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EFFECT OF POLYAMINE COMPOUNDS AND ZEOLITE ON THE ANATOMICAL TRAITS OF DATE PALM OFFSHOOT LEAVES GROWN UNDER HEAVY METAL STRESS CONDITIONS

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SUMMARY

Besides the control treatment, Jabjabb date palm (*Phoenix dactylifera* L.) offshoots received irrigation water contaminated with heavy metals, lead(II) nitrate (Pb[NO3]2) = 300 mg kg^{-1} and cadmium chloride (CdCl2) = 3 mmol. In addition to the control treatment, putrescine (500 mg L^{-1}), coumaric acid (500 mg L^{-1}), and 10 kg of $palm^{-1}$ zeolite entailed application to seedling soil. After nine months of alternately treating the seedlings with pollutants and therapies, preparing tissue slices of the study palm leaves succeeded. Lead and cadmium treatment adversely affected leaf anatomy and decreased epidermal, parenchymal, and vascular bundle markers to the lowest values. The study showed putrescine, coumaric acid, and zeolite improved leaf tissue anatomical properties and recorded the highest values. The enhancers reduced pollution, but putrescine improved the anatomical properties of leaves impacted by heavy metals, most especially lead. This interaction had the highest epidermis thickness, parenchymal cell diameter, primary and secondary xylem diameter, phloem thickness, bundle sheath thickness, vascular bundle length, and width (145.53, 214.17, 90.88, 77.13, 62.12, 30.21, 36.11, and 11.00 micrometers, respectively). By modulating the thickness of leaf tissue cuticles, the heavy metal factor and enhancers boosted the plant's pollution resistance.

Keywords: Date palm (*P. dactylifera* L.), polyamines, zeolite, anatomical characteristics, palm offshoots, heavy elements, stress conditions

Key findings: Heavy metal pollution negatively affected the anatomical characteristics and showed decreased values of epidermal cells, parenchymal cells, and vascular bundles in leaf tissue of the date palm (*P. dactylifera* L.). Bioenhancers, such as putrescine, coumaric acid, and zeolite, improved the anatomical properties of leaf tissues.

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INTRODUCTION

The date palm (*Phoenix dactylifera* L.) belongs to the family Arecaceae, which includes more than 4000 species and around 200 genera. It is one of the most important perennial fruit trees in many countries worldwide. Iraq and the Arabian Gulf region seemed to be its original habitat, specifically Southern Iraq. Iraq has long been notable for its date palm cultivation, production, and marketing worldwide (Bhat and AL-Daihan, 2012; Rasheed and AL-Badri, 2018). However, date palm trees in Iraq have experienced a significant decline in production levels and a sharp decrease in their numbers over the past two decades (Central Statistical Organization, 2021)**.**

Pollution means any undesirable change in the environment's physical, chemical, or biological properties (air, water, and soil) caused by pollutants. Likewise, pollutants are any solid, liquid, or gaseous substance in certain concentrations. Their transfer from different sources in varying quantities causes health and economic damage to humans, other living organisms, and the environment (Al-Wahbi, 2007; Bhatia, 2009). Heavy metal toxicity has become a major and serious phenomenon, with harmful effects on plants' morphological and anatomical structure. It also leads to a decrease in the rate of photosynthesis in plants due to the closure of stomata with the deposition of elements, inhibition of enzyme activities, and disruption of water balance. Moreover, it affects the permeability of cell membranes, in addition to causing disturbances in nutrient absorption (Kabir *et al*., 2010; Taain, *et al*.,2021)**.**

Heavy metals, such as lead and cadmium, are the most dangerous elements polluting soils and water. The presence of these elements in soils leads to a decrease in leaf area and in the synthesis of chlorophyll, inhibiting photosynthesis that eventually decreases carbohydrate content (Singh *et al*., 2011). Exposure of plants to heavy metal stress can potentially cause plant toxicity, which causes delayed seed germination, reduced nutrient absorption, changes in anatomical indicators and metabolic disorders, weak growth and development, production decline, and reduced ability to fix molecular nitrogen (Ashraf and Ali, 2007; Al-Aradi *et al*., 2020). Plants respond to heavy metals, including lead and cadmium, at morphological, biochemical, molecular, and anatomical levels (Nakashima *et al.*, 2009). When plants absorb heavy metals, anatomical changes occur in the leaves, such as a significant decrease in the size of the vascular bundles (wood and phloem) and a reduced size of mesophyll cells (Al-Jabary *et al.*, 2017)**.**

The use of natural enhancers improves the soil's physical, chemical, and fertility properties, thus providing a good environment for plant growth to increase moisture content and provide nutrients, the most important of which is zeolite. The treatment of plants with polyamine compounds, such as spermine, putrescine, and coumaric acid, also boosts the activity of enzymatic antioxidants and concentrations of non-enzymatic antioxidants, as well as augments the anatomical characteristics of the plant (Yamada *et al*., 2002). With the increasing rates of heavy metal pollution, many studies have turned to the use of polyamines for their role in alleviating abiotic stresses, such as drought, salinity, and heavy metal toxicity, to maintain natural growth and economic productivity (Malik *et al*., 2022)**.**

MATERIALS AND METHODS

This study occurred on date palm offshoots of the Jabjabb cultivar, aged 5–6 years, grown in a permanent soil by creating a polluted environment with heavy metals. The experimental soil received contaminated-water irrigation with heavy metals Pb (NO3)2 (300 mg kg^{-1}) and cadmium chloride CdCl2 (3 mmol), aside from the control treatment. Then, some polyamine compounds, putrescine (500 mg L^{-1}) and coumaric acid (500 mg L^{-1}), and zeolite at 10 kg $palm^{-1}$ were successful in their soil application, aside from the control treatment. The offshoots incurred treatment with pollutants and pollution treatments for nine months alternately (one month of treatment with pollutants and the next month with treatments).

Preparation of the paraffin-embedded anatomical sections of leaves followed the method described by Al-Najjar *et al*. (2021) and Al-Najjar *et al*. (2020, 2020b). The fixation process (fixation) proceeded in FAA solution for 48 hours; then, the cut parts underwent passing through ascending concentrations of ethanol, with the samples embedded in paraffin wax at 58 °C. Next, the models' cutting with a rotary microtome to a thickness of 10 micrometers took place before being carried on slides and stained with Safranin dye. After placing in fast green dye, the slides with the samples attained drops of DPX before being covered. Then, the slides entailed studies with measurements taken in micrometers (micrometer μm) using the ocular micrometer lens in an Olympus optical microscope equipped with a camera attached to the calculator.

Statistical analysis

A randomized complete block design (RCBD) layout comprised the factorial arrangement and two factors. The results' assessment used analysis of variance for anatomical structure, engaging the statistical program SPSS. The means also reached analysis, with the significance tested according to the least significant difference (LSD) test at the probability level of 0.05 (Bashir, 2003).

RESULTS AND DISCUSSION

Thickness of the cuticle layer

The results showed the effect of polyamine compounds and zeolite on the thickness of the cuticle layer of date palm seedlings grown under conditions of heavy metal stress (Table 1). The pollution factor affected the thickness of the cuticle layer, with lead recording the highest rate of 10.26 micrometers, revealing a significant difference from the rest of the treatments. The cadmium treatment recorded a rate of 8.98 micrometers, while the control treatment recorded the lowest rate of 4.93. The enhancers also altered the thickness of the cuticle layer, with the control treatment recording the highest value of 9.71 micrometers, giving a significant difference from the rest of the treatments. The putrescine treatment provided the lowest rate of cuticle thickness of 7.26 micrometers, with a significant difference from the zeolite treatment, which recorded a rate of 7.70 micrometers. However, the putrescine treatment did not differ significantly from the coumaric acid treatment.

As for the interaction between pollution treatments and enhancer treatments, the interaction treatment between lead and the control recorded the highest rate of cuticle thickness of 10.60 micrometers, with a significant difference from the rest of the interactions. The interaction treatment between the control and putrescine gave the lowest rate of cuticle thickness of 3.57 micrometers, with a substantial difference from the rest of the interactions, except for the interaction treatments between the control, coumaric acid, and zeolite. It is also noteworthy from the results of the same table that the interaction treatments between lead and putrescine, coumaric acid, and zeolite did not have any relevant differences, recording rates of 10.00, 10.11, and 10.34 micrometers, respectively.

Table 1. Effect of polyamines and zeolites on the thickness of the cuticle layer of the leaves of date palm seedlings grown under heavy metal stress conditions.

Pollutants	Enhancers				Effect rate of Pollutants
	Control	Putrescine	Coumaric acid	Zeolites	
Control	8.00	3.57	3.99	4.15	4.93
Lead	10.60	10.00	10.11	10.34	10.26
Cadmium	10.54	8.22	8.44	8.60	8.95
Effect rate of enhancers	9.71	7.26	7.51	7.70	
$LSD_{0.05}$	Pollutants = 0.316		Enhancers = 0.334		Interaction = 0.41

Table 2. Effect of polyamines and zeolites on the thickness of epidermal cells of the leaves of date palm seedlings grown under heavy metal stress conditions.

Pollutants	Enhancers				Effect rate of Pollutants
	Control	Putrescine	Coumaric acid	Zeolites	
Control	12.54	13.10	13.10	12.81	12.89
Lead	6.16	11.00	9.90	9.60	9.17
Cadmium	5.50	8.80	7.51	7.04	7.21
Effect rate of enhancers	8.07	10.97	10.17	9.82	
$LSD_{0.05}$	Pollutants = 0.3296		Enhancers = 0.3805		Interaction = 0.559

Table 3. Effect of polyamines and zeolites on the diameter of parenchymal cells of the leaves of date palm seedlings grown under heavy metal stress conditions.

Pollutants	Enhancers				Effect rate of Pollutants
	Control	Putrescine	Coumaric acid	Zeolites	
Control	37.80	45.40	42.90	41.30	41.85
Lead	27.90	36.11	35.60	35.40	33.75
Cadmium	24.60	33.40	31.20	29.17	29.59
Effect rate of enhancers	30.10	38.30	36.57	35.29	
$LSD_{0.05}$	Pollutants = 0.843		Enhancers = 0.974		Interaction = 1.223

Epidermal cell thickness

The findings revealed the effect of polyamine compounds and zeolite on the thickness of epidermal cells of date palm seedlings grown under heavy metal stress (Table 2). The pollution factor significantly affected the thickness of epidermal cells, with the control treatment recording the topmost rate of epidermal cell thickness of 12.89 micrometers, considerably different from the rest of the treatments. Cadmium treatment provided the lowest rate of 7.21 micrometers, and lead treatment recorded a rate of 9.17 micrometers.

The enhancers also changed the thickness of epidermal cells, with the putrescine treatment recording the highest value of 10.97 micrometers, significantly different from the rest of the treatments. The control treatment gave the lowest rate of epidermal cell thickness of 8.07 micrometers, notably differing from the zeolite treatment, which recorded a rate of 9.82 micrometers. As for the interaction between pollution treatments and enhancer treatments, the interaction between the control treatment and putrescine and coumaric acid resulted in the premier rate of epidermal cell thickness of 13.10 for both interactions. It also indicated a significant variation from the rest of the interactions. The interaction between cadmium and the control treatment recorded the lowest rate of epidermal cell thickness of 5.50 micrometers. The interaction treatment between cadmium, coumaric acid, and zeolite did not differ significantly, recording rates of 7.51 and 7.04 micrometers, respectively.

Parenchyma cell diameter

The study revealed the effect of polyamine compounds and zeolite on the diameter of the parenchyma cells of date palm seedlings grown under heavy metal stress (Table 3). The pollution factor significantly affected the diameter of the parenchyma cells, with the control treatment recording the broadest rate of parenchyma cell diameter of 41.85 micrometers, differing remarkably from the rest of the treatments. Cadmium treatment provided the lowest rate of 29.59 micrometers, with the lead treatment giving a rate of 33.75 micrometers. The enhancers also influenced the diameter of the parenchyma cells, with the putrescine treatment recording the widest value of 38.30 micrometers, significantly varying from the rest of the treatments. The control treatment displayed the lowest rate of parenchyma cell diameter of 30.10

micrometers, with a significant difference from the zeolite treatment, which recorded a rate of 35.29 micrometers.

Regarding the interaction between pollution treatments and enhancer treatments, the interaction between the control treatment and putrescine recorded the highest rate of parenchyma cell diameter of 45.40, notably varying from the rest of the interactions. The interaction between cadmium and the control treatment expressed the lowest rate of parenchyma cell diameter of 24.60 micrometers. No significant differences appeared between the interaction treatments of lead with putrescine, coumaric acid, and zeolite, which recorded rates of 36.11, 35.60, and 35.40 micrometers, respectively.

Primary wood diameter

Table 4 presents the results of the effect of polyamine compounds and zeolite on the diameter of the primary wood of date palm seedling leaves grown under heavy metal stress (Table 4). The pollution factor significantly affected the diameter of the primary wood, with the control treatment recording the broadest rate of 70.10 micrometers. It notably differed from the rest of the treatments. Cadmium treatment gave the narrow rate of 20.74 micrometers, and lead treatment recorded a rate of 25.20 micrometers.

The impact of enhancers on the diameter of the primary wood is remarkable, with the putrescine treatment recording the highest value of 43.28 micrometers, varying significantly from the rest of the treatments. The control treatment recorded the lowest rate of the diameter of the primary wood, 33.46 micrometers, with a significant difference from the zeolite treatment, which recorded a rate of 35.29 micrometers.

The interaction between pollution treatments and enhancer treatments also considerably altered the primary wood's diameter. The interaction treatment between the control and putrescine recorded the maximum rate of the primary wood diameter at 75.91 micrometers, with a significant difference from the rest of the interactions. The interaction between cadmium and the control treatment indicated the minimum rate of the primary wood diameter at 17.91 micrometers. No weighty differences occurred between the interaction treatments of cadmium with coumaric acid and zeolite, which recorded 21.18 and 20.13 micrometers, respectively. Likewise, no significant distinctions resulted between the interaction treatment of lead with the control and the interaction treatment of cadmium with the control, which recorded rates of 18.33 and 17.91 micrometers, respectively.

Length of the vascular bundle

The results demonstrated the effect of polyamine compounds and zeolite on the length of the vascular bundle of date palm seedling leaves grown under conditions of heavy metal stress (Table 5). The pollution factor significantly affected the length of the vascular bundle, with the control treatment recording the longest rate of 263.71 micrometers, varying remarkably from the rest of the treatments. Cadmium treatment recorded the shortest rate of 166.73 micrometers, and lead treatment gave a rate of 191.23 micrometers .The enhancers also

Table 4. Effect of polyamines and zeolites on the primary wood diameter of the leaves of date palm seedlings grown under heavy metal stress conditions.

Pollutants	Enhancers				Effect rate of Pollutants
	Control	Putrescine	Coumaric acid	Zeolites	
Control	64.13	75.91	72.13	68.22	70.10
Lead	18.33	30.21	27.11	25.13	25.20
Cadmium	17.91	23.72	21.18	20.13	20.74
Effect rate of enhancers	33.46	43.28	40.14	37.83	
$LSD_{0.05}$	Pollutants = 0.843		Enhancers = 0.974		Interaction = 1.687

Table 5. Effect of polyamines and zeolites on the vascular bundle length of the leaves of date palm seedlings grown under heavy metal stress conditions.

Pollutants	Enhancers				Effect rate of Pollutants
	Control	Putrescine	Coumaric acid	Zeolites	
Control	220.66	302.88	280.47	250.81	263.71
Lead	148.18	214.17	209.81	192.77	191.23
Cadmium	137.17	187.33	177.21	165.22	166.73
Effect rate of enhancers	168.67	234.79	222.50	202.93	
$LSD_{0.05}$	Pollutants = 0.722		Enhancers = 0.834		Interaction = 1.444

Table 6. Effect of polyamines and zeolites on the vascular bundle width of the leaves of date palm seedlings grown under heavy metal stress conditions.

Pollutants	Enhancers				Effect rate of Pollutants
	Control	Putrescine	Coumaric acid	Zeolites	
Control	155.45	192.37	177.13	160.71	171.42
Lead	96.66	154.53	143.48	137.71	133.10
Cadmium	88.51	126.25	120.33	110.81	111.48
Effect rate of enhancers	113.54	157.72	146.98	136.41	
$LSD_{0.05}$	Pollutants = 0.847		Enhancers = 0.978		Interaction = 1.693

modified the length of the vascular bundle, with the putrescine treatment recording the highest value of 234.79 micrometers, differing significantly from the rest of the treatments. The control treatment provided the lowest rate of the length of the vascular bundle of 168.67 micrometers.

Similarly, the interaction between pollution treatments and enhancer treatments significantly influenced the length of the vascular bundle. The interaction between the control treatment and putrescine recorded the highest rate of 302.88 micrometers, notably different from the rest of the interactions. The interaction between cadmium and the control treatment expressed the lowest rate of the vascular bundle length at 137.17 micrometers.

Width of the vascular bundle

The results enunciated the effect of polyamine compounds and zeolite on the width of the vascular bundle of date palm seedling leaves grown under conditions of heavy metal stress (Table 6). The pollution factor markedly changed the width of the vascular bundle, with the control treatment recording the highest rate of 171.42 micrometers, varying pronouncedly from the rest of the treatments. Cadmium treatment demonstrated the lowest rate of 111.48 micrometers, and the lead treatment recorded a rate of 133.10 micrometers.

The enhancers also affected the width of the vascular bundle, with the putrescine treatment recording the utmost value of 157.72 micrometers, significantly differing from the rest of the treatments. The control treatment provided the lowest rate of the width of the vascular bundle at 113.54 micrometers. The interaction between pollution treatments and enhancer treatments also notably altered the width of the vascular bundle. The interaction between the control treatment and putrescine gave the highest rate of 192.37 micrometers, significantly different from the rest of the interactions. The interaction between cadmium and the control treatment recorded the lowest rate of the vascular bundle width at 88.51 micrometers.

The cuticle layer in leaves is one of the anatomical traits affected by different stress conditions. The thickness of the cuticle layer increases in the leaves of plants influenced by stress. This is one of the mechanisms of plant adaptation to stress conditions. The increased cuticle layer helps to reduce water loss from the plant, thereby maintaining an internal water balance that helps the plant cope with environmental stresses (Gowayed and Almaghrabi, 2013). Al-Mahmoudi *et al.'s* (2023) study on some aspects of environmental adaptation of date palm seedlings growing under environmental stress conditions showed the thickness of the cuticle layer has an association with the growth rate of the epidermis and mesophyll tissue.

The increased activity and growth of the mesophyll tissue under normal conditions led to the expansion of the cuticle layer, thus reducing its thickness. On the contrary, the cuticle layer thickens when the plant grows in stressful conditions. The epidermis (especially the upper epidermis) plays an important role in isolating heavy metals from mesophyll cells, essential for photosynthesis (Gomes *et al*., 2011). Plants growing under ideal growth conditions have a thicker cuticle layer due to the activity of the mesophyll tissue in photosynthesis and a boost in products of this process (carbohydrates), eventually increasing the size of leaf tissue cells, including epidermal cells. Many studies have shown that exposure of plants to heavy metal stress leads to a decrease in the thickness of the cuticle in leaves due to the deterioration and weak growth of the mesophyll tissue. Consequently, this reduces the products of photosynthesis essential for the growth of epidermal cells (Qaisar *et al*., 2005; Lukovic *et al*., 2012; Al-Saadi *et al*., 2013).

Anatomical changes, such as an increase in the size of vascular bundles, thickness of the phloem, and diameter of the xylem vessels in plant leaf tissues, are some that occur in response to environmental stress. (Parmar *et al*., 2015; Al-Alpresem *et al*., 2023). Heavy metal stress, for example, can cause a significant decrease in the size of vascular bundles. This is due to a decline in the number and thickness of the conducting vessels (xylem and phloem) making up these bundles. This is a plant adaptation to cope with environmental stresses, including heavy metal pollution. Alternatively, it could refer to the relevant impact of heavy metal stress on the components of the phloem (sieve tubes and companion cells) when exposed to various stresses (Vollenweider *et al*., 2006).

This study's results agree with the findings by Al-Jaberi (2017) on the seasonal variation of heavy metal pollution and the effect of cadmium and lead treatment on some anatomical traits of the date palm variety Barhi. His study showed the treatment with heavy metals led to clear changes in the anatomical traits of leaves. These are an increase in the spread of tannin cells and a decrease in the indices of mesophyll tissue and vascular bundle indices.

The use of putrescine reduced the negative effects caused by lead and cadmium pollution factors in leaf tissues. This may be due to the influence of putrescine on these traits, as it participates in cell division and cell development (Kusano *et al*., 2008). Additionally, putrescine or polyamines can act as growth regulators. Their low molecular weight and cationic charge allow them to move between plant tissues quickly, and they have a broad range of effects on growth, including stimulating cell division and improving tissue quality (Gupta *et al*., 2013). Similarly, using natural enhancers, such as zeolite, can improve the soil's physical, chemical, and water properties, causing increased crop yields. Zeolite can also help improve plant growth and development, enhance soil-water retention, and reduce the toxicity of heavy metals (Andry *et al*., 2008). Coumaric acid has proven to reduce the toxic effects of heavy metals and improve the anatomical traits of leaf tissues by activating and remodeling the cell wall due to its high content of non-enzymatic antioxidants and antioxidant-enhancing activity (Liu *et al*., 2020).

CONCLUSIONS

Heavy metal (Pb and Cd) pollution negatively affected the anatomical characteristics and showed decreased values of epidermal cells, parenchymal cells, and vascular bundles. Bioenhancers (putrescine, coumaric acid, and zeolite) improved the anatomical properties of leaf tissues, recording the highest values of various parameters.

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