THE USE OF CHEMICAL SOIL ADDITIVES TO OVERCOME THE ADVERSE EFFECTS OF CADMIUM ON GROWTH AND CHEMICAL COMPOSITION OF *Tagetes erecta* L. PLANTS

By

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B.Sc. Agric. Sci. (Ornamental Horticulture), Fac. Agric., Cairo Univ., 2005
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THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

DOCTOR OF PHILOSOPHY

In

Agricultural Sciences
(Ornamental Horticulture)

Department of Ornamental Horticulture
Faculty of Agriculture
Cairo University
EGYPT

2015

APPROVAL SHEET

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ABSTRACT

This study was carried out in the Experimental Field of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2012 and 2013. The aim of this study was to investigate the possibility of reducing the harmful effect of cadmium soil pollution on the growth and flowering of *Tagetes erecta* (African marigold) plants by the addition of nickel, FeNa+EDTA and salicylic acid. Plants were grown in 20 cm pots filled with clay + sand (1:1, v/v, weight= 4.5 kg soil) and were treated twice with cadmium, in the form of cadmium acetate $[(CH_3COO)_2Cd.2H_2O]$, as a soil drench at 0, 5, 15 and 25 mg Cd/ kg soil, in addition to three different chemicals (nickel as nickel sulphate $[NiSO_4.H_2O]$) as a soil drench at 6 and 12 mg Ni/ kg soil, EDTA+Fe Na $[C_{10}H_{12}N_2O_8.FeNa]$ as a foliar spray at 30 and 60 ppm, and salicylic acid $[C_7H_6O_3]$ as a foliar spray at 50 and 100 ppm). Control plants received the Cd treatments only. The recorded results showed that Cd at 25 mg Cd/ kg soil gave the lowest values for the studied vegetative growth and flowering characteristics. In most cases, the different chemical additives (except salicylic acid at 100 ppm and EDTA+FeNa at 60 ppm) decreased the plant height, stem diameter, number of leaves, number of branches and number of flower heads per plant, as well as the fresh and dry weights of leaves, stems, roots, shoots and flower heads, as well as dry matter accumulation of roots and shoots. Also, Cd caused a higher reduction of weight in roots than in shoots, thus resulting in an increase in the shoots:roots ratio at the highest concentration of Cd. Regarding the chemical composition, increasing Cd concentration up to 25 mg reduced chlorophylls (a, b, and a+b), carotenoids, free sugars, free amino acids and nutrient elements, but increased the chlorophyll a/b ratio, as well as the concentration of total soluble phenols and the activities of the antioxidant enzymes. On the other hand, combining the Cd treatments with the use of chemical additives (especially salicylic acid at 100 ppm) caused some reduction in the harmful effects of Cd. Meanwhile, heavy metals (Ni and Cd) concentration were always increased by the Cd treatments, alone or when combined with chemical additives, except Fe in the leaves, which was increased only with Cd at 5 mg /kg soil. From the results of this study, it was concluded that salicylic acid can be applied at 100 ppm in the nursery or the production field to increase yield of *Tagetes erecta* (African marigold) under cadmium stress.

Keywords: *Tagetes erecta*, salicylic acid, EDTA+Fe, cadmium acetate, nickel sulphate, African marigold, chemical additives.

ACKNOWLEDGMENT

First of all I would like to express my deepest thanks to "Allah" for helping me to carry out and complete this work.

I wish to express my sincere thanks, deepest gratitude and appreciation to ***Prof. Dr. Hazem Abdelgalil Mansour*** *and* ***Prof. Dr. Effat Ismail El-Maadawy,*** *Professors of Ornamental Horticulture, Faculty of Agriculture, Cairo University, for their supervision, continued support, valuable advice, constructive criticism and their guidance throughout the course of the study, and during the revision of the manuscript of this thesis.*

Great thanks are also due to ***Prof. Dr. Ahmed Hussein Hanafy****, Professor of Plant Physiology, Faculty of Agriculture, Cairo University, for suggesting the problem, supervision, guidance and continuous support throughout the whole course of this investigation.*

Deep gratitude is extended to all staff members of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, and special deep appreciation is given to my father and my mother and my husband and all my family for their help and encouragement.

Finally I wish to express my sincere thanks to everyone who gave me a helping hand through this study.

CONTENTS

INTRODUCTION

African marigold (*Tagetes erecta* L., family: Asteraceae, "Compositae") is a species of the genus Tagetes (Bailey, 1960), native to Mexico and Central America. This plant is a hardy annual plant that reaches heights of 50–80 cm, and is an erect, branched plant. The leaves are green, mostly alternate, oblong- shaped to odd-pinnate, compound, with dentate margins. Blooms naturally occur in golden, orange, yellow and whitish colors, often with maroon highlights. Foliage and flowers are aromatic when bruised or crushed. The main components of the oil are limonene, linalool, tagetone, ocimene; α-d-phellandrene and linalyl acetate. The plants need full sun, and grow best in fertile, moist, well-drained soil with a lot of mulch. Plants grow so rapidly that there is little reason to start seed indoors. Taller varieties should be sited in locations sheltered from strong winds and heavy rains. African marigold is propagated by seeds, which are sown in early spring.

In general, African marigold is usually used in masses in the flower bed or in the annual border with other bright colored flowers. The rugged marigolds are perfect for containers where they combine well with other plants, and most of marigold flowers are good and long lasting as cut flowers. African marigold is grown today as a commercially important source of carotenoid pigments. The principal pigments present in the flowers are xanthophylls, particularly lutein, which account for 80 to 90% in the form of esters of plamitic and myristic acids. The ground blossom meal, (petal meal) or its extract, is usually saponified for better absorption, and is added to poultry feed to ensure a good colouration of egg yolks and broiler skin, especially in the absence of well-pigmented yellow maize in the feed (Dole and Wilkins, 2005).

Heavy metals are a group of elements with an atomic mass greater than 5 g/cm^3. Some heavy metals are also classified as “trace elements” because they occur in the earth’s crust at concentrations of not more than 0.1% (1000 mg/kg) and, in fact, most have average concentrations of less than 100 mg/kg. Heavy metals are sometimes referred to as “toxic metals”, since they include elements (such as Pb, Cd and Hg) that can cause toxicity problems. However, the name “toxic metals” is generally considered to be inappropriate because all trace elements are toxic to living organisms when present in excess. However, some such as Co, Cr, Cu, Mn, Mo and Zn are essential (in small, but critical concentrations) for the normal healthy growth of either plant, animals or both, although they are toxic at high concentrations (Alloway, 1995).

The increasing use of wide varieties of heavy metals in both the industrial and agricultural sectors has caused a serious concern of environmental pollution. At high concentrations, heavy metals cause severe damage to plants (Mohan and Hosetti, 2005; Sinhal, 2007; Sinhal *et al.*, 2007; Gupta *et al.*, 2008a and b and Pandey *et al.*, 2007).

Cadmium (Cd) is a highly toxic pollutant released into the environment by both anthropogenic and natural sources. Its presence in the soil, including agricultural lands, is considered a serious environmental issue, mainly because of its entry in the human food chain, and its dangerous effects on living organisms. Although Cd is not essential for plant growth, it is readily taken up by roots and accumulated in plant tissues at high levels (Prasad, 1995). Excess of cadmium causes a number of toxic symptoms to the plants, viz. growth retardation, inhibition of photosynthesis, induction and inhibition of enzymes, altered stomatal action, efflux of cations and generation of free radicals (Chen and Kao, 1995). Also, cadmium has been shown to affect various aspects of metabolism in different plant systems (Shah and Dubey, 1997).

There are several methods that can limit access of heavy metals into the plants, e.g. introducing organic substances to the soil, some chemical treatments, or even liming. The selected chemicals should have low environmental impact but high efficiency (Makino *et al.*, 2007). Strong metal chelating agents, neutral salts and strong acids have been used (Davis, 2000), especially ethylenediaminetetraacetic acid (EDTA), which can be an efficient mean to remove Cd from contaminated soils (Abumaizar and Smith, 1999), although its extraction efficiency depends on many factors, such as liability of heavy metals in the soil, the strength of EDTA, electrolytes, pH and soil matrix [Brown and Elliott, 1992; Papassiopi *et al.*, 1999). Also, chelating organic acids are able to dislodge the exchangeable fractions of heavy metals (Peters, 1999); likewise other chelating compounds such as citric acid and salicylic acid (Naidu and Harter, 1998).

Cd is an element of group IIB in the periodic table, and its atomic number is 48. It shows chemical similarity with the other elements of group IIB, especially with nickel (Ni). Cd and Ni are elements having similar geochemical and environmental properties; their chemical similarity can lead to competition between them during plant uptake and transport from roots to the aerial parts (Das *et al*., 1997).

This study was conducted to investigate the effect of different rates of chemical treatments, using additives such as a Ni (as nickel sulphate), salicylic acid or EDTA+FeNa on the vegetative growth, flowering and chemical composition of marigold (*Tagetes erecta*) plants grown under Cd stress. The information provided by this study may help in successfully reducing the harmful effects of cadmium.

REVIEW OF LITERATURE

The results of some previous investigations concerning the effects of Cd (as cadmium acetate), as well as different doses of chemical additives (Ni as nickel sulphate, salicylic acid and EDTA+FeNa) and their interactions are summarized in the following:

1. Effects of different levels of Cd

a. Effects on growth

Many researchers have studied the effect of Cd on the vegetative growth of several plant species**. Woolhouse (1983)** reported that Cd causes inhibition and abnormalities of general growth in many plant species, including the following symptoms: (i) roots are mucilaginous, browning, and decomposing; (ii) reduction of shoots and root elongation, (iii) rolling of leaves and chlorosis can occur. The main reason indicated is disordered division and abnormal enlargement of epidermis and cortical cell layers in the apical region. The changes in the leaf include alterations in chloroplast ultrastructure and low content of chlorophylls, which cause chlorosis, and restrict activity of photosynthesis. On the other hand, **Poschenrieder *et al*. (1989)** proposed theories for explaning the effect of cadmium on plants: First, cadmium (Cd) can interfere with mineral nutrition by hampering the uptake and translocation of essential elements. The second is that Cd can inhibit photosynthesis and plant growth. Finally, Cd may exert detrimental effects on the overall cell metabolism via alterations in (i) the behaviour of key enzymes of important pathways, (ii) membrane composition and function, and (iii) by lowering the control of the cell redox state, which ultimately causes oxidative stress

Grunhage and Jager (1985) reported that *Allium porrum* and *Pisum sativum* showed no visible symptoms of metal toxicity in soil contaminated with Cd as $CdCl_2$ at 0.1 and 1μmol/l. However, dry matter production was reduced in relation with increasing the concentration of Cd.

Wilson (1988) concluded that cadmium stress (50 mg/kg soil) thereby mostly reduced leaf and root growth of *Lupinus albus* plants and increased at least relatively dry matter allocation into the shoot fraction leading to a rising shoot to root-ratio.

Dang *et al.* (1990) treated onion (*Allium cepa*) and fenugreek (*Trigonella fonum-graecum*) plants with Cd at 0, 50, 100, 200, 400 mg/kg soil, and found that there was a slight decrease in the yield of both crops at 50 mg/kg Cd. At 100 mg /kg Cd and above, the decrease was significant; no growth was observed at 400 mg /kg Cd.

Salim *et al*. (1992) treated *Vicia faba* with Cd at the rates of 1.8-12.5 ppm as foliar application, and 3.1-12.5 ppm as root drench. The results indicated that foliar application affected the growth more adversely than the root application.

Aidid and Okamoto (1993) on *Impatiens balsamina*, concluded that the growth elongation rate of stem cells was inhibited by the application of heavy metals such as Cd^{2+}, and that this may be due to their suppression of cell wall extensibility. They added that the major rate-limiting factor for cell elongation growth was the cell wall extensibility. Furthermore, Cd^{2+} was found to be more toxic than other heavy elements such as Cr, Cu, Mn, Ni, Pb and Zn.

According to **Wagner (1993),** Cd concentration in the soil solution of uncontaminated soils is in the range of 0.04- 0.32 µM Cd, while moderately polluted soils contain 0.32-1.00 µM Cd. Taken up in excess by crop plants, Cd directly or indirectly inhibits physiological processes, such as respiration, photosynthesis, cell elongation, plant water relationships, nitrogen metabolism and mineral nutrition, resulting in poor growth and low biomass.

In addition, **Jalil *et al.* (1994)** reported that treatment of wheat plants with 0.1 and 0.5 Cd µM in sand culture decreased shoot and root biomass, root length and leaf area.

Prasad (1995) studied the toxic symptoms of Cd in plants, and also the Cd tolerance manifestations being evolved by plants. Excess Cd causes a number of toxic symptoms in plants, e.g. growth retardation, inhibition of photosynthesis, induction and inhibition of enzymes, altered stomatal action, changes in the water relations, efflux of cations and generation of free radicals. Plants must therefore attempt to adapt themselves to environments contaminated with excess Cd. Some of the prevalent mechanisms of Cd-tolerance are: accumulation, sequestration, synthesis of Cd-binding complexes (phytochelatins) and their stabilization by sulphide ions, damage rescue by heatshock proteins and phytochelatin constituting organics. Identification of Cd ligands present in cytosol and vascular tissue emerges as one of the priority areas of investigation.

Varennes *et al.* (1996) studied the effect of Cd on the growth of *Alyssum pintodasilvae*. The results showed that Cd application at 5, 10, 15, 20, 25 mgl^{-1} (as cadmium carbonate) to the media led to a significant decrease in dry matter yield.

Zheljazkov and Nielsen (1996) studied the effect of Cd on *Mentha piperita* and *Mentha arvensis*. They found that, when cadmium was incorporated into the soil as an aqueous solution of cadmium chloride ($CdCl_2$), in doses of 30 and 40 mg Cd kg^{-1} of soil, the yields of fresh herbages were reduced by 9-16%.

Aery and Jagetiya (1997) tested the relative toxicity of Cd on the growth performance of barley (*Hordeum vulgare* L.). They found that application of Cd as cadmium sulphate ($CdSO_4$) at 0.5 and 1mg/ kg soil resulted in a significant decrease in growth over the controls, with the roots showing a greater decrease in dry matter accumulation than the shoots.

Michalska *et al.* (1998) studied the effect of cadmium (300 or 600 ppm) on different cultivars of lettuce grown in a nutrient solution, and noticed visible symptoms of toxicity in some cultivars. The analysis of variance of fresh and dry weights indicated that cadmium reduced plant growth.

Taleva and Djonova (1998) tested different rates of Cd (0, 5, 10, 20 and 40 mg/ kg soil) added as $CdCl_2$ to *Allium cepa* plants. They found that increasing rate of Cd than 5 mg/kg decreased the plant development.

Zayed *et al.* (1998) investigated the Cd accumulation potential in duckweed (*Lemna minor* L.) by supplying the plants with different