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Combination Effect Between Lead and Salinity on Anatomical Structure of Date Palm *Phoenix dactylifera* L. Seedlings

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ABSTRACT

The study was conducted to evaluate the effect of lead (Pb) stress alone or in combination with salinity on the anatomical structure of roots and leaves of Date palm seedlings. Pb was added to soil at 100, 300 and 600 mg/kg concentrations as a pure aqueous solution or mixed with saline solution at 200 mM. Compared with the control, the microscopic study of root tissues showed that all treatments caused a significant increase in the thickness of epidermis, endodermis and pericycle, whereas the cortex thickness and diameters of the vascular cylinder, protoxylem and metaxylem decreased significantly. However, only the phloem diameter was affected significantly by 600 mg/kg Pb with or without salinity. Compared with the control, results on leaf tissues revealed that treatment with 300 and 600 mg/kg Pb alone or in combination with salinity led to a significant increase in the thickness of cuticle layer, upper epidermis and lower epidermis. Results also showed a significant increase in the diameter of tannin and palisade cells when treated with 100 mg/kg Pb with or without salinity. Small vascular bundle diameter decreased significantly in seedlings exposed to Pb at all examined concentrations with or without salinity.

INTRODUCTION

Date palm *Phoenix dactylifera* L. belongs to the Arecaceae family (Abass, Hassan, & Al-Jabary, 2015). Approximately 120 million of this plant species are distributed in over 30 countries worldwide, producing about 7.5 million tons of fruits annually; it has been cultivated for its sweet fruits and as an ornamental tree (Abd Rabou & Radwan, 2017; Johnson, Al-Khayri, & Jain, 2013). Date palm trees are common in arid and semiarid regions (Alhammadi & Kurup, 2012), which suffer from salinity due to insufficient rainfall coupled with over-irrigation using brackish or saline groundwater (Al-Khashman, Al-Muhtaseb, & Ibrahim, 2011). Moreover, these regions suffer from heavy metal pollution (Al-Jabary, Neama, & Abass, 2016). Lead (Pb) is an extremely toxic heavy metal that disturbs various plant physiological processes (Jaishankar, Tseten, Anbalagan, Mathew, & Beeregowda, 2014). Individually, salinity and Pb stress have been the subject of intensive research on plants other than Date palm. With regard to Date palm, Alhammadi & Kurup (2012) reviewed the literature in relation to the salinity effect on Date palm; in addition, they reported the morphological, physiological, biochemical and molecular changes in Date palm in response to salinity stress. The negative effect of Pb on Date palm was reported (Abass, Neama, Al-Jabary, & Abass, 2016), and these effects were represented as the decline in photosynthetic pigments, total soluble protein and free amino acids contents; meanwhile, their study showed that total soluble carbohydrates, anthocyanin and proline contents increased, and Pb at 276 mg/kg soil affected the genetic stability of Date palm. Plants respond to heavy metals, including Pb, at the morphological, biochemical, molecular and anatomical levels (Nakashima, Ito, & Yamaguchi-

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Shinozaki, 2009). Anatomical changes may occur when plants absorb heavy metals across the roots, and a remarkable decrease in the size of root tissue cells, which results in the shrinkage of root diameter, is one of the most prominent changes observed (Batool, Hameed, Ashraf, Ahmad, & Fatima, 2015). Pb shows an adverse effect on root anatomical structure (Zarinkamar, Ghelich, & Soleimanpour, 2013). Moreover, Pb-induced anatomical changes in leaf and these changes include the reduction in size of mesophyll parenchyma, vascular bundle, xylem vessel diameter and epidermal cell size (Batool, Hameed, Ashraf, Ahmad, & Fatima, 2015). Regarding the effect of Pb on the Date palm anatomical structure, a unique study conducted by Naema, Abass, & Al-Jabary (2017) indicated a significant reduction in the size of vascular bundles and conducting elements (xylem and phloem), thickness of mesophyll and size of parenchyma cells in Date palm leaves under Pb stress. Anatomical changes under salinity stress were previously reported in various studies. Younis et al. (2014) observed a decrease in the xylem and phloem area in the roots and leaves of *Gazania harlequin* L. In the field, the reductions in cell size and vascular tissue of maize roots and leaves have been reported (Farhana, Rashid, & Karmoker, 2014).

Plants are subjected to a combination of different abiotic stresses. Salinity and heavy metals are important abiotic stress factors for plants (Gul, Nawaz, Azeem, & Sabir, 2016). Hatje et al. (2003) indicated the increased metal mobility in the saline soil, which resulted in increased metal accumulation in plant parts. Meanwhile, Kadukova & Kalogerakis (2007) reported that Pb accumulation decreased in the root of *Tamarix smyrnensis* Bunge under high salinity, but low salinity showed no effect on Pb accumulation. In addition, Kadkhodaie, Kelich, & Baghbani (2012) observed that the increase of salinity led to the increase of Pb availability in soil, which subsequently increased its accumulation in sunflower and sudangrass roots; by contrast, no accumulation was observed in stems and leaves. Acosta, Jansen, Kalbitz, Faz, & Martínez-Martínez (2011) suggested the competition between Pb and Ca^{+2} for absorption sites in soil as the main mechanism for the regulation of Pb mobility. However, only a few studies were performed on the combination effects of heavy metals and salinity on different plant characteristics. The results of Huang, Wei, Yang, Dai, & Zhang (2007) showed a decline in chlorophyll content in four barley *Hordeum vulgare* genotypes under the combined effect of cadmium d and salinity and a significant decrease in the biomass and length for all genotypes. Their study also showed disrupted micronutrient accumulation in roots and leaves. Combination effect of copper and salinity on anatomical organization of leaves and roots of wheat (*Triticum aestivum* L.) have been studied. The study showed that under combined stresses, the thickness of upper and lower epidermis increased, whereas the size of conduct bundles, which include xylem vessels, and root tissues decreased. In addition, the exodermal thickness also decreased while, the endodermis thickness increased (Atabayeva et al., 2013).

On the effect of combination of cadmium and copper with salinity on the two maize cultivars (*Zea mays* L.), Gul, Nawaz, Azeem, & Sabir (2016) reported a reduction in fresh and dry weights of roots and chlorophyll content in leaves. During the stress, K^+ concentration increased in shoots of both cultivars and they concluded that the effect of combined stresses was drastic compared with that of individual stress.

Given the lack of previous studies on the combined effect of Pb and salinity on Date palms, the study was conducted at the Palm Research Centre, Basrah University/Iraq, during the spring of 2018 to identify the effect of Pb at different concentrations (i.e. 100, 300 and 600 mg/kg) solely and in combination with salinity on the anatomical changes in the roots and leaves of Date palm seedlings.

MATERIALS AND METHODS

This experiment was conducted at Date Palm Research Centre, Basrah University, Iraq from April to August 2019. Twenty one Date palm seedlings have been selected and cultured in plastic pot containing silty clay soil. The soil properties were pH = 7.54, EC = 2.31 dS/m, organic matter content 5.48%, Cation Exchange Capacity (CEC) = 21.51 cmol/kg. $Pb(NO_3)_2$ (Sigma Aldrich, USA) was used to prepare aqueous solutions at three concentrations i.e. 100, 300 and 600 mg/kg. Pure NaCl (Himedia, India) was used to prepare saline solution at 200 mM. Every examined concentration of Pb was added to soil with irrigation water alone, as well as mixed with saline solution. While, the control seedlings were irrigated with Pb and salt free water.

Randomized Complete Block Design (RCBD) was used as an experimental design with three replicates for each treatment. The treatments were applied as follow: (1) control treatment (irrigation with water only); (2) Pb at 100: irrigation water contains Pb at 100 mg/kg; (3) Pb at 100+ salinity: irrigation water contains Pb at 100 mg/kg and NaCl at 200 mM; (4) Pb at 300: irrigation water contains Pb at 300 mg/kg; (5) Pb at 300+ salinity: irrigation water contains Pb at 300 mg/kg and NaCl at 200 mM; (6) Pb at 600: irrigation water contains Pb at 600 mg/kg; (7) Pb at 600+ salinity: irrigation water contains Pb at 600 mg/kg and NaCl at 200 mM

The seedlings were irrigated according to field capacity; each treatment was added with 500 ml every week.

Anatomical Analysis

Cross sections of Date palm root and leaf were prepared according to Willey (1971). The segments of root and first expanding leaf was fixed in FAA (each 100 ml consist of 5 ml of formaldehyde + 5 ml of acetic acid + 90 ml of 70% ethyl alcohol) for 24 hours then washed with ethyl alcohol subsequently in gradual increasing concentrations (70, 80, 90, 95%). The duration was one hour for each concentration and followed by overnight in absolute alcohol. The samples were then transferred to a mixture of absolute alcohol and xylene in different proportions (1:3, v/v), (1:1, v/v), (3:1, v/v) (alcohol: xylene), as well as absolute xylene for half an hour per proportion. Followed by transferring samples into a mixture of xylene and paraffin wax (1:1 v/v). The samples were heated in oven at 60^{o}C and left in paraffin wax overnight at same temperature degree. At the same temperature degree, pure paraffin was poured in plastic molds containing the samples. After labeled and cooled in running water for 24 hours, the samples were ready for cutting. A table microtome was used to obtain cross sections with 10-15 micrometer thickness per slide, then placed in water bath at 50-60^{o}C for 5 min. After transferring the samples into glass slides, drops of Mayer solution (mixture of egg albumin and glycerin) put into the glass slides. The glass slides were then placed in the oven for 90 minutes at 50-60°C. The glass slides were transferred to Coplin Jar containing absolute xylene for 60 minutes and the process was repeated three times. The process was then finally passed in ethyl alcohol at several concentrations (100, 95, 90, 80, 70 and 50 %), for 15 minutes in each descending concentrations. The samples staining was carried out using safranin solution for 30 minutes. Safranin solution was prepared by dissolving 1 g of safranin in 100 ml of 70% ethyl alcohol. The staining was also conducted using fast green solution (dissolved 1 g of fast green dye in 100 ml absolute alcohol) for 15 seconds. The slides was passed in xylene for 5 minutes and the process was repeated three times and left to dry for 5 minutes. One drop of DPX (Distrene Plasticize Xylene) was added per slide then covered by slide cover and continued by heating the slides on heat plate before examining by microscopic analysis. Olympus microscope (Japan, model: CX31RBSFA; S.N:OMOO418) with a camera was used to examine anatomical sections. The thickness of epidermis, endodermis, pericycle also diameter of vascular cylinder, metaxylem, protoxylem and phloem were measured as root anatomy characteristics. While leaf anatomy characteristics included thickness of cuticle layer, upper epidermis and lower epidermis and diameter of tannin cells palisade cells, small vascular bundle, fiber bundle. The study also examined large vascular bundle characteristics which included dimension of vascular bundle, distance between bundles and diameter of protoxylem, metaxylem and phloem.

The data were statistically analyzed using One Way ANOVA using SPSS (V.22). The mean comparison was carried out using least significant difference (LSD) at% 5 probability level.

RESULTS AND DISCUSSION

Anatomical Root Features

Table 1 shows the effect of Pb alone or in combination with 20 mM salinity on some anatomical root characteristics of Date palm seedlings. The cross sections of Date palm seedling roots were presented on Fig. 1a–g.

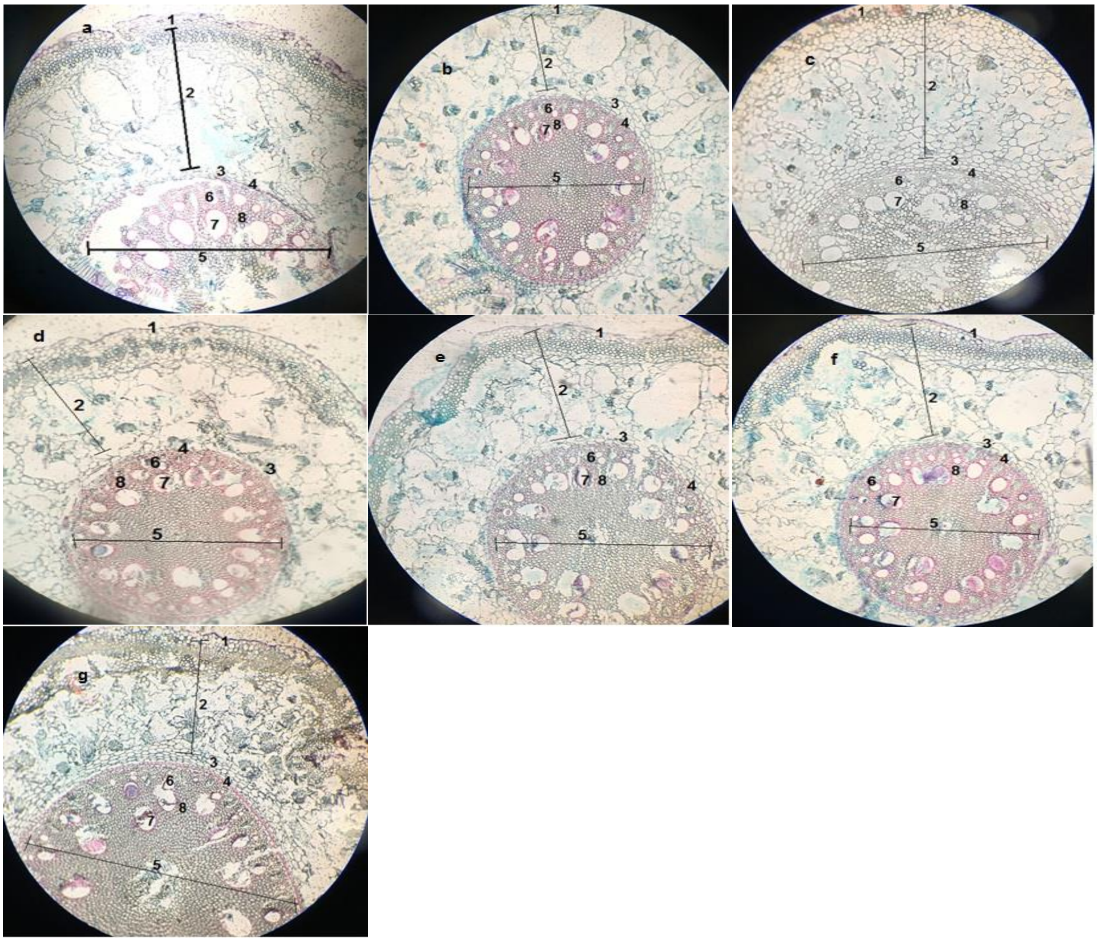
Epidermal Thickness

The epidermal thickness reached 22 µm in the control, whereas single 100 mg/kg Pb led to a significant increase in epidermal thickness of 25 µm. In combination with salinity, the treatment induced the increase of epidermal thickness up to 28 µm. In addition, an increased thickness up to 30 µm was reported with Pb stress at 300, 600 mg/kg and the combination of 300 mg/kg and salinity. The highest increase of epidermal thickness with the value of 35 µm was observed at 600 mg/kg Pb combined with salinity.

Table 1. Effect of single Pb or combined with salinity on some anatomical root characteristics of Date palm seedlings

Treatment**)	Epidermis Thickness (µm)	Endodermis Thickness (µm)	Cortex Thickness (µm)	Pericycle Thickness (µm)	Vascular Cylinder Diameter (µm)	Protoxylem Diameter (µm)	Metaxylem Diameter (µm)	Phloem Diameter (µm)
Control	22*)	14	900	10	1550	70	160	20
Pb at 100	25	20	855	13	1360	70	130	20
Pb at 100 + salinity	28	18	850	13	1270	68	120	20
Pb at 300	30	20	760	15	1230	60	120	20
Pb at 300 + salinity	30	20	710	20	1200	45	100	18
Pb at 600	30	20	680	20	980	38	100	17
Pb at 600 + salinity	35	20	640	20	920	40	80	13
LSD	1.13	2.25	23.23	1.27	38.29	7.28	9.57	2.11

Remarks: *) Value (mean, n = 3) significantly difference at $P < 0.05$; **) The concentration unit of Pb was mg/kg



Remarks: a. control; b. Pb at 100 mg/kg; c. Pb at 300 mg/kg; d. Pb at 600 mg/kg; e. Pb at 100 mg/kg with salinity at 200 mM; f. Pb at 300 mg/kg with salinity at 200 mM; g. Pb at 600 mg/kg with salinity at 200 mM. Which: 1. Epidermis; 2. Cortex; 3. Endodermis; 4. Pericycle; 5. Vascular cylinder; 6. Protoxylem; 7. Metaxylem; 8. Phloem

Fig. 1. Cross sections of Date palm seedling treated with different concentrations of lead with or without salinity (X40)

Endodermal Thickness

Root endodermal thicknesses was induced by the application of Pb in any applied concentrations compared to control. The increase was observed from 14 µm (control) to 20 µm in any Pb treatments, except combination of 100 mg/kg Pb and salinity with the value of 18 µm.

Cortical Thickness

Results indicated a gradual decrease in cortical thickness during the Pb exposure of Date palm seedlings. The reductions were severe when Pb stress combined with salinity. When singly applied, 100 mg/kg Pb reduced cortical thickness from 900 µm (control) to 855 µm and 850 µm when combined with salinity. When the concentration increased up to 300 mg/kg, the reduction was more severe up to 760 µm in single application and 710 µm in combination with salinity. The highest reductions were observed at 600 mg/kg Pb when apllied ini combination with salinity. The value reached 640 µm compared to 680 µm when applied in single treatment.

Pericycle Thickness

Exposure of Date palm seedlings to Pb increased the pericycle thickness significantly. Pericycle thickness increased from 10 µm (control) to 13 µm at 100 mg/kg Pb was applied either single or in combination with salinity. Applied in single 300 mg/kg Pb, pericycle thickness increased up to 15 µm. In combination with salinity, 300 and 600 mg/kg Pb gave similar effects to increase pericycle thickness with the value of 20 µm.

Vascular Cylinder Diameter

The vascular cylinder diameter of Date palm seedlings decreased significantly from 1550 µm (control) to 1360 and 1270 µm at 100 mg/kg Pb alone and in combination with salinity, respectively. Greater reduction of cylinder diameter was observed at 300 mg/kg Pb, with the values of 1230 µm when applied single and 1200 µm in combined application with salinity. The severe reduction was observed at 600 mg/kg Pb which reached up to 920 µm when applied in combination with salinity.

Protoxylem Diameter

No significant effect on the protoxylem diameter was observed between control and 100 mg/kg Pb when applied both solely or in combination with salinity (Table 1). The decreases of protoxylem diameter were merely observed on 300 mg/kg Pb single or with combination with salinity that reached 60 and 45 µm, respectively. The highest decrease were observed on 600 mg/kg Pb, on both single and combination with salinity.

Metaxylem Diameter

All examined Pb concentrations when applied alone or combined with salinity led to significant decreases on metaxylem diameter compared with the control treatment. The highest decrease of metaxylem diameter was observed when 600 mg/kg Pb combined with salinity that reached only 50% from the control.

Phloem thickness

There was no significant decrease phloem diameter on 100 and 300 mg/kg Pb in both on single and with combination with salinity compared to control treatment (Table 1). The decrease of phloem diameter was merely observed at 600 mg/kg Pb and the highest decrement was detected when applied in combination with salinity.

The obtained results revealed that in root tissues, most of the studied traits were influenced by the presence of Pb either applied alone or in combination with salinity. However, the protoxylem and phloem thicknesses were unaffected by the lowest Pb level in this study (100 mg/kg) when applied alone or in combination with salinity. The combination of 600 mg/kg Pb and salinity was the most effective technique in all anatomical root traits, which led to the increased thickness of epidermis, endodermis and pericycle by as much as 28.57%, 30% and 50%, respectively. However, this combined treatment decreased the diameter of vascular cylinder, protoxylem, metaxylem and phloem to as low as 40.64%, 42.58%, 50% and 35%, respectively, and the cortex thickness by as much as 28.88%. The increase in epidermal and endodermal thickness may reflect the heavy metal tolerance mechanism, which considers the most important locations to accumulate heavy metals in plant roots (Batool, Hameed, Ashraf, Ahmad, & Fatima, 2015; Emamverdian, Ding, Xie, & Sangari, 2018). Moreover, this adaptation protects root cells against penetration of toxic metals from the environment (Zarinkamar, Ghelich, & Soleimanpour, 2013). Pericycle thickening under Pb stress was previously reported by Alves, de Jesus, de Almeida, Souza, & Mangabeira (2014); Gomes, de Sáe Melo Marques, de Oliveira Gonçalves Nogueira, de Castro, & Soares (2011); and Tupan & Azrianingsih

(2016). Other studies also reported the increased epidermal and endodermal thickness under salinity stress (Al Hassan, Gohari, Boscaiu, Vicente, & Grigore, 2015; Céccoli, Ramos, Ortega, Acosta, & Perreta, 2011). Heavy metals are transferred after absorbed by the roots through two pathways: apoplastic and symplastic. In symplastic pathway, heavy metals are transported across the root cortex to plant storage tissues (Shahid et al., 2017). Reduction in cortical thickness under heavy metal stress was previously reported by Gomes, de Sáe Melo Marques, de Oliveira Gonçalves Nogueira, de Castro, & Soares (2011) similar phenomena were also observed under salinity stress (Atabayeva et al., 2013). Reduction in conductive element size, particularly the metaxylem, is one of the strategic plant responses to heavy metals stress and such adaptation reduces the translocation of heavy metals to aerial parts (Alves, de Jesus, de Almeida, Souza, & Mangabeira, 2014; Gomes, de Sáe Melo Marques, de Oliveira Gonçalves Nogueira, de Castro, & Soares, 2011; Singh et al., 2015; Tupan & Azrianingsih, 2016).

Anatomical Features of the Date Palm Leaf

Table 2 presents the effect of three Pb concentrations applied alone or in combination with salinity on the anatomical characteristics of Date palm seedling leaves. The data were derived from the cross-sections analysed using light microscope and illustrated in Fig. 2a–2g and Fig. 3a–3g.

Cuticle Layer Thickness

Cuticle layer thickness increased significantly when the seedlings were exposed to 300 and 600 mg/kg Pb either alone or in combination with salinity compared with control. The highest effect on cuticle layer thickness, which increased from 2.10 µm (control) to 4.50 µm was observed under 600 mg/kg Pb with salinity. Notably, 100 mg/kg Pb applied alone or with salinity showed no significant effect on the increment of cuticle layer thickness from the control.

Upper Epidermal Thickness

The thickness of upper epidermis of Date palm seedlings increased significantly when exposed to 300 and 600 mg/kg Pb alone or in combination with salinity. The effect of Pb alone was greater than when combined with salinity. Moreover, 600 mg/kg Pb applied alone exhibited more significant effects compared with 300 mg/kg Pb. The upper epidermal thickness of control plant was 4.89 µm, which increased to 10.23 and 11.62 µm when the seedlings were exposed to 300 and 600 mg/kg Pb, respectively.

Lower Epidermal Thickness

With the same trend of upper epidermal thickness results, the lower epidermal thickness showed no significant difference in the control (3.69 µm) and under 100 mg/kg Pb applied alone (4.33 µm) or in combination with salinity (3.39 µm). Meanwhile, 300 mg/kg Pb significantly increased the upper epidermal thickness when applied alone (8.35 µm) or in combination with salinity (7.09 µm). The highest increase was recorded in seedlings exposed to single 600 mg/kg Pb (9.99 µm). However, the combined effect of Pb at this concentration with salinity was lower than the effect of Pb alone (8.39 µm).

Tannin Cell Diameter

Pb with 100 mg/kg concentration showed no significant effect on tannin cell diameter, either alone or when combined with salinity. A significant change in tannin cell diameter was observed when the Date palm seedlings were exposed to 300 mg/kg Pb only (26.92 µm) and in combination with salinity (22.38 µm). The highest tannin cell diameter reached 28.92 µm, which was recorded under 600 mg/kg Pb treatment.

Palisade Cell Length

The palisade cell length was unaffected when 100 mg/kg Pb was added to the soil, either alone or in combination with salinity compared to control. While, 300 and 600 mg/kg Pb led to significant increase in the length of palisade cells with the presence or absence of salinity compared with control and 100 mg/kg Pb alone or combined with salinity.

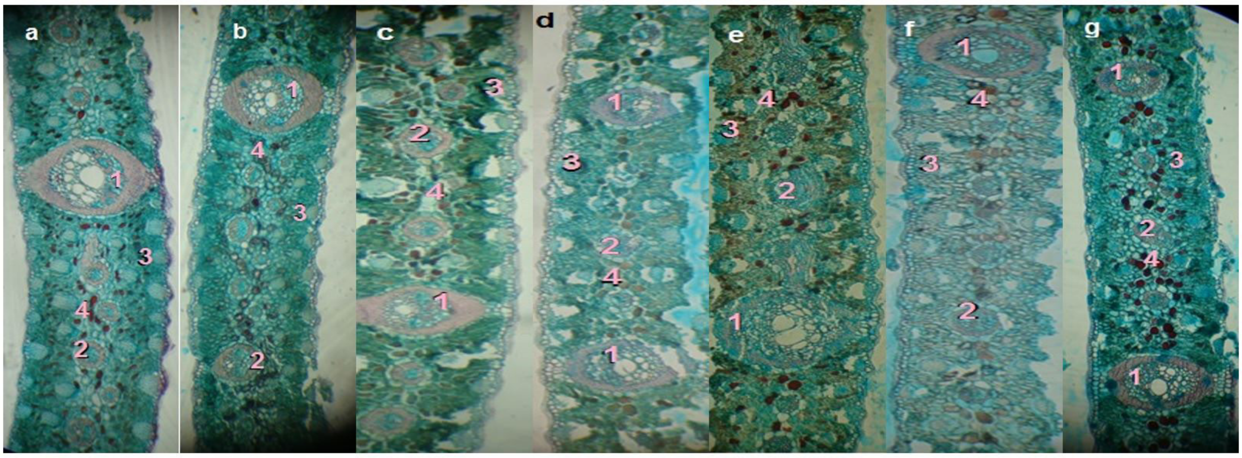
Diameter of Small Vascular Bundles

TThe diameter of small vascular bundles in Date palm seedlings decreased in 100, 300 and 600 mg/kg Pb-treated seedlings without significant differences with the control. However, the values decreased significantly when salinity was existed in all concentration levels of Pb compared with the control. The diameter of small vascular bundles decreased from 46.20 µm (control) to 39.20, 37.40 and 35.30 µm in 100, 300 and 600 mg/kg Pb in combination with salinity, respectively.

Table 2. Effect of Pb alone or combined with salinity on some anatomical leaf characteristics of Date palm seedlings

Treatment**)	CL Thickness (µm)	UE Thickness (µm)	LE Thickness (µm)	TC Diameter (µm)	PC Length (µm)	SVB Diameter (µm)	FB Diameter (µm)
Control	2.10*)	4.89	3.69	17.52	8.00	46.20	20.88
Pb at 100	2.07	5.59	4.33	17.23	8.7	43.00	14.00
Pb at 100 + salinity	2.12	4.75	3.39	16.52	9.11	39.20	22.60
Pb at 300	2.80	10.23	8.35	26.92	15.8	42.60	36.00
Pb at 300 + salinity	3.09	9.23	7.09	22.38	15.89	37.40	33.60
Pb at 600	4.20	11.62	9.99	28.92	17.98	41.80	33.81
Pb at 600 + salinity	4.50	11.09	8.39	26.53	17	35.30	32.70
LSD	0.23	1.23	1.17	2.03	3.19	5.07	2.16

Remarks: *) Value (mean, n=3) significantly difference at P<0.05; **) The concentration unit of Pb was mg/kg; CL: Cuticle layer; UE: Upper epidermis; LE: Lower epidermis; TC: Tannin cells; PC: Palisade cells; SVB: Small vascular bundle; FB: Fiber bundle.



Remarks: a. Control; b. Pb at 100 mg/kg; c. Pb at 300 mg/kg; d. Pb at 600 mg/kg; e. Pb at 100 mg/kg with salinity at 200 mM; f. Pb at 300 mg/kg with salinity at 200 mM; g. Pb at 600 mg/kg with salinity at 200 mM. Which: 1. Large vascular bundles; 2. Small vascular bundles; 3. Fiber bundles; 4. Tannin cells.

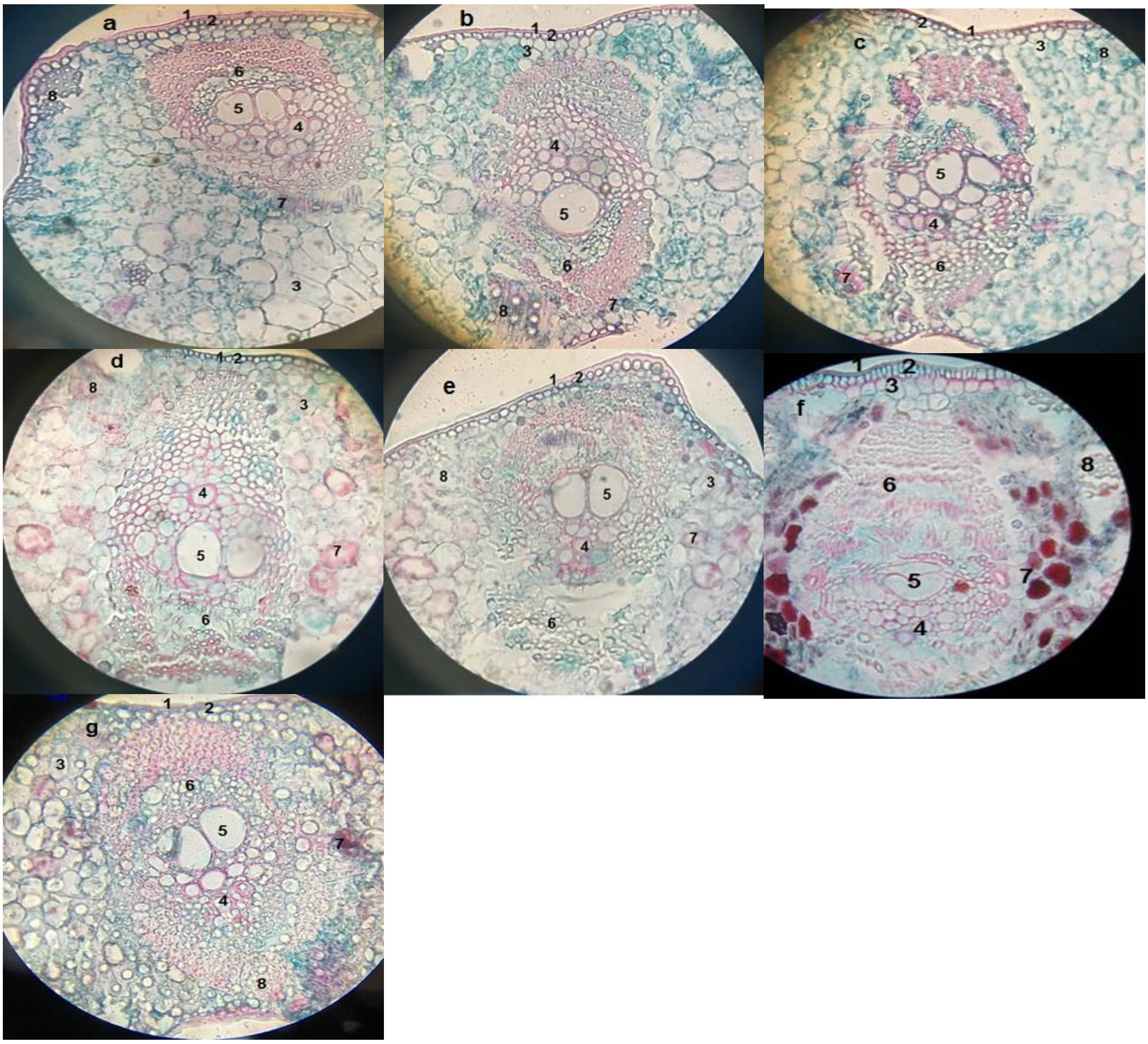
Fig. 2. Cross sections of Date palm seedling leaf treated with different concentrations of lead with or without salinity (X10)

Fibre Bundle Diameter

Inconsistent results were observed for the fibre bundle diameter, which decreased from 20.88 µm (control) to 14.00 µm (100 mg/kg Pb). At 300 mg/kg Pb, however, the fibre bundle diameter increased significantly to 36.00 µm from other treatments. Moreover, single 600 mg/kg Pb led to an increase in this anatomical trait to 33.81 µm. In terms of the fibre bundle diameter results under both Pb and salinity, no significant effect was observed between 100 mg/kg Pb with salinity and control. Meanwhile, 300 and 600 mg/kg Pb with salinity significantly increased the fibre bundle diameter to 33.60 and 32.70 µm, respectively.

Characteristics of Large Vascular Bundles

Table 3 shows the results obtained based on the cross-sections of Date palm seedlings in Fig. 3(a–g). The data reveal the influence of 100, 300 and 600 mg/kg Pb applied alone or in combination with salinity on the large vascular bundle characteristics. The applied Pb at all levels either alone or combined with salinity significantly affected all observed parameters, with the exception of the metaxylem diameter of seedlings exposed to 100 mg/kg Pb alone or combined with salinity and bundle width and phloem diameter of plants treated with 100 mg/kg Pb alone. Results showed that 600 mg/kg Pb led to a decrease in all examined traits, and this decrease was augmented when accompanied by salinity. The combination effect of 600 mg/kg Pb and salinity caused the reductions in bundle length from 210.20 µm to 123.22 µm, bundle width from 132 µm to 84.20 µm and distance between bundles from 500.00 µm to 293.00 µm. Moreover, in the control seedlings, the protoxylem, metaxylem and phloem diameters reached 30.60, 60.40 and 86.60 µm, which were reduced to 7.90, 31.90 and 44.80 µm when exposed to the combination of 600 mg/kg Pb and salinity, respectively.



Remarks: a. Control; b. Pb at 100 mg/kg; c. Pb at 300 mg/kg; d. Pb at 600 mg/kg; e. Pb at 100 mg/kg with salinity at 200 mM; f. Pb at 300 mg/kg with salinity at 200 mM; g. Pb at 600 mg/kg with salinity at 200 mM. Which: 1. Cuticle; 2. Epidermis; 3. Palisade cells; 4. Protoxylem; 5. Metaxylem; 6. Phloem; 7. Tannin cells; 8. Fiber bundles

Fig. 3. Cross sections of Date palm seedling leaf treated with different concentrations of lead with or without salinity (X40).

Table 3. Effect of Pb alone or combined with salinity on large vascular bundles traits of Date palm seedlings

Treatment[**)]	Bundle Length (µm)	Bundle Width (µm)	Distance Between Bundles (µm)	Protoxylem Diameter (µm)	Metaxylem Diameter (µm)	Phloem Diameter (µm)
Control	210.20[*)]	132.00	500.00	30.60	60.40	86.60
Pb at 100	200.00	128.00	418.00	27.40	58.20	82.80
Pb at 100 +salinity	192.15	120.46	395.00	25.00	55.40	80.40
Pb at 300	179.45	104.50	365.00	18.60	50.00	70.2
Pb at 300 +salinity	171.45	93.32	358.00	14.40	46.40	66.8
Pb at 600	131.00	90.43	302.00	10.60	37.30	47.70
Pb at 600 +salinity	123.22	84.20	293.00	7.90	31.90	44.80
LSD	9. 85	6.64	11.02	2.67	5.48	6.03

Remarks: [*)] Value (mean, n = 3) significantly difference at $P < 0.05$; [**)] The concentration unit of Pb was mg/kg

Based on the comparison between the effect of 600 mg/kg Pb applied solely or in combination with salinity, no significant difference was observed between these treatments in terms of the effect on upper epidermal thickness, palisade cell length and fibre bundle diameter. Meanwhile, the combined treatment increased the cuticle layer thickness to 6.66% compared with individual treatments. By contrast, individual treatments increased the lower epidermal thickness, tannin cell diameter and small vascular bundle diameter by as much as 16.01%, 8.26% and 15.55%, respectively, compared with combined treatments. Studies reported the increase in cuticle layer thickness, upper epidermal thickness and lower epidermal thickness under Pb stress (Gomes, de Sáe Melo Marques, de Oliveira Gonçalves Nogueira, de Castro, & Soares, 2011; Zarinkamar, Ghelich, & Soleimanpour, 2013) and salinity stress (Céccoli, Ramos, Ortega, Acosta, & Perreta, 2011; Karjunita, Khumaida, & Ardie, 2019). Plants thicken their upper and lower epidermis to minimize water loss by transpiration (Singh et al., 2015) and prevent toxic metals from reaching the photosynthetic tissues (Vollenweider, Cosio, Günthardt-Goerg, & Keller, 2006).

Plants also produce phenolic compounds, which include tannins, as a non-enzymatic defence mechanism to mitigate the harmful or toxic effects of free radicals and reactive oxygen species formed by heavy metals (Michalak, 2006). Based on the results on large vascular bundle traits, all traits decreased significantly under 300 and 600 mg/kg Pb stress applied alone or combined with salinity. Moreover, the combination action was more drastic compared with the individual effects of Pb. Compared with the control, the 600 mg/kg Pb applied alone or combined with salinity respectively reduced the bundle length (37.67% and 41.37%), bundle width (31.49% and 36.21%), the distance between bundles (39.60% and 41.40%), protoxylem diameter (65.35% and 74.18 %), metaxylem diameter (38.24% and 47.18%) and phloem diameter (44.91% and 48.26%). Such decreases in vascular bundle dimensions can lead to diminished water entry, which also lowers transpiration rate (Batool, Hameed, Ashraf, Ahmad, & Fatima, 2015; Gomes, de Sáe Melo Marques, de Oliveira Gonçalves Nogueira, de Castro, & Soares, 2011). Pb translocation through xylem may form deposits, thereby inhibiting the growth of vascular bundles and decreasing their diameter (Vollenweider, Cosio, Günthardt-Goerg, & Keller, 2006). Similar results were also reported for plants under salinity (Atabayeva et al., 2013; Bastías, González-Moro, & González-Murua, 2015; Ola et al., 2012) and Pb stresses (Gomes, de Sáe Melo Marques, de Oliveira Gonçalves Nogueira, de Castro, & Soares, 2011; Naema, Abass, & Al-Jabary, 2017; Singh et al., 2015; Zarinkamar, Ghelich, & Soleimanpour, 2013).

CONCLUSION

The results of this study derived from cross sections showed anatomical changes in root and leaves of Date palm exposed to 100, 300 and 600 mg/kg Pb alone or in combination with salinity. Results reported an increase in dermal thickness and reduction in conductive elements of roots and leaves. These findings suggested the effect of Pb was most effective at high concentrations compared with lower concentration and the combination of two stresses gave less variation on the anatomical changes on root and leaf of Date palm seedlings.

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