



Effect of Cadmium concentrations on the Vegetative Growth, Flowering and Chemical Constituents of *Tagetes erecta* L. Plant

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Abstract: This investigation was carried out at Experimental area of National Research Centre, Dokki, Giza, during the two successive seasons of 2012 and 2013. The objective of this study is to investigate the effect of cadmium concentrations on vegetative growth, flowering and chemical constituents of *Tagetes erecta* L. plants. Results indicated that cadmium concentrations at 80 ppm decreased all vegetative growth and flowering parameters (plant height, branches number /plant, fresh and dry weight of herbage, flowers number /plant and dry weight of flowers) compared with the control plants. In this respect, cadmium at 20 and 40 ppm slightly increased carbohydrates percentage and didn't affect essential oil percentage. Catalase and peroxidase enzymes increased by increasing cadmium concentrations, but decreased superoxidase enzymes. Additionally, all macroelements (nitrogen, phosphorus and potassium percentage) in roots and herbage decreased by increasing cadmium concentrations. The same results were obtained by cadmium concentrations on microelements content (manganese, iron, zinc and copper). On the contrary, cadmium content increased by increasing cadmium concentrations. This study recommends that *Tagetes erecta* L. plants can tolerate cadmium concentrations up to 40 ppm.

Keywords: *Tagetes erecta* L, marigold plants, cadmium, growth, chemical constituents

Introduction

Tagetes erecta L. plant belongs to the family composite (Asterceae) commonly known as Marigold, it is one of the most important ornamental plants and its importance comes from its use in landscaping and as cut flowers¹. Its flowers are used in several industries such as processed food, confectionary drugs and pharmaceuticals and also in poultry² *Tagetes erecta's* oil is also used in insecticidal and antifungal activities³, the plant as a whole is used medicine as it is very useful in treating skin diseases and burning sensations where its flowers with some oil are applied on the infected area.

Heavy metals exist as rare elements in the soil. Only certain metals are usually present in high concentrations in forms that are not easily available. As a result of human activity, the release of heavy metals in biological forms can damage or change the natural and even the synthetic (man-made) ecosystems. Heavy metals' chemical forms in soil solutions depend mostly on the metal element concerned, on pH of the soil and on the presence of other ions, etc. Heavy metal ions exert a lot of toxic action on the enzymes. Enzymes inhibiting may have been caused by the process of making groups of catalytically active protein denaturation, some of the common features of pollution of soil by heavy metals are soil respiration and impeded litter

decomposition. Additionally, the toxic effect of heavy metals on the mitochondria in the soil and extracellular soil enzymes was also revealed in a number of other studies⁴.

Cadmium (Cd) is a metal contaminant that causes great concern in the environmental agricultural system⁵. Cadmium accumulation may result in a number of physiological, biochemical structural changes⁶ as it changes the uptake of nutrients, causes stomatal opening inhibition by interacting with water balance of the plant, it disturbs the Calvin cycle enzymes, the photosynthesis process, carbohydrates and antioxidants metabolism processes as well as lower the productivity of the plant⁵.

This work is to investigate the effect of cadmium on the growth and chemical constituents of *Tagetes erecta* L. plant and feasibility of using this element to improve plant's quality.

Materials and Method

A pot experimental trial was carried out during the two successive seasons of (2012 and 2013) at the screen of National Research Centre, Cairo, Egypt. It was intended to study the effect of cadmium element on the growth and chemical constituents of *Tagetes erecta* L. plant. Marigold seeds were obtained from Horticulture Research Institute, Agric. Res. Centre, Ministry of Agricultural Egypt. The seeds were grown on pots filled with sandy soil in March for both seasons. The investigated soil characterized by sand 78.33%, silt 4.43%, clay 17.24% with pH 7.81, EC 2.18 dS/m, CaCO₃ 2.25%, OM 0.55%, Ca 11.61, Mg 4.80, Na 4.64, Cl 1.80, HCO⁻ 1.40, SO⁼ 8.5 meq/L, N 19.60, P 65.80, K 165.64 meq/100g.

The inner surfaces of pots were lined with a polyethylene sheet. Each pot contained 12 kg of air-dried soil. Plants were thinned to a maximum of five per pot, after a week of germination. Each treatment including the control was replicated five times. Cadmium as cadmium nitrate Cd (NO₃)₂ was used after dissolving to prepare to determined concentration using distilled water and added as surface and repeated twice every week till the end of the season. Concentrations used were as follows: Control, Cd 20ppm, Cd 40 ppm and Cd 80 ppm. The pots were arranged in a randomized complete blocks design, with 4 treatments (control plus three cadmium treatments) replicated three times, which each replicate (block) consisting of 5 plants / treatment. All plants were supplied with commercially fertilizer Kristalon (NPK, 19:19:19) produced by Phayzen Company, Holland. It used at rate of 5.0 g/pot in three equal doses after 4, 8 and 16 weeks from sowing.

The plant herbage was harvested by cutting above 6cm over the soil surface and the following data were recorded, plant height, number of branches/plant, fresh and dry weight of herbage/plant, No. of flowers and dry weight of flowers/plant.

Chemical Analysis: Carbohydrate was extracted from drying finely ground leaves (Powdered) at flowering stage according to⁷ and estimated the total carbohydrate calorimetrically by the phenol sulphuric acid method⁸. The essential oil percentage of fresh herb samples was extracted by water distillation for 6hr and then dried over anhydrous sodium sulphate and determined according to⁹. The recorded data (means of the two growing seasons) were statistically analyzed according to the procedure of¹⁰, while the means of the studied treatments were compared using L.S.D test at 0.05 of probability.

Macro and micro elements were determined in dried herbage and roots at flowering stage according to the official and modified methods of analysis¹¹. Cadmium percentage was determined using the Atomic Absorption Spectrophotometer Zeiss FMD₃ according to¹². Catalase activity was measured spectrophotometrically according to¹³. Peroxidase and Superoxidase activity was measured as a biochemical marker according to¹⁴. The physical and chemical properties of the tested soil were determined according to¹⁵.

Results and Discussion

Vegetative growth:

The growth parameters as affected by cadmium treatments are shown in Table (1). The lowest values of plant height, number of branches/plants, fresh and dry weight of herbage, number of flowers /plant and dry weight of flowers were obtained from the plants treated with the highest concentration of cadmium (80ppm), while the highest values occurred by treated with 20ppm cadmium. Numerically, plant height, number of

branches/plant, fresh and dry weight of herbage, number of flowers/plant and dry weight of flowers were decreased by (15.74, 18.0,10.97, 26.76, 29.66 and 40.38 %) as a results of treated with cadmium having the concentration of 80 ppm in comparison with untreated plants. On the other hand, application plants with 20 ppm cadmium promoted all growth parameters compared with control plants. The averages were (1.2%) in plant height, (22.0%) in number of branches/plant, (0.37 %) in fresh weight of herbage, (2.11%) in dry weight of herbage and (3.85%) in flowers dry weight in the plant treated with cadmium at concentration of 20ppm compared with control plants.

Table 1 : Effect of different concentrations of cadmium on plant height, number of branches/plant, fresh and dry weight of herb, number of flowers and dry weight of flowers (gm) /plants of *Tagetes erecta L.* plant

Character Treatment	Plant height(cm)	Number of branches	Fresh weight of herb (g)	Dry weight of herb (g)	Number of flowers	Dry weight of flowers (g)
Control	50.2	5.0	80.2	14.2	14.5	5.2
Cd 20 ppm	50.8	6.1	80.5	14.5	14.5	5.4
Cd 40 ppm	50.0	6.0	78.1	13.2	13.7	5.0
Cd 80 ppm	42.3	4.1	71.4	10.4	10.2	3.1
L.S.D at 5%	1.3	0.8	2.1	1.4	1.8	0.7

The most common effect of Cd toxicity in plants is stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways¹⁶. In our study, varied concentrations of Cd affected fresh weight of *Tagetes erecta L*. The reduction in the growth in *Tagetes erecta L* could also be attributed to the suppression of the elongation growth rate of cells, because of an irreversible inhibition performed by Cd on the proton pump responsible for the process¹⁷. Parameters such as fresh weight of herbage as well as number of flowers/plant were used as useful indicators of metal toxicity in plants.

Total carbohydrates content:

The results obtained in Table (2) showed that total carbohydrates percentage slightly increased by Cd at 20 and 40 as a compared to the control plants .The increments were (3.17 and 3.17%) by Cd at 20 and 40 ppm respectively. Whereas, Cd at 80ppm reduced total carbohydrates percentage, the decrease was (21.13%) compared with untreated plants.

Low starch export from the mesophyll and higher starch accumulation in damaged leaves of *tagests erecta L.* may result both in the higher resistance of their photosynthetic apparatus and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebis phosphate carboxylase¹⁸.

Essential oil content:

From the given data in Table (2) it could be concluded that essential oil percentage not affected by Cd at 20 and 40 ppm compared with the control plants .While Cd at the high concentration (80 ppm.) decreased essential oil percentage as compared with the other treatments. The decrease of the essential oil percentage was (37.14%) compared with the control plants.

Enzymes content:

Catalase enzymes content of *Tagetes erecta L.* plant, as affected by the different cadmium concentrations are revealed in Table (2). Catalase enzyme content was increased by increasing cadmium concentrations, the increments were (0.56, 2.51 and 3.35%) by cadmium at 20, 40 and 80 ppm, respectively compared with the untreated plants.

Table 2: Effect of different concentrations of cadmium on the percentage of carbohydrates, essential oil, and catalase, peroxidase, superoxidase enzymes of *Tagetes erecta* L. plant

Treatment	Carbohydrates %	Essential oil %	Catalase	Peroxidase	Superoxidase
Control	28.4	0.35	358	60.3	48.2
Cd 20ppm	29.3	0.35	360	60.4	25.4
Cd40ppm	29.3	0.35	367	67.2	27.2
Cd80ppm	22.4	0.22	370	73.4	29.3
L.S.D. at 5%	2.3	0.04			

In the same Table similar result was obtained by cadmium concentrations on peroxidase enzyme. All concentrations of cadmium increased peroxidase enzyme content; the content was gradually increased by increasing cadmium concentrations. The increment by cadmium 20, 40 and 80 ppm were (0.17, 11.44 and 21.72 ppm) respectively, compared with the control.

The results in the same table indicated that the superoxidase content was affected in response to the different cadmium concentrations. It is obvious from the data that the superoxidase content was markedly decreases when the plants were treated with the following concentrations: 20 ppm Cd gave (47.3 %), 40 ppm Cd gave (43.57 %) and 80 ppm Cd gave (39.21%) as compared with untreated plants which gave the highest superoxidase content. These findings are in accordance with those obtained by^{19,20}. Their findings indicated that these defensive systems consist of metabolites like tocopherol, glutathione, ascorbate, etc. and activated oxygen enzymatic scavengers for example catalases, peroxidases and superoxidase dismutases.

Nutrient contents:

The results obtained in Table (3) showed that cadmium concentrations decreased all macroelements in roots and herbage of *Tagetes erecta* L. plants. Plants treated with cadmium at 20,40 and 80 ppm decreased nitrogen percentage in roots compared with the control, The reductions were (7.34,20.24 and 32,94 %) respectively, as compared with the untreated plants. The same results were observed in herbage, nitrogen% decreased by increasing cadmium concentrations. The reductions were (4.70,10.18 and 21.14) for (20,40 and80 ppm cd) respectively compared with the control plants. The reduction in nitrogen content under cadmium concentrations agrees with the results of²¹ they showed that heavy metals have caused disturbance in the nitrogen metabolism process.

Additionally, It is obvious from the data that phosphorus percentage was markedly decreased in roots when the plants were treated with the following concentrations of cadmium 20 ppm giving(4.1%) 40ppm(3.8%) and 80ppm giving (2,4%). The untreated plants gave the highest percentage of phosphorus (4.5%).Cadmium concentrations had the same effect on herbage. It is clear from the data of phosphorus percentage on herbage was decreased when plants were treated with cadmium at 20ppm gave (6.3%), 40ppm gave (6.0%) and 80ppm gave (5.2%) compared with the control plants gave (7.4%). The inhibition of mobilization of phosphorus from soil to root system probably led to the decrease in phosphorus content²². This is in comparable with results of²³ in barley and²⁴ in rye grass.

Regarding the effect of cadmium concentrations on potassium percentage of Marigold plants, the response of potassium percentage to cadmium concentrations had almost the same trend as nitrogen and phosphorus percentage in roots and herbage. Potassium percentage gradually decreased by increasing cadmium concentrations as compared with the untreated plants. The reductions of potassium percentage in roots and herbage were (16.05 and 16.85 %) for 20 ppm Cd (25.51 and 24.31 %) for 40 ppm Cd and (50.21 and 44.20%) respectively compared with the control. These results are in consonance with finding of^{25, 26}

Table 3: Effect of different concentrations of Cadmium on the percentage of nitrogen, phosphorous, and potassium in the root and shoot of *Tagetes erecta* L. plant

Treatment	Root			Herbage		
	Nitrogen%	Phosphorus%	Potassium%	Nitrogen%	Phosphorus%	Potassium%
Control	5.04	0.45	2.43	8.94	0.74	3.62
Cd 20ppm	4.67	0.41	2.04	8.52	0.63	3.01
Cd 40 ppm	4.02	0.38	1.81	8.03	0.60	2.74
Cd 80 ppm	3.38	0.24	1.21	7.05	0.52	2.02

Concerning the effect of cadmium concentrations on microelements of roots and herbage, the data in Tables (4 and 5) indicated that manganese content was decreased in plants treated with any cadmium concentrations. The plants treated with 20ppm followed by 40ppm and 80ppm produced (57.3 and 70.5ppm), (51.3 and 67.8ppm) and (44.1 and 57.3ppm) in roots and herbage, respectively. The result in harmony with those of ^{27, 28}.

Data on iron content of marigold plants as affected by different concentrations of cadmium. The plants treated by adding 20 ppm cd gave (285 and 301 ppm), followed by 40 ppm Cd gave (280 and 289 ppm) and 80 Cd ppm (270 and 270 ppm) in roots and herbage respectively. Generally, iron content decreased by increasing cadmium concentrations. The untreated plants gave the highest value of iron content (290 and 305 ppm) in roots and herbage, respectively. This effect was previously revealed by ^{29, 30}.

Table 4: Effect of different concentrations of cadmium on the content of manganese, iron, zinc, copper and cadmium in the root of *Tagetes erecta* L. plant

Treatment	Root				
	Mn ppm	Fe ppm	Zn ppm	Cu ppm	Cd ppm
Control	60.0	290	43.5	18.3	0.81
Cd 20ppm	57.3	285	32.3	15.2	0.87
Cd 40 ppm	51.3	280	28.7	14.4	0.90
Cd 80 ppm	44.1	270	22.8	10.0	0.93

Table 5: Effect of different concentrations of cadmium on the content of manganese, iron, zinc, copper and cadmium in the herbage of *Tagetes erecta* L. plant.

Treatment	Herbage				
	Mn ppm	Fe ppm	Zn ppm	Cu ppm	Cd ppm
Control	80.4	305	50.0	31.3	0.58
Cd 20ppm	70.5	301	48.0	29.4	0.63
Cd 40 ppm	67.8	289	44.0	25.2	0.66
Cd 80 ppm	57.3	270	36.0	20.1	0.78

Regarding the effect of cadmium concentrations on zinc content of roots and herbage, the data in Tables (4 and 5) indicated that, zinc content of roots and herbage was decreased in plants treated with any concentration of cadmium. The plants treated with 20 ppm followed by 40 ppm and 80 ppm resulted in (32.3 and 48.0 ppm), (28.7 and 44.0 ppm) and (22.8 and 36.0 ppm) in roots and herbage, respectively. The highest value of zinc content was obtained by untreated plants (43.5 and 50.0 ppm) in roots and herbage, respectively. These results were confirmed with those obtained by ^{31,32}.

The same trend was obtained by cadmium concentrations on copper content of marigold roots and herbage plants. The response of copper content of roots and herbage to cadmium concentrations revealed a negative effect on copper content, where using 20 ppm, 40 ppm and 80 ppm Cd were producing (15.2 and 29.4 ppm), (14.4 and 25.2 ppm) and (10.0 and 20.1 ppm) copper content in roots and herbage, respectively. Similar results were obtained by several others such as ^{24,26}.

On the other hand, the adverse effect was obtained by cadmium concentrations on cadmium content in roots and herbage of marigold plants. Cadmium content gradually increased by increasing cadmium

concentrations. The average cadmium content ranged between (0.81 and 0.93 ppm) in roots and (0.58 and 0.78 ppm) in herbage. Similar trend was obtained by^{33,34,35}.

References

1. Nau, J., 1997. *Tagetes* (marigold), pp: 763-766, In: Ball, V, (ed.). Ball nedbook, Ball Publishing, Batavia, IL.
2. Ram, M., S.N. Garge, G. Ram and S. Kumar, 2000. Plant population and yields in *Tagetes pataula* and *Tagetes erecta*. *J. of Herbs, spices & Medicinal Plants*, 7(3): 1-5.
3. Balbaa, Laila, K., G. Abd El Aziz, Nahed and A.A. Youssef, 2008. Physiological effects of stigmasterol and Nicotinamide on growth, flowering, oil yield and some chemical compositions of *Tagetes erecta* L. plant. *J. of Appl. Sci. Res.*, Co: CC-CC: 1-7.
4. Tyler, G.; Pahlsson, A. M. ; Bengtsson, G., Baath. E and Transvik, L. (1989): Heavy metal ecology and terrestrial plants, micro-organism and invertebrates; a review, *water, Air and Soil Pollution* 447, 187-215.
5. Toppi. L. S. and Gabrielli, R. (1999): Response to cadmium in higher Plants. *Environmental and Experimental Botany*, Vol. 41 No. 2. 105-160.
6. Khan, N. A.; Anjum, N. A; Nazar, R. and Iqbal, N. (2009): Increased Activity of ATP_Sulfurylase and Increased Contents of Cysteine and Gluthathione Reduce High Cadmium-Induced Oxidative Stress in Mustard Cultivar with High Photosynthetic Potential. *Russian Journal of Plant Physiology*. 56 (5): 670-677.
7. Herbert, D., P.J. Phipps and R.E. Strange, 1971. Determination of total carbohydrate. *Methods Microbiol.*, 5B: 209-344
8. Montgomery, R., 1961. Further studies of the phenol-sulphuric acid reagent for carbohydrate. *Biochim. Biophys. Acta*, 48: 591-593.
9. Gunther, E., 1961. *The essential oils*, 4th Vol.1, D.V. Nostrand Corn. Inc. New York.
10. Snedecor, G.W. and W. Cochran, 1980. *Statistical Methods*. 7th Edn, Jowa State Univ., Press, Jowa, USA.
11. AOAC, 1984. Association of Official Analytical Chemists –Official methods of analysis of the association of official analytical chemists. 21ed. Washigton D.C.
12. Jackson , M.L.,(1973) : *Soil chemical analysis .Advanced Course Publ . by the author, Dept . Soil Sci .,Univ .of Wisconsin , Madison ,Wisconsin .*
13. Dhindsa, R.S., Plumb-Dhindsa, P., Thorpe, T.A.: Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. - *J. exp. Bot.* **32**: 93-101,1981.
14. Reuveni, R., M. Shimoni, Z. Karchi and J. Kue, 1992. Peroxidase activity as a biochemical marker for resistnce of muskmelon (*Cucumis melo*) to Phytopathol., 82: 749-753
15. Jackson, M.I., 1967. *Soil chemical analysis printice hall of India private limit new Delhi*.
16. Arduini I., Godbold D.L., Onnis A. (1996): Cadmium and copper uptake and distribution in Mediterranean tree seedlings. *Physiol. Plant.*, 97: 111–117.
17. Aidid S.B., Okamoto H. (1993): Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *Impatiens balsamina* to lead, cadmium
18. Stiborová M., Ditrichová M., Březinová A. (1987): Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley and maize seedlings. *Biol. Plant.*, 29: 453–467.
19. Demiral , T and I.Turkan , (2005) : Comparative lipid peroxidation , antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. *Environ. Exp.,Bot.*,53(2): 47-57.
20. Mandhania ,S.; S. Madan and V.Sawhney ,(2006) : Antioxidant defense mechanism under salt stress in wheat seedlings . *Boil . Plant*, 227,227-231.
21. Stayonova and Doncheva (2000) : The effect of zinc supply and succinate treatment on plant groth and mineral uptake in pea plants, *Braz. J. Plant and Physiol*, 14(2): 1-9.
22. Lin, A. C.; H. Su and S. C. Wu, (1975): Translocation and accumulation of mercury and cadmium and their effect on the uptake of phosphate (P12) in rice plants. *J. Agric. China*, 90: 34-43.
23. Chernykh, N. A., 1991 : Alernations of the concentrations of certain elements in plants by heavy metals in the soil. *Soviet soil Sci.*, 23(6):45-53.

24. Bonnet, M.; O. Camares and P. Veisseire, (2000): Effect of Zinc and influence of *Acremonium lolii* on growth parameters, chlorophyll (a), Fluorescence and antioxidant enzyme activities of rye grass (*Lolium perenne* L. cv Apollo) J. Exp. Bot., 51 (346): 945-953
25. Vijayarangan,P. ,(2005) :Iron and manganese status of blackgram cultivars under nickel stress. Geobios , 32:11-13.
26. Thamayyanthi, D.; P. S. Sharavanan and B. jayaprasad, (2013): Phytoremediating capability biochemical changes and nutrient status of marigold (*Tagetes erecta* L.), Plant under cadmium stress. International Journal of Research I Plant Science. 3(4):57-63.
27. Palove-Balang, P. ; Kisova, A., Pavlovkin, J and and Mistrik, I. (2006): Effect of Manganese on *Codium* Toxicity in Maize seedlings. Plant Soil and Environment. 52(4): 143-149.
28. Zornoza,P; Sanchez-Pardo ,B.and Carpena,R.R.O.,(2010):Interaction and accumulation of manganese and Cadmium in the manganese accumulator *Lupinus albus*.Journal of Plant Physiology.167(13):1027-1032.
29. Nada, E. ; Ferjani, B. A. ; Ali, R. ; Imed, B. R. B. M. and Makki, B. (2007): Cadmium induced growth inhibition and alteration of Biochemical parameters in Almond Seedlings grown in solution culture. Acta Physiologiae Plantarum. 29(2): 57-62.
30. Quershi, M. I.; D'Amici, G. ; Fagioni, M. ; Rinalducci, S. and Zolla, L. (2010): Iron stabilizes thylakoid Protein-Pigment Complexes in Indian Mustard during Cd-Phytoremediation as revealed by BN-SDS-PAGE and ESI-MS/MS, Journal of Plant Physiology, 167(10): 761-770.
31. Kramer, U. ; Talke, I. N. and Hanikanne, M. (2007): Transition Metal Transport. FEBS Letter. 581 (12): 2263-2272.
32. Street, R. A.; Kulkarni, M. G. ; Strik, W. A. ; Southway, C. and Van Stadden, J. (2010): Effect of Cadmium on growth and micronutrients Distribution in Wild Garlic (*Tulbaghia violacea*). South African Journal of Botany. 76 (2): 332-336.
33. Prasad,M .N.,(1995): Cadmium toxicity and tolerance in vascular plants. Environ. Expt, Bot.,35,525-545.
34. Arun,K.S.,C.Carlos ,L.Z. Herminia and S.Avudainayagam .(2005):Chromium toxicity in plants. Environ.Int. 31:739-753.
35. XinChen,J.Wang,Y.Shi,M.Q . Zhao and G. Y.Chi (2011): Effects of cadmium on growth and photosynthetic activities in pakchoi and mustard . Botanical studies .52 :41-46.
