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Effects of Cadmium and Combined Cadmium-Zinc Concentrations on Rooting and Nutrient Uptake of Cowpea Seedlings Grown in Hydroponic

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Abstract: A hydroponic experiment was carried out to investigate the effect of cadmium treatments at (0.3, 0.6, 0.9 and 1.2 mg/l), zinc at (2, 4, 6 and 20 mg/l) and combined cadmium-zinc at (0.6 mg/l Cd plus 2, 4, 6 and 20 mg/l Zn) on rooting, nutrient uptake in cowpea seedling. The results showed that increasing Cd supply markedly reduced the rooting of seedling. Increased Cd accumulation in cowpea seedling was observed as Cd concentration increased in culture media. Significant antagonistic effects of Zn on Cd concentration in the tissues occurred at levels above 4 mg/l in culture media. Zinc concentrations in the tissues decreased with increasing rates of applied Cd. Increasing Cd and Zn additions to the media resulted in increases in the concentration of Cd and Zn in plant tissues. It could be concluded that the reduction in Cd uptake caused by Zn addition might result from the competitive transport and absorption interaction between these two ions.

Key words: Cadmium stress · Cadmium-zinc interaction · Cowpea · Nutrient uptake · Rooting

INTRODUCTION

Cadmium is released into the environment by industrial processes and phosphate fertilizers [1]. In 1993, the International Agency for Research on Cancer classified cadmium among the known human carcinogens. Cadmium is absorbed by inhalation and ingestion and has a very long biological half-life (>25 years). It induces tumorgenesis in lung, prostate, kidney and stomach [2, 3]. It is a non-essential metal for the plants and humans, enters crops through roots, accumulates in plants and affects human health [1]. Cadmium is a widespread environmental pollutant with high toxicity to human, animals and plants and the accumulation of Cd in plant cell generally results in functional alteration of the physiological pathways and the plants exposed to Cd showed growth inhibition, photosynthesis, water and nutrient uptake [4]. Studies carried out in different plant species have revealed that cadmium affects various physiological and biochemical processes in plants. Also, Somashekaraiah *et al.* [5] found that Cadmium accumulation caused a reduction in photosynthesis. While, inhibition of various enzyme activities such as the enzymes involved in CO_2 fixation was reported by Greger and Ogren [6] and De Filippis and Ziegler [7].

As a consequence, Cd-exposed plants showed various symptoms of injury such as chlorosis, accompanied by a lowering of photosynthetic rate, growth inhibition, browning of root tips [8], impedes respiration [9], reduces mitochondrial electron transport [10], induces high vacuolization in cytoplasm and nuclei and increases disintegration of organelles [11]. Cd toxicity has been found to interfere with electron transport chains or block antioxidant enzymes structures, leading to accumulation of H_2O_2 and oxidative damage, membrane leakage and finally cell death [12]. Among other effects, Cd^{2+} produces oxidative stress possibly by generating free radicals and reactive oxygen species (ROS) in plant and animal cells [13, 14]. On the other hand, the tolerance of crops to Cd toxicity depends on crops ability to absorb Cd. It is important to know the difference of Cd toxicity resistance among genotypes and the potential of Cd accumulation as well as their physiological responses to Cd toxicity. Cadmium is

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easily taken up by plants and translocation to different plant parts. Generally, visual toxicity symptoms and impaired growth occur only at relatively high internal Cd concentrations [15]. Concerning, zinc, Alloway [16] stated that the zinc is required in small amounts but critical concentrations to allow several key plant physiological pathways to function normally. These pathways have important roles in photosynthesis and sugar formation, protein synthesis, fertility and seed production, growth regulation and defense against disease. Where zinc is deficient, these physiological functions will be impaired and the health and productivity of the plants will be adversely affected, resulting in lower yields (or even crop failure) and frequently in poorer quality crop products. Ziaeyan and Rajaie [17] stated that the formation of male and female reproductive organs and pollination process are disturbed in Zn deficiency which may be attributed to the reduction of indole acetic acid (IAA) synthesis. Abd El-Hady [18] stated that, zinc is one of the essential micronutrients for the normal healthy growth and reproduction of crop plants. It is required in relatively small concentration in the plant tissues (5-100 mg/kg). In general zinc have main role in synthesis of proteins, enzyme activating, oxidation and revival reactions and metabolism of carbohydrates. By utilizing of fertilizers contain zinc and other micronutrients, performance on quality of crops is increasing and with shortage of this elements due to decline in plant photosynthesis and destroy RNA, amount of solution carbohydrates and synthesis of protein decreased and then performance and quality of crop will be decreased [19]. The relationship between Cd and Zn in plants pertaining to uptake, translocation and remobilization of these ions is very complex. Both antagonistic and synergistic Cd-Zn interactions have been reported by Bingham [20]. Cadmium and Zn have similar ionic structures and electro negativities; however, they have different ionic radii (Zn^{2+} = 0.074 nm, Cd^{2+} = 0.097 nm). These differences could be comparatively related to plant selectivity [21]. The reduction in Cd uptake caused by Zn fertilization might be resulted from the competitive transport and absorption interaction between these two ions [22].

The aim of this study is to evaluate the effect of cadmium stress and different levels of Zn on rooting, uptake of some nutrients in cowpea seedling under hydroponic culture and studying the effect of different levels of Zn culture media addition on reducing the physiological impact caused by Cd and their effects on growth analysis.

MATERIALS AND METHODS

Plant Materials and Experimental Design: Three hydroponic experiments were carried out to investigate the effect of cadmium treatments at (0.3, 0.6, 0.9 and 1.2 mg/l) as Cadmium chloride ($CdCl_2$), Zinc at (2, 4, 6 and 20 mg/l) and cadmium-zinc interaction at (0.6 mg/l Cd plus 2, 4, 6 and 20 mg/l Zn) Zinc sulfate on cowpea as a test crop. Seeds of cowpea were planted in a moist clay soil and germinated for obtaining cuttings. The cuttings were taken after 6 days old cow-pea planting. The cuttings were prepared by excising the seedlings root system 3 centimeters below the cotyledonary node, thus, the cutting was of 3 centimeters of hypocotyls plus the epicotyls including a pair of primary leaves. Five cuttings were used per a vial which also contained the Cd or Cd + Zn to be tested plus 20 ml of 1\4 MS medium (Murashige and Skoog basal medium nutrient solution).The cowpea cuttings were then placed at room temperature and 16/8 h light/dark cycles under 9000 Lux fluorescent lights. The average number and length of roots per cutting of the five cow-pea cuttings used per vial was obtained after 5 days. After 5 days of growth under Cd stress conditions, the plants were taken and washed in distilled water, then representative portions were wet digested by using a mixture of $HClO_4$ and H_2SO_4 at a rate of 3:1 to determine P, K and micronutrients; total P was determined by ascorbic acid method; total K was determined by using flame photometer method according to Page *et al.* [23]. The micronutrients (Fe, Mn, Zn and Cd) were determined by Inductively Coupled Plasma Spectrometer (ICP) plasma 400.

Tolerance Index: The Cd tolerance indexes were measured according to the following equation of described by Das *et al.* [24].

$$\text{Tolerance indexes} = \frac{\text{Growth (dry matter) increase in Cd level}}{\text{Growth (dry matter) in nutrient solution without Cd}} \times 100$$

Statistical Analysis: The experiments were arranged in a completely randomized design. Differences among Cd concentrations, as well as interactions between these variables, were tested using the SPSS statistical program. Statistical variance analysis of the data with three replicates was performed using ANOVA and compared with least significant differences (LSD) at the 5% level.

RESULTS AND DISCUSSION

Effect of Cadmium Treatments on Some Plant Growth Parameters: The effect of cadmium treatments on dry matter accumulation of plant, number of roots, root length and tolerance indexes are presented in Table 1. The mean number of roots and length of roots were significantly decreased with increasing cadmium concentration. The reduction was parallel with increasing Cadmium concentrations (Table 1). Cowpea seedlings dry weights and tolerance indexes were decreased with increasing cadmium concentrations. The rates of reduction were significantly recorded with 0.9 and 1.20 mg/l Cadmium chloride concentrations. This might be attributed to the effect of cadmium toxicity on cell division and/or cell expansion and might be through its effect on DNA and RNA synthesis, heavy metals are known to promote protein denaturation [25] and to increase the hydrolytic activities of proteases, RNAase and DNAase enzymes. Lee *et al*. [9] and Munzuroglu and Zengin [26] showed that the increase incadmium concentration caused a greater inhibition of germination, root and coleoptiles growth.

Furthermore, any change in the growth rate which results from increasing Cd supply must be dependent on the change in the rate of net photosynthesis that reduces the supply of carbohydrates or proteins and consequently decreases the growth of the plant. The consequence of root growth inhibition by cadmium will result in low nutrient and water uptake, low rate of transpiration and as a result low shoot growth rate [27]. These findings are confirmed with the findings of Erdem *et al.* [28], who studied the effect of cadmium stress on growth and mineral composition of two tobacco cultivars, he found that, the Cd application significantly decreased the dry matter accumulation of tobacco due to the high Cd concentration (2.5 and 10 mg/kg) which was probably related to the change in biomass distribution between shoots and roots. While, Wagner [29] stated that the reductions in dry matter accumulation of mustard plants have been attributed to the direct effect of higher Cd concentrations in plant tissue and not through an indirectly induced deficiency of other nutrients.

Effect of Cadmium Treatments on Some Nutrients Uptake: Data in Table 2 show that increasing Cd^{2+} concentrations resulted in an increase of Cd uptake by seedling, reaching a maximum 39.50 mg/ plant at 1.2 mg/l Cd^{2+} treatment. Accumulation of Cd in seedling was proportional to the increase in the concentration of Cd^{2+} in media growth, while other nutrients content in plants were decreased with increasing cadmium concentration excluding iron when compared with the control. As shown in Table 2, the different concentrations of Cd^{2+} treatments increased tissue Fe content in the Cd^{2+} treatment compared to the control. Cd^{2+} led to marked reduction in P, K, Mn and Zn in cowpea seedling. This might be attributed to the level of nutrients absorbed by plants in relation to the amount of available nutrients in the growth media. The uptake of nutrients increases for some nutrients or decreases for the others depending on antagonistic or synergistic (interactions) effects among plant nutrients. In this concern, Moustakas *et al.* [22] stated that the cadmium and zinc are elements having similar geochemical and environmental properties; their chemical similarity can lead to interaction between Cd and Zn during plant uptake, transport from roots to the aerial parts, or accumulation in edible parts. Also, Erdem *et. al.* [28] stated that, the decreased in K concentration under Cd treatment may be due to the effects on the ATPase, responsible for the proton gradient needed to take up K^+ actively. On the other hand, iron content was increased with increasing cadmium concentration, while the concentration of copper, phosphorus and potassium were decreased with higher concentration of cadmium as compared to the control. These results are in harmony with those obtained by Liu *et al*. [30] and Hernandez *et al*. [31], who found that Fe in pea plants was higher than that recorded in the control plants after the treatment with 50 μM Cd. The relationship between Fe and Cd uptake is significant as reported by Lombi *et al*. [32] where Cd uptake was significantly enhanced by Fe deficiency in the Ganges ecotype. Also, Rudio *et al*. [33] mentioned that the rice cv. Baha accumulated large quantities of Cd and Ni when growing for 10 days in nutrient solution containing these heavy metals, also induced a decrease in K, Ca and Mn content in the plants. Also, Yang *et al*. [34] seated that the concentrations of Zn, Fe, Cu, Mn, Ca and Mg were decreased with increasing external Cd levels at differed plant species compared to control

Effect of Zinc Treatments on Some Plant Growth Parameters: The effect of zinc treatments on dry matter accumulation, number of roots, length of roots and tolerance index of plant are presented in Table 3. Data showed that, the dry matter of plant was affected by zinc treatments; the highest values of dry matter, number of roots, length of roots and tolerance index were 1.25 g/plant, 6.83 root/plant, 12.07 cm and 100.01 % at 2 mg/l Zn and decreased to 1.01 g/plant, 1.62 root/plant, 9.23 cm

Table 1: Effect of cadmium treatments on some plant growth parameters.

Treatments	Dry weight(g/plant)	Number of roots/plant	Root length (cm)	Tolerance index %
Control (1\4 MS)	1.23	7.40	10.43	100.00
0.3 mg/l Cd	1.22	3.90	8.12	99.18
0.6 mg/l Cd	1.22	3.20	2.30	99.18
0.9 mg/l Cd	0.98	2.70	0.84	79.67
1.2 mg/l Cd	0.91	0.20	0.21	73.98
LSD 0.05	0.25	0.49	0.36	2.05

Table 2: Effect of cadmium treatments on some nutrients uptake.

	Nutrients (mg/plant)					
Treatments	Cd	Zn	P	K	Mn	Fe
Control (1\4 MS)	0.001	64.51	417	902	46.2	245
0.3 mg/l Cd	11.00	61.70	398	889	43.00	262
0.6 mg/l Cd	21.00	45.31	382	814	46.25	355
0.9 mg/l Cd	35.50	49.20	388	871	44.00	250
1.2 mg/l Cd	39.50	53.12	349	714	41.12	247
L.S.D 0.05	3.71	2.54	13.67	20.45	3.10	9.60

Table 3: Effect of zinc treatments on some plant growth parameters.

Treatments	Dry weight (g/plant)	Number of roots/plant	Root length (cm)	Tolerance index %
Control (1\4 MS)	1.24	6.15	11.67	100
2 mg/l Zn	1.25	6.83	12.07	100.01
4 mg/l Zn	1.23	5.92	8.12	99.19
6 mg/l Zn	1.14	4.95	6.77	91.93
20 mg/l Zn	1.01	1.62	9.23	81.45
LSD 0.05	0.08	0.28	0.80	5.53

Table 4: Effect of zinc treatments on some nutrients uptake.

	Nutrients (mg/plant)					
Treatments	Zn	Cd	P	K	Mn	Fe
Control (1\4 MS)	72.40	0.001	403	895	42.50	273
2 mg/l Zn	98.16	0.001	369	812	34.52	251
4 mg/l Zn	117.13	0.001	371	818	37.10	229
6 mg/l Zn	112.51	0.001	352	795	34.56	218
20 mg/l Zn	142.70	0.001	367	803	29.47	154
LSD 0.05	4.95	NS	17.04	25.09	5.75	14.97

and 81.45 % at 20 mg/l Zn, respectively. These reductions may be attributed to the effect of zinc as an essential trace element for many physiological processes, but they became toxic at high concentration levels [8]. Such response may be due to that zinc is known to be as an activator of several enzymes in plants and is directly involved in the biosynthesis of growth substances such as auxin which produces more plant cells and more dry matter [35].

Effect of Zinc Treatments on Some Nutrients Uptake: Data presented in Table 4 show that the zinc uptake was increased in plant by increasing concentration of the treatments when compared with control. The value of zinc uptake was 72.40 mg/plant in the control and increased to 142.70 with 20 mg/l zinc treatment. While, the values were 403, 895, 42.50 and 273 mg/plant for P, K, Mn and Fe, respectively in the control then; decreased to 367, 803, 29.47 and 154 g/plant for the previous nutrients at 20 mg/l zinc concentration. Similar results were obtained by Rajaie *et al.* [36], who found that the application of $ZnSO_4$ decreased the concentration of N, P, K, Mn and Cu in plant shoots of lemon plant. They added that, there was no significant decrease in plant uptake of Mn and Cu with Zn addition. The reduction in the shoot concentration of these nutrients may be due to dilution effect or antagonistic relationship between Zn and other micronutrients. Loneragan and Webb [37] reported that antagonistic relationship between Zn and other cationic micronutrients (Mn and Cu) appears to be as a result of competition at the absorption sites of plant root. While, Menser [38] found that P content in roots and leaves of soybean plants was decreased with increasing zinc up to 70 μM/l. Also, Norvell and Welch [39] stated that the increase of Zn concentration in growth media decreased Fe concentrations in the barley shoots.

Table 5: Effect of interaction between cadmium and zinc on some on plant growth parameters.

Treatments	Dry weight (g/plant)	Number of roots/plant	Root length (cm)	Tolerance index %
Control (1\4 MS)	1.24	6.15	11.67	100
0.6 mg/l Cd	1.23	3.46	5.85	99.19
0.6 mg/l Cd + 2 mg/l Zn	1.24	5.72	7.39	100
0.6 mg/l Cd + 4 mg/l Zn	1.23	6.88	5.46	99.19
0.6 mg/l Cd + 6 mg/l Zn	0.94	4.62	4.66	75.80
0.6 mg/l Cd + 20 mg/l Zn	0.72	1.36	4.53	58.06
LSD 0.05	0.10	0.33	0.58	5.13

Table 6: Interaction effect between cadmium and zinc treatments on some nutrients uptake.

	Nutrients(mg/ plant)					
Treatments	Cd	Zn	P	K	Mn	Fe
Control (1\4 MS)	0.001	72.4	403	895	42.50	273
0.6 mg/l Cd	23.71	69.12	395	860	41.32	291
0.6 mg/l Cd + 2 mg/l Zn	25.39	74.15	417	852	36.70	314
0.6 mg/l Cd + 4 mg/l Zn	16.62	82.33	422	870	39.00	350
0.6 mg/l Cd + 6 mg/l Zn	17.21	91.21	434	835	35.52	347
0.6 mg/l Cd + 20 mg/l Zn	21.43	125.8	380	712	32.12	372
LSD 0.05	1.62	4.92	8.90	17.88	4.17	8.12

Interaction Effect Between Cadmium and Zinc Treatments on Some Plant Growth Parameters: The effect of zinc on cadmium toxicity and its effect on dry matter, number of roots, length of roots and tolerance index in cowpea plant are presented in Table 5. Data showed that, the effect of zinc treatments on cadmium toxicity could eliminate the inhibitory effects of cadmium especially at low concentration (2 and 4 mg/l Zn) on seedling. This difference may play a role in plant selectivity for Zn, in other words, the reduced uptake of Cd as a result of Zn addition. In this concern, Adriano *et al.* [15] and Nan *et al.* [40] found that Zn supply can inhibit Cd adsorption and thereby cause a low Cd concentration in plants. The highest values of dry matter, length of roots and tolerance index were recorded with the low concentration of zinc (0.6 mg/l Cd +2 mg/l Zinc) and control. But the mean number of roots increased with (0.6 mg/l Cd +4 mg/l Zinc), when compared with 0.6 mg/l Cd only. On other hand, it was decreased with the treatments of 0.6 mg/l Cd +6 mg/l Zinc and 0.6 mg/l Cd +20 mg/l Zinc for all growth parameters. Other studies, Das *et al.* [8], Abdel-Sabour *et al.* [21] and Nan *et al.* [40] have been conducted to establish the existence of Cd-Zn interaction in soil plant systems

Interaction Effect Between Cadmium and Zinc Treatments on Some Nutrients Uptake: Data in Table 6 show that the interaction between cadmium and zinc treatments on cadmium and zinc uptake. Application of zinc treatments on cadmium accumulation could eliminate the inhibitory effects of cadmium on seedling. Data revealed that the cadmium uptake was decreased with increasing zinc concentration from 2 ppm to 20 ppm compared with the control. These decreases may be playing an important role in plant selectivity for Zn. In other words, the reduced uptake of Cd as a result of Zn addition in our work might be a result of competitive transport and absorption interaction between these two ions. Some researchers found that Zn supply can inhibit Cd adsorption and thereby cause a low Cd concentration in plants [40]. Also Abmann *et al*. [41] showed that inhibition of Cd uptake by Zn is ascribed to competition of increasing Zn levels (being dominating ion) with Cd for membrane binding sites and transport systems. Madhoolika and Rajesh [42] treated carrot plant with two Cd concentrations (10 and 100 μM) and two Zn concentrations (100 and 300 μM). They pointed out that bioaccumulation of Cd in root and leaf was greater at the low metal application rates of Cd and Zn in combination than the higher rates. Zn was found in some cases to depress Cd uptake, indicating some kind of interaction between these metals [43, 44].

CONCLUSION

In conclusion, the mean values of dry matter of seedling, number of roots, length of roots and tolerance index were significantly decreased with increasing Cd hydroponics culture applied either alone or combined with Zn.

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