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Antioxidant Glutathione Promote *Mours spp*. seedlings tolerance to heavy metal stress

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Abstract

Among the pollutants in Basrah governorate that passively influence human health and highly contaminate the environment, lead and cadmium are listed as the most existing due to oil industry. Crops that grown in the contaminated area pose a risk for human health when consumed as food. Mulberry *Mours spp*. is a fruit tree that is cultivated in temperate regions like Basrah city and may be widely affected by the polluted environment which notably reduce the trees growth and their fruits quality. Current study investigated the recovery role of antioxidant glutathione at (0, 25, 50) mg/L on growth and biochemical parameters of mulberry seedlings that are affected by heavy metal stress including lead at (0, 5, 10) mg/L and cadmium at (0, 75, 150) mg/L. The results exhibited that foliar application with glutathione particularly at 50 mg/L ameliorated the vegetative growth and biochemical traits post heavy metal pollution. These parameters included seedling height, number of leaves, number of branches, leaf area index, fresh weight and biomass of roots as well as the leaves content of chlorophyll, carbohydrates, moisture, lead and cadmium. Our findings indicated that glutathione played a remarkable role in promoting mulberry seedlings growth and increasing their tolerance against heavy metal stress.

Keywords: *Mours spp*., Glutathione, Pollutants, Lead, Cadmium, Heavey metal stress

1- Introduction

Morus spp. belongs to the Moraceae family, it is fruit tree, grows to big size and its branches variously spread according to the varieties. Mulberry tree is a deciduous fruit tree, could be reached more than ten meters and grown in subtropical and temperate regions (Ibrahim, 1996). The leaves are simple, large and used to raising the silkworms, while tree wood is used in construction and furniture industry (Abdul Karim, 2020). Pollution is defined as either the entry of new elements into the ecosystem that were not originally present or the increase of these elements levels (Wn et al., 2004). Among these elements that widely enter the ecosystem and cause high pollution are heavy elements. Heavy elements including cadmium and lead are classified as the most passive environmental pollutants due to their high degree of stability and remain for unlimited periods.

Cadmium is considered a toxic element for humans, animals and plants. It concentrates in vital parts of the human body such as a liver, spleen and kidney after combining with some proteins. Some studies have indicated that it replaces the zinc in some body enzymes and disrupts their vital functions (Smirjakova, 2005).

Lead is a common air pollutant, it is released to the environment from car exhausts, electric power generators, water pipe manufacturing, printing inks, and pesticides (Tripathg et al., 2022). Human activities increase the toxic level of leads, Pistic and his team reported that 95% of Cadmium in the air is produced due to human activities (Pistic et al., 2013). This element is highly toxic due to its high atomic mass its accumulation in the tissues of living organisms and its non-biodegradability (Mohanty et al., 2016). The main source of both pollutants, cadmium and lead in Basrah is the oil industry

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Many methods have been developed to reduce the harmful effects of heavy elements contamination, including antioxidants. Glutathione is an important antioxidant compound in the defense system of plants and animals. It is a tripeptide (glutamyi-cystenyi-glycinc) and one of the most abundant forms of organic sulfur in plants. Glutathione exists in two forms: either oxidized in the form of GSSG or reduced in the form of GSH. (Tandogan and Ulusn, 2006). It is considered a strong antioxidant and has a high ability to scavenge free radicals and increase the efficiency of metabolic processes, resulting in enhancing plants tolerance under stress conditions (Rai et al., 2023).

We aimed in the current study to explore the mitigation efficiency of glutathione on mulberry seedlings that grown under cadmium and lead stress in Basrah governorate

2- Materials and Methods

This study was conducted in the plant shade of the Department of Horticulture and Landscape Diesgn / College of Agriculture / University of Basrah during the 2023-2024 season. The varieties of mulberry seedlings have been obtained from one of the private nurseries in Basrah governorate. Seedlings transferred to 5 kg pots with substrate 1:1 of soil to peatmoss. The soil composition was estimated in the laboratories of the Marine Chemistry Department, Marine Science Center (Table 1).

2.1. Experimental parameters

2.1.1. Vegetative traits

The vegetative growth parameters including the seedlings heigh, the number of branches and leaves and leaf area were assessed after completing the application of all treatments.

Leaf area cm^2 was estimated according to Dovnic (1965) and following the equation:

$S = (G \times s)/g$

S = leaf area (cm^2)

G = fresh leaf weight (g)

s = average area of selected square (cm^2)

g = average fresh weight of selected square (g)

2.1.2. Biochemical traits

1- Total chlorophyll content in leaves (mg/100g FW): Estimated according to the method described by Goodwin (1976).

2- Total soluble carbohydrates in leave (mg/100g FW): Estimated according to the phenol-sulfuric acid method following Dobois et al.(1956).

3- Cadmium and lead levels in leaves (mg/g): Estimated according to Kalra (1998) using the Atomic Absorption device model SHEMADZUAA7000.

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

attribute	unit	value
PH	DS Siemens. M-1	1.83
Organic matter	g/kg	7.50
Nitrogen	mg/kg	8.32
Phosphorus	mg/kg	30.20
Potassium	mg/kg	2.61
Calcium	g/kg	164.2

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Soil texture		
Sand	%	81.2
Silt	%	11.2
Clay	%	7.00
Soil Texture	loamy sand	

2.1.3. Experimental Design

The factorial experiment was designed as a Randomized Complete Block Design (RCBD). The first factor was represented by two types of pollutants, lead and cadmium, at three concentrations (0, 75, 150) mg/L and (0, 5, 10) mg/L respectively. The second factor was represented through antioxidant glutathione at three concentrations (0, 25, 50) mg/L. Thus, the number of treatments was 15 treatments with three replicates, The statistical program GenStat was used to analyze the results, and the difference between the averages was compared using the LSD test at a probability level of 5% (Al-Rawi and Khalaf Allah, 2000).

3- Results and discussion

The result of table (2) show that pollutants negatively affected vegetative traits of mulberry seedlings compared to control plants. The treatment with cadmium at 10 mg/L and lead at 150 mg/L reduced the seedlings heigh to 83.87 cm and 82.71 cm, respectively compered to untreated plant that recorded 124.61 cm. However, foliar application with antioxidant glutathione at 50 mg/L mitigated the contamination effect of heavy elements and increased seedlings heigh, reaching 110.62 cm, with a significant difference from the rest of the treatments. The interaction between glutathione and pollutants recorded the highest value 140.47 cm in 0 and 50 mg/L glutathione-treated plants and lowest value 77.85 cm in 10 mg/L cadmium and 25 m/L glutathione-treated plants.

The results of Table (2) indicate that the leaves number reduced due to heavy metals contamination to 25.11 leaf/plant in 150 m/L cadmium-treated plants and 24.11 leaf/plant in 10 mg/L lead-treated plant compared to control plants which reached to 37.78 leaf/plant. Spraying glutathione at 50 mg/L had an alleviative effect in increasing leaves number and reducing the damage of pollutants recording 35.93 leaf/plant compared to the control which recorded 24.53 leaf/plant. The interaction between the two treatments was significant, 0 mg/L pollutants and 50 mg/L glutathione-treated plants obtained the highest values, reaching 51.67 leaf/plant, while 150 mg/L lead-treated plants and 10 mg/L cadmium-treated plants obtained the lowest values, reaching 21.00 leaves and 20.33 leaves, respectively.

The results of the same table showed that the number of branches in the seedling decreased significantly due to the effect of pollutants. While the untreated plants recorded the highest branches, reaching 5.00 branch/plant, Plants treated with the 75 and 150 mg/L lead had the lowest branches, reaching 2.66 branch/plant. The treatment with glutathione promoted the number of branches recording the highest number of branches in the seedling, reaching 3.80 branch/plant in 50 mg/L glutathione-treated plants. As for the interaction treatments, Plants treated with 0 mg/L pollutants and 50 mg/L glutathione had the highest number of branches in the seedling, reaching 6.33 branch/plant.

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Table (2). Effect of spraying with glutathione on some vegetative characteristics of mulberry seedlings growing under lead and cadmium pollution stress

Pollutants Cd + Pb	Glutathione mg/L	Plant height	Leaves Number	Number of branches	Leaf area	fresh root weight	Dry root weight
0	0	103.13	29.67	4.00	122.69	15.06	3.743
	25	130.24	32.00	4.667	131.72	17.77	4.533
	50	140.47	51.67	6.333	169.66	23.99	6.227
75 pb	0	72.96	26.00	2.00	119.32	12.33	3.350
	25	84.03	31.67	2.667	125.39	14.71	4.097
	50	91.15	36.33	3.333	130.66	19.02	5.360
150 pb	0	90.41	21.00	2.00	102.41	11.15	3.033
	25	103.37	25.67	2.667	105.78	12.38	4.010
	50	112.90	29.67	3.333	112.14	13.93	4.547
5 Cd	0	96.90	25.67	2.333	124.19	13.73	4.423
	25	112.53	31.00	3.333	130.46	15.50	4.930
	50	120.70	34.33	3.667	135.67	16.76	5.180
10 Cd	0	85.86	20.33	2.000	98.99	6.16	2.750
	25	77.85	24.33	2.333	107.23	7.61	3.033
	50	87.89	27.67	2.333	112.45	9.03	3.873
0.05 LSD	3.627	3.004	0.824	3.148	1.626	0.5169	
Average pollutant impact	0	124.61	37.78	5.000	141.36	18.94	4.831
	75	102.23	31.33	2.667	125.12	15.35	4.269
	150	82.71	25.11	2.667	106.78	12.48	3.863
	5	110.04	30.33	3.11	130.10	15.33	4.844
	10	83.87	24.11	2.222	106.22	7.60	3.219
0.05 LSD	2.094	1.734	0.476	1.818	0.939	0.298	
Average effect of glutathione	0	89.85	24.53	2.467	113.52	11.69	3.460
	25	101.60	28.93	3.133	120.11	13.59	4.121
	50	110.62	35.93	3.800	132.12	16.55	5.037
0.05 LSD	1.622	1.343	0.368	1.408	0.727	0.2312	

Table (2) includes the results of the effect of pollutants on leaf area. It is clear from the results that leaf area decreased with increasing the pollutants concentration. Plants that received 150 mg/L lead and 10 mg/L cadmium recorded the lowest leaf area without a significant difference between them, reaching 106.73 cm^2 and 10.22 cm^2 respectively. While, control plants obtained the highest value for leaf area, reaching 141.36 cm^2. Spraying glutathione increased the leaf area reaching to highest value (132.12 cm^2) in 50 mg/L-treated plants and ameliorated the passive effect of pollutants on leaf area. The combination between two factors showed that plants treated with 0 mg/L pollutants and 50 mg/L obtained the highest leaf area, reaching 169.66 cm^2. While plants treated with 10 mg/L of cadmium and 0 mg/L of glutathione recorded the lowest leaf area 98.99 cm^2.

The results of Table (2) also show the effect of pollutants on the fresh and dry weight of roots in mulberry seedlings. The results exhibited that the control plants recorded the highest value for fresh and dry weight, reaching 18.94 g and 4.83 g respectively, while the cadmium pollution treatment of 10 mg/L achieved the lowest value for fresh and dry weight, reaching 7.60 and 3.21 g respectively. For the effect of the antioxidant glutathione, sprayed plants with 50 mg/L of glutathione were superior and recorded 16.55 g for fresh root and 5.03 g for roots biomass. The two-way interaction between the study factors was significant, plants without pollutants and treated with 50 m/L of glutathione significantly increased their fresh and biomass of roots, reaching 23.99 g and 6.22 g respectively.

The results of table (2) presented the harmful effect of heavy elements on most of the vegetative traits of the seedlings, as high concentrations of cadmium and lead caused a decrease in the rate of plant heigh, leaves number, branches number, leaf area, and fresh and biomass of roots. Increasing the concentrations of heavy elements reduced the efficiency of the photosynthesis process which decreased in cell divisions and the number of cells and consequently diminishing in the most vegetative traits (Arile and DeBellis, 2020). In addition, cadmium and lead contamination negatively affected on the roots growth which reduced nutrient uptake from soil. Also increasing heavy metals concentration in pots soil reduced nutrient availability and microorganisms ability of organic matter decomposition which caused nutrients lack (Chibnik and Obiora, 2012). The results indicate the positive effect of foliar application with glutathione in reducing the damage of heavy metal pollution and regulated the vegetative growth. The recovery role of glutathione may be contributed to its antioxidant action in scavenging the free radicals and serving as a hormonal messenger that encourage the accumulation of nutrients in the leaves. Also, glutathione could be increased the seedlings ability to withstand stress by activating ATP, boosting the flexibility of cell walls and maximizing the efficiency of nutrient absorption, which was positively reflected in vegetative growth of mulberry seedlings (Marder, 2004 and Kusano et al., 2008).

From results in table (3), high concentrations of pollutants negatively affected the chemical characteristics of seedlings. Contamination with cadmium at 10 mg/L passively reduced carbohydrates portion to 10.53%, and chlorophyll to 2.404 mg/100 g compared to untreated plants that obtained 33.23% and 6.16 mg/100 g for carbohydrates and chlorophyll respectively. Spraying with the antioxidant glutathione had a positive effect in increasing the content of leaves from carbohydrates and chlorophyll and reducing the harmful effect of heavy element stress. Plants treated with 50 mg/L glutathione had the highest values, reaching 23.13% and 4.56 mg/100g for carbohydrates and chlorophyll, respectively. The combination between pollutants and antioxidant displayed increasing in carbohydrates and chlorophyll in leaves of plants treated with 0 mg/L of pollutants and 50 mg/L of glutathione recording 36.23% and 7.68 mg/100g, respectively. While cadmium 10 g/L and 5 mg/L-treated plants with 0 mg/L of glutathione recorded the lowest content of both traits 9.2% and 1.54 mg/100g.

The results of the table also show that the percentage of leaves water decreased significantly due to the heavy metals stress, as the cadmium 10 mg/L and 5 mg/L-treated plants obtained the lowest percentage, reaching 35.67% and 41.55% compared to control plants, reaching 65.46%. For the effect of glutathione, plants with 50 mg/L treatment obtained had the highest value for water content in leaves, reaching 56.90%. The effect of the dual interaction included the highest value, reaching 70.68% in leaves of 0 mg/L and 50 mg/L of pollutants and glutathione respectively, plants treated with 10 mg/L cadmium and 0 mg/L glutathione recorded 30.16 %.

Table (3) also included leaves content of lead and cadmium post treatments, as the concentration of the two elements increased in leaves in pattern. Plants treated with 150 mg/L lead recorded the highest concentration of lead in the leaf reaching 1.18 mg/L, and the cadmium element recorded its highest concentration in the leaf and reached 1.92 mg/L at a concentration of 10 mg/L cadmium. It is clear from the results that spraying with glutathione was able to reduce the stress of heavy elements with increasing spray concentration, as the treatment of 50 mg/L glutathione recorded the highest values for lead and cadmium and reached 0.72 and 0.74 mg/L for the two elements, respectively. As for the effect of the interaction, the interaction treatment (0 + 50) mg/L lead recorded the highest value for lead and reached 1.24 mg/L, and the treatment (0 + 10) mg. The highest concentration of cadmium in the leaves was 2.50 mg/L.

The results show that the high concentration of pollutants negatively affected most of the chemical properties under experimental conditions which may be attributed to the toxicity of heavy elements to the plant depending on their availability and ability to be absorbed by the plant. The heavy elements when are present in high quantities could be reduce the absorption of necessary elements and replace them in the chlorophyll, which consequently diminish the chlorophyll efficiency and its vital roles (Shah et al., 2009 and Dahash, 2023) In addition, the exposure to high concentrations of elements, especially cadmium, generates osmotic stress, as the relative water content in the leaves decrease and transpiration increase (Rizwan et al., 2016). Furthermore, the increase in the percentage of heavy elements Cd, Pb caused a reduction in the percentages of useful nutrients in the leaves and caused a decrease in water absorption by the plant which effect on metabolic process. Heavy metals may be caused increase the accumulation of free radicals ROS in the cell leading to destruction of the plasma membrane, protein and membrane of lipids (Isma et al., 2019).

Table (2). The effect of spraying with glutathione on some chemical traits of mulberry seedlings growing under lead and cadmium pollution stress

Pollutants Cd + Pb	Glutathione mg/L	Carbohydrates %	Chlorophyll mg/100g	Moisture content%	Pb element concentration mg/L	Cd concentration mg/L
0	**0**	30.79	4.43	64.32	0.047	0.553
	25	32.67	6.38	61.22	0.630	0.296
	50	36.23	7.68	70.83	0.0427	0.216
75 pb	**0**	25.27	2.307	40.53	1.0300	0.600
	25	22.31	3.833	53.50	0.956	0.420
	50	30.52	4.017	60.39	0.780	0.356
150 pb	**0**	15.89	1.830	38.37	1.246	0.710
	25	10.25	2.947	45.18	1.700	0.540
	50	10.45	4.007	55.34	0.943	0.296
5 Cd	**0**	10.26	1.543	38.17	0.126	7.866
	25	21.12	2.213	40.33	0.081	1.573
	50	26.33	3.890	46.14	0.065	1.190
10 Cd	**0**	9.27	1.843	30.16	0.047	2.150
	25	10.20	2.150	35.09	0.0377	1.97
	50	12.10	3.220	41.77	0.0310	1.656
0.05 LSD	1.088	0.1988	1.795	0.055	0.047	
Average pollutant impact	**0**	33.23	6.167	65.46	0.378	0.355
	75	26.03	3.386	51.48	0.994	0.478
	150	12.20	2.928	49.63	1.184	0.515
	5	19.24	2.549	41.55	0.090	1.543
	10	10.53	2.404	35.67	0.038	1.925
0.05 LSD	1.088	0.114	1.036	0.032	0.047	
Average effect of glutathione	**0**	18.29	2.391	42.31	0.778	1.188
	25	19.31	3.505	47.06	0.461	0.960
	50	23.13	4.563	56.90	0.372	0.743
0.05 LSD	1.405	0.088	0.803	0.024	0.036	

4- Conclusion

We concluded from a current study that antioxidant glutathione mitigated the harmful effect of heavy metals particularly cadmium and increased mulberry seedlings tolerance to their stress by promoting the vegetative growth and leaves content of total chlorophyll and carbohydrates. In addition, glutathione reduced the leaves content of cadmium and lead toxicity and increased their content of moisture. Our findings attract attention to the recovery role of glutathione in reducing the toxicity effect of heavy metals on crops growth which get along with sustainability direction by utilizing eco friend substances and reducing pollution influence on our environment.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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Author Contribution

K.M., N.O. designed the research, K.M. and N.O. conducted the fieldwork and lab experiments, and analyzed the data. The manuscript was written and edited by K.M., F.A., W.N., and M.A. All authors read and approved the final manuscript.

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