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Interaction between Cd & K in Some Growth Parameters and Peroxidase Activity of Tomato (*Lycopersicon esculentum*L.) Seedlings

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Abstract

Tomato (*Lycopersicon esculentum* L. var. Al-Wejdan) seedlings at 35 days old supplied with $CdCl_2$ (0, 1, 10, 50, &100 mg/kg soil), KCl (0, 20, 40, &60 mg/kg soil) as well as the combinations between these concentrations. Seedlings at 65 day old showed increasing in stem length and diameter by a combination Cd, 100+K, 60 significantly. Whereas, no significant changes in leaves numbers. Seedlings at 95 day old showed no significant changes in stem length or it diameter whereas, leaves numbers increased by combinations Cd,50+K,20 and Cd,50+K,40. Leaf area was stimulated by K (60mg), Cd (100mg) and by many combinations. There was inhibition in chlorophyll content at Cd (100mg) and at a combination (Cd,1+K,20). Peroxidase activity was inhibited by K at (20 & 60 mg), Cd at 50mg, with all combinations contain K (20mg) except a combination Cd,1+K,20 and increased by Cd at 1mg. There were no significant changes at another combinations comparing with control.

Introduction

Heavy metals are very important environmental pollutions. Many of them are toxic even at low concentrations .Unlike many other pollutants, heavy metals persist longer in ecosystems because they cannot be destroyed biologically (Yurekli & Porgali, 2006). Cadmium (Cd) is a widespread heavy metal in the environment & it is regarded as a non-essential element and has along half-life which is extremely persistent in the environment (Wagner, 1993; Salt *et al*., 1995 and Salt *et al*., 1998). Harmful effects of Cd possibly belong to its ability to inactivate enzymes through reaction with the SH-groups of portions (Fuhrer, 1982). Many researchers refer the changes of fresh and dry weights, leaf area, root length and shoot height as well as antioxidant enzymes activates to Cd treatment, suggesting that Cd can be added to the list of oxidative stresses(Hatata & Abdel-Aal,2008). Peroxidase in plants plays crucial role in all physiological events related to diminishing growth, such as lignification, cross-connection of cell wall polysaccharides, oxidation of IAA, cell elongation, and phenol oxidation (Mocquot *et al*., 1996). It's evident that many heavy metals induce the appearance and development of free radical reactions, including the induction of peroxidase activity (Stoeva&Bineva, 2003).

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Potassium (K) is an important macronutrient and the most abundant cation in plant tissues (Mengel & Kirkby, 1979). K nutrition has been shown to decrease the uptake of Cd as observed in wheat (Zhao *et al*., 2003). At the same time, element uptake by roots and shoot was negatively affected by raise in Cd concentrations (Haouri*et al*., 2012). K is important osmoregulation in guard cells (Talbott & Zeiger, 1996). Under salt stress, plants maintain a high concentration of K^+ and low concentration of Na^+ in the cytosol. Intracellular K^+ and Na^+ homeostasis is important for activates of many cytosolic enzymes, and for maintaining membrane potential and an appropriate osmoticum for cell volume regulation (Zhu, 2003). A low plants K status triggers expression of high affinity K^+ transporters, up regulates some K channels, and activates signaling cascades, some of which are similar to those involved in wounding and other stress responses (Ashley *et al*., 2006). The aim of the current study is whether K nutrition may protect plants from Cd toxicity by investigation the effects of interaction between K & Cd on some growth parameters and peroxides activity of tomato plant.

Materials & Methods

Plant material & treatments: seedlings of tomato (*Lycopersiconesculentum*L.var. Al-Wejdan) with 35 day old were grown in earthen pots (24 cm diameter, 18 cm height). Each filled with 4 kg of soil. The soil was sandy loam in texture with pH=7.07, E.C. = 21.5 ms/cm, & organic carbon = 0.69. The soil was mixed with appropriate amounts of $CdCl_2$ to supply a 0, 1, 10, 50, & 100 mg Cd/kg soil, & of KCl to supply a 0, 20, 40, & 60 mg K/kg soil. There were combinations between these concentrations. The treatment was replicated 3 times. The pots were kept in naturally illuminated plastic house (day/night temperature 25/20 ± 5°C & relative humidity 70 ± 5%). The place of experiment was in department of Biology, College of Science, Babylon University.

Growth Parameters: Length and diameter of stem & leaves number measured at 2 growth stages (65 and 95 day old). Leaf was measured by a leaf area meter (KP90N, Placom). Total chlorophyll was estimated by chlorophyll meter (SPAD-502, Konnica Minolt),it is newer methods are no destructive, very quick, and now possible to use in the field depending on the Markwell *et al.* (1995). Peroxidase activity was estimated according to Birecka *et al*. (1973).

Statistical Analysis: Data were analyzed statistically by two-way analysis of variance (ANOVA). The least significant difference (L.S.D) calculated to identify significant differences between treatment at P =0.05.

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Results

At 65 day old, there was enhancing of stem length at high concentrations of K (40 &60 mg/kg soil), also by low concentrations of Cd (1 & 10 mg/kg soil). All combinations have no significant effects except the promontory effect at Cd,10+K,20 & Cd,100+K,60 and the inhibitory effect at Cd,1+K,60 (table 1). All concentrations of Cd and K have no significant effects on stem diameter except the lower concentrations of Cd (1 & 10mg\kg) (table 2). In addition, all combinations have no significant effects except the promontory concentrations at Cd, 100+K, 40 & 60 and the inhibitory effect at Cd, 10+K, 40. There was no significant response in leaves numbers as shown in table 3.

Table 1: Stem length (cm) of tomato plant (65 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations [L.S.D. ≥2.17, (*P*=0.05)]

K / Cd	0	20	40	60
0	14.0	12.3	17.3	17.0
1	17.2	16.0	15.7	11.5
10	17.0	17.2	13.0	13.0
50	15.3	16.7	15.7	14.6
100	12.0	13.5	14.2	17.0

Table 2: Stem diameter (mm) of tomato plant (65 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations [L.S.D. ≥0.63, (*P*=0.05)]

K / Cd	0	20	40	60
0	4.3	4.7	4.0	4.2
1	5.0	4.3	4.2	4.0
10	5.0	4.3	3.7	4.5
50	4.3	4.2	4.7	4.7
100	3.8	4.2	5.0	5.3

Table3: Leaves numbers of tomato plant (65 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations

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[L.S.D. no significance, (*P*=0.05)]

K / Cd	0	20	40	60
0	7.3	6.7	7.0	8.0
1	7.3	7.3	7.0	7.3
10	8.3	7.7	6.7	7.7
50	7.0	7.7	7.0	7.7
100	7.0	7.0	7.0	8.0

At 95 day old, plants exhibit no significant increases in stem length and stem diameter as shown in tables 4& 5 respectively. Leaves numbers as shown in table 6 increased significantly by K(40 mg/Kg soil) and by combinations (Cd,10+K,40) , (Cd,50+K,20), and (Cd,50+K,40). Whereas, decreased significantly by Cd, 10+K, 60.

Table4: Stem length (cm) of tomato plant (95 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations

[L.S.D. no significance, (*P*=0.05)]

K / Cd	0	20	40	60
0	48.0	55.0	55.0	52.7
1	56.0	52.0	44.3	50.0
10	54.0	52.7	49.0	55.3
50	50.0	48.0	49.0	55.3
100	48.7	51.7	49.0	51.7

Table5: Stem diameter (mm) of tomato plant (95 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations

[L.S.D. ≥no significance, (*P*=0.05)]

K / Cd	0	20	40	60
0	8.0	6.1	7.0	8.7
1	7.0	9.3	7.7	7.7
10	6.7	8.3	7.3	7.3
50	6.3	8.3	8.0	9.0
100	9.0	7.7	7.0	7.7

Few treatments caused increasing in leaf area especially the highest concentration of K (60mg/Kg soil) as well as Cd at 50 & 100mg/kg as shown in table7. The interaction

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between Cd & K has been raised the leaf area significantly, the highest was at Cd, 50+K, 40. By Cd (100mg/Kg soil) and combination (Cd, 1+K, 20) chlorophyll content inhibited significantly as shown in table8.

Table6: Leaves numbers of tomato plant (95 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations [L.S.D. ≥ 1.8, (*P*=0.05)]

K / Cd	0	20	40	60
0	12.0	13.0	14.0	12.3
1	12.7	13.3	12.7	12.3
10	13.7	11.7	15.0	10.3
50	13.0	15.3	14.7	13.0
100	13.0	13.7	12.0	13.0

Table7: Leaf area (cm^2) of tomato plant (95 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations [L.S.D. ≥47.8, (*P*=0.05)]

K / Cd	0	20	40	60
0	134.5	133.4	109.9	220.6
1	180.3	154.1	189.2	186.1
10	184.0	185.6	98.5	107.4
50	136.0	127.8	236.3	108.3
100	191.0	140.3	157.1	149.3

Table8: Chlorophyll content (mole/cm^2) of tomato plant (95 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations [L.S.D. ≥ 7.09, (*P*=0.05)]

K / Cd	0	20	40	60
0	52.3	48.8	48.2	50.8
1	50.4	44.7	45.7	52.4
10	48.8	46.2	50.5	54.2
50	45.7	54.5	54.0	47.2
100	40.9	47.3	45.9	52.2

Table 9 shows inhibition of peroxidase activity with increasing Cd concentration particularly at 50mg (% inhibition ≈ 85%). Almost combinations have no effect except

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treatments of K (20mg) & Cd (100mg) and combinations between (Cd, 10-100+K, 20) caused inhibition of peroxidase activity more than 50%.

Table9: Peroxidase activity (U/g fresh weight) of tomato plant (95 day old) treated with different concentrations of KCl & $CdCl_2$ (mg/kg soil) with their combinations [L.S.D. ≥ 1.67, (*P*=0.05)]

K / Cd	0	20	40	60
0	3.79	1.8	2.3	0.99
1	5.57	2.92	2.87	5.12
10	3.9	1.52	3.62	2.69
50	0.58	0.89	2.66	3.51
100	2.48	1.89	3.34	3.01

Discussion

As a consequence of industrial development, the environmental pollution is increasing rapidly with heavy materials. Cd treatment led to inhibition of plant growth rate. Plants possess homeostatic mechanisms that allow them to keep correct concentration of essential metal ions in cellular compartments and to minimize the damaging effects of an excess of non-essential ones (Mickalak, 2006). In this study, there was enhancement of stem length of tomato at the 65 day old by K at high concentrations (40 & 60mg) when Cd was zero only (table 1). Indeed, it improves the importance of K in cell elongation. This increasing in elongation of stem cells corporate with increase or in no change diameter of stem by almost concentrations except combination (Cd, 10+K, 40) that reduce stem diameter to 3.7mm as shown in table 2. While, leaves numbers were not affected significantly by all concentrations (table 3). In this stage of growth, there was enhancement by K with almost combinations. Marchner (2002) referred to induce the cell elongation and maintains osmoregulation of plant cell by K.

The enhancement effect of Cd was clear at low concentrations (1 & 10mg/kg) for stem length (table 1), stem diameter as shown in table 2, and leaves numbers (table 3). These results indicated that unnecessary element for plants as Cd may be alleviated the activity of some biological processes of plant. Nyitrai *et al*. (2002) concluded that low concentrations of Pb& Ni supplied with nutrient solutions or sprayed on leaves of maize &mung bean caused simulated effects.

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Plants at 95 day old which is represent the end of vegetative growth, there was a uniformity in stem lengths(table 4) in contrast with control, as well as, in stem diameter as shown in table 5. The leaves numbers increased by K (40mg) and many combinations (table 6). These results indicated the important of K in growth refreshment.

Generally, the combinations differed in their effects, (Cd, 100+K, 60) caused increasing in stem length and diameter significantly at 65 day old (tables 1&2), and caused no changes in these parameters significantly at 95 day old (tables 4 & 5). Similar results were obtained Saddiqui *et al*., (2012). They showed that plants subjected to Ca & K alone as well as in combination was efficient to restore the altered plant growth induced by Cd toxicity in *Vicia faba*.

Table 7 shows increasing in leaf area by K &Cd at high concentrations as well as few combinations of them. At the same time, chlorophyll content (table 8) decreased by Cd at 100mg and not changes significantly by other concentrations. Some studies have a similar results, for example, Abdel-Latif (2008) referred to Cd exposure significantly decreased the total chlorophyll content of *Triticum eastivum*, which belong to decrease in carotenoids that protect chlorophyll from photo oxidative destruction. While, Haouri *et al*., (2012) refers to take place a limitation in stem length, dry weight of tomato shoot, roots, and chlorophyll content by Cd toxicity. These results were similar to the present study especially at high concentrations of Cd & for the combinations that contain low concentrations of K (20mg) as shown in table 8.Increasing in leaf area at Cd 100mg with decreasing in chlorophyll content may be attributed to avoid the Cd toxicity by enlargement the cell size or increasing of the thickness of cell wall comparing to the destruction of chlorophyll molecules.

Peroxidase activity decreased gradually with increasing Cd concentrations particularly at 50mg (table 9). Peroxidase is considered to play an important role in heavy metals stress (Mocquot *et al*., 1996). Cd causes oxidative stress properly through indirect mechanisms such as interaction with oxidative defense, disruption of the electron transport chain or induction of lipid peroxidation (Smeets *et al*., 2005).Siddiqui *et al*.,(2012) proved that Cd decreased the peroxidase activity whereas, K caused increasing it's activity. Depending on Siddiqui *et al*. the increased activity of antioxidant enzyme maybe due to the active role of K, this activates more than 50 enzymes. In the present study, the combinations between Cd and high concentrations of K (40&60mg) cause significant increase in peroxidase activity compared to control. It indicates that subsequent application of K to Cd-stressed plants may neutralize the adverse effect of Cd and resulted in considerable improvement in the activity of this enzyme compared to their individual treatment. As shown in table 9, the low concentrations of Cd

stimulated the antioxidant enzyme activity & decreased at high concentration for both Cd & K individually. This stimulation could play a significant role in protecting cells against Cd-induced oxidative stress (Scebba *et al*., 2006 & Nikolic *et al*., 2008). The combinations with low K concentrations (20mg) shows inhibitions of peroxidase activity which reflects a high level of (ROS), which is already high in plant exposed to stress conditions. This result suggests the improvement of K-nutritional status of the plants might be of great importance for survival of crop plants under environmental conditions (Cakmak, 2005).

Conclusions

Potassium can neutralized the toxicity effect of cadmium, thereafter, it's possible for the contaminated soils by Cd to be treated by the addition of K to enhance the crops yield production via reduction of oxidative stresses that occurred by heavy metals.

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الخلاصة

جهزت بادرات نباتات الطماطة (*Lycopersicon esculentum* L.) صنف الوجدان بعمر 35 يوم بتراكيز مختلفة من كلوريد الكادميوم(0، 1، 10 و50 ملغم/كغم تربة) وكلوريد البوتاسيوم (0، 20، 40 و 60 ملغم/كغم تربة) مع توليفات من هاتين المادتين وبالتراكيز اعلاه . بينت النتائج زيادة معنوية في طول الساق وقطره في النباتات بعمر 65 يوم بتأثير التوليفة Cd,100+K,60 ملغم/كغم تربة، ولم يحصل تغير معنوي في عدد الأوراق. أما في مرحلة النمو الثانية (بعمر 95 يوم) فلم يحصل تغيير معنوي في طول الساق وقطره، بينما ازداد عدد الأوراق بالتوليفتين ((Cd, 50+K, 20 و((Cd, 10+K, 40. حفزت المساحة الورقية بالتراكيز العالية لكل من البوتاسيوم والكادميوم ومعظم التوليفات، في حين سبب التركيز العالي للكادميوم (100ملغم) انخفاض معنوي في محتوى الكلوروفيل. ثبطت فعالية إنزيم البيروكسيديز بتأثير البوتاسيوم (20 و60ملغم)والكادميوم بتركيز (50ملغم)، وزادت بتأثير الكادميوم(1 ملغم) فضلا عن انخفاضها بتأثير كل التوليفات الحاوية على البوتاسيوم بتركيز (20 ملغم) عدا التوليفة Cd,1+K,20. ولم تؤثر بقية التوليفات معنويا في فعالية الأنزيم عند المقارنة مع عينة السيطرة.