

Influence the antioxidant ascorbic on some vegetative and root growth indicators of *Calendula officinalis* L.) grown under Chromium contaminated soil conditions(

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I. Abstract

The beginning of the twenty-first century witnessed a clear superiority in various fields of scientific and technological progress in our contemporary life and in almost all fields. However, this was accompanied by global concern about the excessive or misuse of our planet's precious natural resources and environmental damage. Technological advancements and their negative effects have led to widespread contamination pollution in the natural environment. Therefore, ecosystems are facing numerous risks. The severe and widespread pollution of land and water resources with metalloids and heavy metals raises widespread environmental and societal concerns. Excessive concentrations of heavy elements in agricultural soil present a critical threat to global food supplies, human health, and plant life. Soil pollution occurs due to the large expansion in the volume of industrial, agricultural and technological production, in which heavy metals are used in their processes, increasing air, soil and water pollution. Moreover, technological development has produced complex electronic waste containing toxic metals such as lead and cadmium, which leak into the environment in harmful or excessive quantities when disposed of improperly. The health, quality and production of plants are negatively affected when concentrations of hazardous metals in agricultural lands reach dangerous values. Major toxic metals, for example zinc, arsenic, chromium, mercury, nickel, copper, as well as lead and cadmium, are found everywhere in the environment, and their high levels lead to changes in soil properties and harm plants, reducing their productivity.

II. Introduction

Chrysanthemum, which belongs to the Asteraceae family, is considered an important ornamental and medicinal plant because it contributes to many industrial applications. It is used in the pharmaceutical industry due to its antimicrobial properties, in addition to its effectiveness as a stimulating substance that is anti-oxidant, anti-spasmodic. It is an anti-tumor agent, due to its many active ingredients, such as carotenoids and flavonoid antioxidants (Ukiya *et al.*, 2006). Toxic elements are considered among the most dangerous pollutants of the environment today as a result of industrial development in many countries. Their increase has caused the development of many symptoms of serious diseases due to their toxic effects. This has a negative impact on human health due to their transfer to plants that grow in polluted soil, which leads to their entry into the Energy transfer path and then it poses health risks to humans and plant health due to their non-decomposition and accumulation in the soil, which is the main storehouse for them, especially agricultural soil. This has raised great concern among environmental specialists (Sumiahadi and Acar, 2018). The sources of human pollution has exacerbated the spread of these common environmental pollutants such as cobalt, copper, iron, lead and cadmium, and others,



in the ecosystem are various activities that result from burning coal, oil, blacksmithing, metal smelting, cement manufacturing and mining. Sometimes, heavy metal pollution results from the use of wastewater for agricultural irrigation, leading to increased concentrations (Adelikane and Abijondi, 2011). Among the 11 most dangerous metals are As, Cu, Co, Cd, Mn, Pb, Hg, Cr, Ni, Sn, and Ti. These substances originate from natural sources in the environment and exist in trace, non-toxic quantities, particularly in the Earth's crust, in which, along with other elements, contribute to the planet's ecological balance (USDA and NRSC, 2000; Obodai *et al.*, 2011). L-ascorbic acid, among the best important non-enzymatic antioxidants that is water-soluble, with a concentration exceeding 2 mmole in chlorophyll pigment. It is found in all parts of the plant, as well as in cell walls, and performs vital functions in plant growth and development and normal functions by regulating many cellular processes, such as cells division and differentiation, and aging, as well as stimulating the formation of nucleic acids and proteins, acting as a powerful electron donor and serving as a cofactor in many key enzymes in plants (Venkatesh and Park 2014). A study by Gabash *et al.*, (2023) indicated impact of treatments application different of lead on *Tagetes erect* L. A meaningful decrease was observed within studied vegetative and root growth indicators, while, the study conducted by Al-Amri *et al.* (2018) confirmed that treating wheat plants growing under the influence of lead poisoning by treating them with ascorbic acid using different concentrations led to a reduction in oxidative damage resulting from lead poisoning and improved vegetative and root growth indicators. The results of the carried out by Fatima *et al.* (2020) also indicated that the treatment fenugreek plants with ascorbic acid gave positive results in vegetative and root growth indicators compared to other plants growing under the influence of copper at concentrations of (100, 250, 500) mmole. This study provides a clear view of the possibility of reducing the harmful effects of chromium stress on the vegetative and root growth indicators of chrysanthemum plants growing under metal stress, and to find the best treatments that work to build a defense system that stimulates the formation of antioxidants in plant tissue cells to limit the negative effects of chromium stress. In order to positively influence and encourage root and vegetative growth of the plant.

II. Materials and Methods

This field experiment was conducted in a plant shade part of the garden unit at the College of Agriculture / University of Basra through the 2024-2025 growing period to examine the effectiveness of using ascorbic acid on some indicators of vegetative and root growth of chrysanthemum plants growing under soil conditions contaminated with chromium. The seeds of the local variety *Calendula officinalis* L. Var. Lemon were sown in a cork dish on 15/9/2024 containing a mixture of soil and peat moss. After three weeks the seedlings were transferred to new pots with capacity of 15 cm, and after about a month, they were transferred to larger pots with a diameter of 25 cm filled with soil contaminated with potassium dichromate ($K_2Cr_2O_7$) at a concentration of (50 mg kg^{-1}) as a source of contamination, at a rate of one plant per pot. The horticultural service methods were carried out, which included irrigation, weeding and hoeing whenever necessary, and the compound fertilizer NPK (20-20-15) was added at a concentration of 3 g/L. After 10 days of pots contamination, treatments were applied to the seedlings, which consisted of ascorbic acid at concentrations of (0, 25, 50, 100) mg/L.

Table (1) shows some of the physical and chemical characteristics of the used soil and peat moss.

No.	Traits	Values
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1	E.C.	2.53
2	PH	8.11
3	The amount of moisture retention is	50%
4	Organic matter (mgkg^{-1})	7.8
5	Nitrogen (mgKg^{-1})	24
6	Phosphorus (mgKg^{-1})	1.56
7	Potassium (mgKg^{-1})	7.28
8	Sand%	71.14
9	Silt%	10.22
10	Clay%	18.64
11	Soil texture	Loamy sand

First: The studied vegetative growth indicators included:

1- Whole Plant Height (cm): The height of each plant in the experimental unit was measured from the surface of the potting soil to the top of the plant, using a metric measuring tape, and the average was recorded for each treatment.

2- Number of leaves (plant leaf⁻¹): Calculated the total number of leaves for each plant in the experimental unit and its average was recorded.

3- Number of lateral branches (plant branch⁻¹): All branches formed on each plant in the experimental unit were counted and their average was recorded.

4- Main stem length (cm): Measured the main stem length of each plant in the experimental unit from the surface of the pot soil to the top of the plant using a metric measuring tape, and record the average for each treatment.

5- Main stem diameter (mm): Measure the main stem diameter of each plant in the experimental unit at a distance of 3 cm from the surface of the pot soil using a Vernier Caliper and record its average.

6- Fresh weight of the vegetative group (g): Estimate the fresh weight of the vegetative mass of each plant in the experimental unit using the direct weight method using a sensitive balance then record its average.

7- Dry weight of the vegetative group (g): Estimate the dry weight of the vegetative group after completing the measurement of the fresh weight of the vegetative group. Place it in an electric oven at a temperature of 60°C for 48 hours and until the weight stabilizes, take its dry weight.

Second: The studied root growth indicators included:

The roots were separated from the green mass and washed well with running water to get rid of any remaining potting soil. They were then placed on filter paper to get rid of excess water droplets and left to dry a little. The following root mass indicators were calculated:

1- Roots length (cm): Measure the longest roots of each plant in the experimental unit using a ruler from the area where the root connects to the stem to the end of the root, then record its average.

2- Number of roots (plant root⁻¹): The number of roots was calculated for each plant in the experimental unit and then its average was recorded

3- Fresh weight of roots (g plant⁻¹): Estimate the fresh weight of the roots for each plant in the experimental unit using the direct weight method using a sensitive balance, then record its average.

4- Dry weight of roots (g plant⁻¹): Estimate the dry weight of the roots after completing the measurement of the fresh weight of the roots Place them in an electric oven at a temperature of 70°C for 48 hours and until the weight stabilizes, take their dry weight.

Third: Digestion and estimation of Chromium in roots, stems, and leave:

Plant samples (Roots, Stems, and Leaves) were collected from the studied treatments. They were dried in an electric oven at 60°C for 72 hours, finely ground using a ceramic mortar, and digested by placing 0.5 g of the samples in a test tube with hydrochloric acid (HCl) and nitric acid (HNO₃) in a 1:3 ratio. The samples were then placed on a hot plate at 250°C until the color was completely removed, The samples were then diluted to 50 ml with deionized distilled water and the chromium concentration was measured using a Shimadzu AA-7000 atomic absorption spectrophotometer, as suggested by Ehsan *et al.*, (2013).

III.Statistical design

The study experiment was designed according to a completely randomized design (CRD) with one operator and four treatments, each treatment contained 3 replicates, and each replicate consisted of 5 seedlings. The results obtained were analyzed using one-way ANOVA with the ready-made statistical program (Genstat 23.1) to analyze the data of the indicators under study. To compare the differences between the mean of the treatments, the least significant difference (LSD) test was used at a probability level of 0.05 (Al-Rawi and Khalaf Allah, 2000).

IV.Results and Discussion

1-Vegetative growth indicators

The data in Table (2) show significant effects on the average whole plant height, number of leaves and lateral branches, main stem length and diameter, and fresh and dry weight of the vegetative group.

It has been observed that plants treated with high concentration ascorbic acid (100 mg/L) showed superiority into averages of the limited parameters that were studied comparison with plants of the



remaining treatments and reached (29.12 cm, 100.33 leaves plant⁻¹, 19.00 branches plant⁻¹, 19.76 cm, 8.88 mm, 55.12 g, 9.62 g) respectively. While the control treatment with concentration (0 mg/L) gave the lowest averages, which reached (21.27 cm, 60.66 plant leaves⁻¹, 9.33 plant branches⁻¹, 11.35 cm, 5.56 mm, 27.76 g, 5.75 g) respectively.

2- Root growth indicators:

The results in Table (3) also showed significant effects on the average length, number and weight of fresh and dry roots, and plants treated with high concentration ascorbic acid (100 mg/L) gave superior averages in the calculated parameters comparison with plants in other treatments, reaching (15.02 cm, 34.33 root plant⁻¹, 6.02 g, 0.536 g) respectively, while the control treatment at a concentration of (0 mg/L) gave the lowest averages, reaching (10.15 cm, 18.66 roots plant⁻¹, 3.52 g, 0.339 g) respectively.

3- Chromium concentration in plant parts:

Figure

(1) indicates the concentrations of chromium into different parts of the chrysanthemum plant. The data in the figure showed that the roots gave the highest concentration comparison with the stems with leaves.

The results

in Table (2) indicate a significant increase in all studied vegetative growth indicators with increasing concentrations of ascorbic acid for all treatments. The results indicated that treatment with high ascorbic acid concentration (100 mg/L) gave an increase in the averages for all indicators of the studied plants comparison with the control treatment (untreated). This can be explained by the fact that the higher the concentration of ascorbic acid, the more effective it is in reducing chromium toxicity and thus increasing the essential nutrient content (Ca, N, P, K, Mg). water content and amino acids, especially cysteine, ascorbic acid promotes cell division and elongation and improves the plant's defense mechanisms. In addition, it acts as an antioxidant and, along with other antioxidant enzymes such as glutathione, catalase and superoxide₀dismutase prevents the formation of H₂O₂ and malondialdehyde. This reduces oxidative stress and protects cells from free radicals, which are responsible for plant deterioration during its growth, development, and aging stages.

This result is consistent with the results of many researchers who indicated that the use of ascorbic acid led to an improvement in the studied vegetative characteristics in the in the Fenugreek plant (*Trigonella foenum-gracecum*) (Fatima *et al.*, 2020) and with Ullah *et al.*, (2016) on barley (*Hordeum vulgare*) seedlings and with Alhasnawi *et al.*, (2016) on rice (*Oryza sativa*) plants. The results in Table (3) show a significant increase in some of the studied root growth indicators with increasing concentrations of ascorbic acid. The high ascorbic acid treatment at the concentration (100 mg/L) recorded the highest averages in all traits compared to the control treatment. The effect for this may be that the application of ascorbic acid led to the regulation of the nutritional conditions of the



elements Calcium, Nitrogen, Phosphorus, Potassium, Magnesium in plants growing under the influence of chromium stress, and thus these essential elements mitigate the harmful effect of various environmental stressors through the development of plants' physiological and molecular capabilities (Siddiqui *et al.*, 2012 ; Arshad *et al.*, 2016). In addition to increasing the effectiveness of antioxidant enzymes that work to reduce high oxidative capacity (oxidative stress), this causes the prevention of damage to the cell walls of root cells, which is positively reflected in increasing the plants' tolerance to chromium toxicity, as increasing the absorption of nutrients and improving the water content. They play a vital role, either directly or indirectly, in the process of mitosis, cells expansion, and differentiation, because all nutrients are the main components of many metabolically active compounds that regulate various physiological functions, stimulating detoxification mechanisms and secretion of chromium into the extracellular space (Marschner, 2002). Our results in this study are similar to those found by Fatima *et al.* (2020) on the use of ascorbic acid on Fenugreek (*Trigonella foenum-gracecum*) plant grown under different concentrations of copper, as the results showed an improvement in root growth indicators such as root length, fresh and dry weight with the addition of ascorbic acid, which reduces oxidative stress and improves growth under heavy metal stress conditions (Urso and Clarkson, 2003). As for the increase in chromium concentrations in root tissues, this indicates that the roots are the main part in accumulating chromium, as the application of ascorbic acid may have led to the regulation and improvement of nutritional conditions and thus reduced the harmful effect of chromium. This is what caused the activation of chromium absorption through the roots and its accumulation in the different plant organs. The variable and cumulative concentration of chromium within the same part of the plant depends on the chromium available in the root zone and the concentration applied, While the differences in chromium concentrations in the studied plant parts may be explained by the ability of chrysanthemum plants to adapt to chromium-contaminated conditions. Ascorbic acid is a weak substance in chelating metals and thus enhances chromium absorption (MacFarlane *et al.*, 2003; Allue *et al.*, 2014).



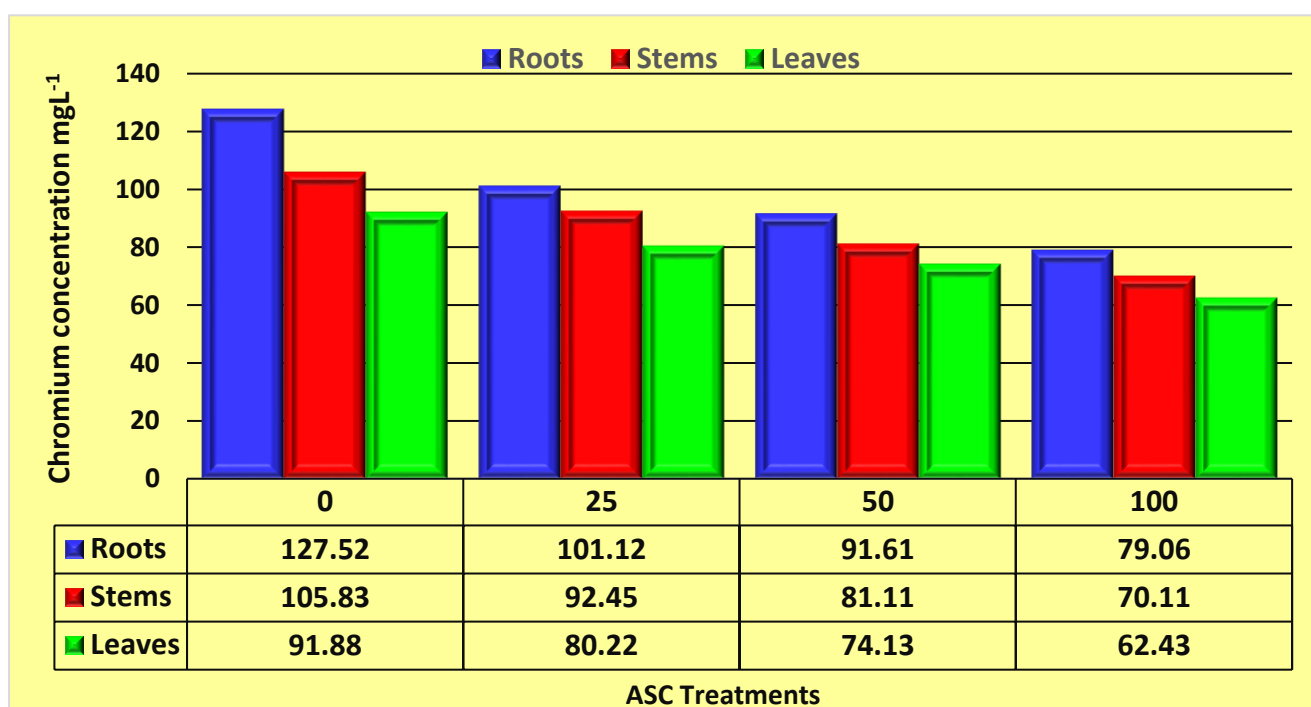
Table (2) Effect of ascorbic acid concentration on some vegetative growth indicators of chrysanthemum plants.

Treatments (mg L ⁻¹)	Plant height (cm)	Number of leaves (plant leaf ⁻¹)	Number of lateral branches (plant branch ⁻¹)	Main stem length (cm)	Main stem diameter (mm)	Fresh weight of the vegetative group (g)	Dry weight of the vegetative group (g)
0	21.27	60.66	9.33	11.35	5.56	27.76	5.75
25	23.56	76.33	13.66	13.95	6.35	39.21	6.61
50	26.43	87.00	16.33	16.12	7.01	46.65	7.06
100	29.12	100.33	19.00	19.67	8.88	55.12	9.62
L.S.D	1.99	6.35	2.31	2.54	0.912	4.11	0.981



Table (3) Effect of ascorbic acid concentrations on some root growth indicators of chrysanthemum plants.

Treatments mg L ⁻¹) (Roots length (cm)	Number of roots (plant root ⁻¹)	Fresh weight of roots (g plant ⁻¹)	Dry weight of roots (g plant ⁻¹)
0	10.15	18.66	3.52	0.339
25	12.98	24.00	4.73	0.462
50	13.57	27.66	5.11	0.493
100	15.02	34.33	6.02	0.536
L.S.D	1.04	2.13	0.874	0.113

Figure 1 Chromium concentrations in different parts of the chrysanthemum plant (mg kg⁻¹)

V. Conclusion

The research results showed that the high concentration of chromium negatively affects plants, as evidenced by the significant decrease in the studied vegetative and root growth indicators in plants not

treated with ascorbic acid. Treatment with the antioxidant ascorbic acid also improved the studied vegetative and root growth characteristics, which had a positive impact on increasing the plant's tolerance to nickel stress conditions, especially when ascorbic acid concentrations were gradually increased, which was reflected in the phenotypic appearance of the plants. Roots are the main plant parts in which the largest amount of chromium was concentrated.

VI. Referneces

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