anthocyanins [Xiang *et al.* (2001)], as well as it leads to an increase in enzymatic activities [Mamdouh (1995)], and works on the synthesis of the protein that forms enzymes and hormones [Gilbert *et al.* (1990)]. And glutathione is a non-enzymatic antioxidant that works to get rid of stress by linking glutathione with molecules and then enzymes bind to the outer surface of glutathione [Roubier *et al.* (2008)]. We conclude from this study that treatment with the antioxidants citric acid and glutathione led to a reduction in pollution stress with heavy metals and oil pollutants, and this in turn leads to an increase in the tolerance of date palm trees, Al-Sayer cultivar, to abiotic stresses and an improvement of the studied physiological characteristics, which is positively reflected on the characteristics of fruit growth.

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