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Mitigating the Harmful Effect of Cadmium on *Eucalyptus gomphocephala* by Using Mycorrhiza Fungi

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This study was carried out at the experimental field of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, during the two successive seasons of 2014 and 2015. The present study investigated possible role of mycorrhiza fungi in alleviating cadmium toxicity of *Eucalyptus gomphocephala* plants. The plants were cultivated in 30 cm plastic pots and filled with mix of clay and sand (1:1 by volume) and treated with cadmium chloride ($CdCl_2.H_2O$) as soil drench at concentrations (0, 50, 100 and 200 ppm), and/or mycorrhiza fungi which was added at two rates (5 and 10 g/pot). The results revealed that cadmium-stressed *Eucalyptus gomphocephala* plants treated with mycorrhiza fungi had increased levels of photosynthetic pigments (chlorophyll a, b and carotenoids) and total hydrolysable carbohydrate as compared with the control plants in the two seasons, and stimulated significantly some of the growth characteristics (plant height, number of leaves/plant, and fresh and dry weights of leaves) as compared to Cd-stressed and the control plants in the two seasons. The results of the present investigation clearly manifested that the addition of the mycorrhiza fungi (10 g/pot), to *Eucalyptus gomphocephala* plants grown in soil contaminated by cadmium up to 200 ppm, boosted plants to overcome or even reduce cadmium toxicity and thus obtained relatively better vegetative growth, as well as better chemical constituent.

Keywords: *Eucalyptus gomphocephala*, cadmium chloride, mycorrhiza fungi.

INTRODUCTION

Eucalyptus gomphocephala family Myrtaceae (Brooker and Kleingi, 2004). Heavy metals are significant environmental pollutants; contamination of soil with heavy metals is an increasing problem globally (Jalbani et al. 2009).

Cadmium is among the heavy metal contaminants and is considered as a major environmental concern to the agriculture systems as its resides in soils for over thousands of years (Sanita di Toppi and Gabbrielli, 1999). Cadmium is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water. It can accumulate to toxic levels in the tissues of plants used for human and animal nutrition (Valerie and Feller, 2005). Cd is a non essential metal, and toxic to many plants species at low concentrations (Schutzendubel and Polle, 2002). Although it has unknown physiological functions, plant cannot prevent its uptake since it occurs via metal transports required for essential elements (Verbruggen et al. 2009).

Cadmium is readily taken up by the cells of different plant species (Liu et al. 2007) and induces many morphological, physiological, biochemical and structural changes in plants, such as water imbalance, inhibition of seed germination, inhibition in photosynthesis, reduction of growth especially the root growth, disturbances in mineral nutrition, and sugar metabolism and therefore, strongly influences biomass production (Sanita di Toppi and Gabbrielli 1999, Moussa 2004) and finally can cause plant death (Kahle 1993). Harmful effects produced by Cd^{2+} might be explained by its ability to inactivate enzymes possibly through reaction with the SH-groups of proteins (Gouia et al. 2004). Cd decreases photosynthetic rate due to reduced chlorophyll content and the enzymatic activity involved in CO^2 fixation (Greger and Ögren 1991).

The mycorrhizal relationship benefits both the host plant and the fungi. The fungi increase the plant's ability to take up phosphorus and micronutrients from the soil and provide protection from certain root diseases, and they also improve the plant's ability to access water from the surrounding soil, which helps keep the plant hydrated in dry soil conditions (Kheyrodin, 2014). The arbuscular mycorrhizal symbiosis belongs to the strategies plants have developed to cope with adverse environmental conditions including contamination by heavy metals such as cadmium (Aloui et al. 2009).

The current study focuses on testing the effect of mycorrhiza fungi on some growth characteristics (plant height, number of leaves/plant, and fresh &dry weights of leaves), photosynthetic pigments (chlorophyll a, b and carotenoids) and total hydrolysable sugar of *Eucalyptus gomphocephala* in soil polluted by cadmium.

MATERIALS AND METHODS

The present study was carried out at the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt, during the two successive seasons of 2014 and 2015. Mycorrhiza was obtained from Microbiology Department, National Research centre. Uniformed seedlings of *Eucalyptus gomphocephala* (10–15 leaves and height 25–30 cm) were obtained from the nursery of Forestry Department, Horticulture Research Institute, Agriculture Research Centre. The seedlings were transplanted individually on March, 2014 and 2015 in 30 cm plastic pots, filled with the mixture of clay and sand (1:1 by volume). The physical and chemical properties are shown in Table (1), using the methods described by Jackson (1973). In both seasons, the established plants were treated with Cd as cadmium chloride ($CdCl_2.H_2O$), added as a soil drench after two weeks from transplanting at the four different concentrations Cd (0, 50, 100 and 200ppm), and supplied separately and/or with three different rates of mycorrhiza fungi (0, 5 and 10g/pot). The mycorrhiza fungi were mixed with the media before transplanting and covered with sand. All the plants were held under the open field condition for 9 months continuously. Common cultural practices were followed including regular watering, hand picking of weeds, as well as the available commercial fertilizer used through the two experiments work was the fertilizer kirstalon (NPK 19:19:19) at 3.0 g/pot in monthly intervals after 30 days from transplanting.

The experiment was a complete randomized design, with 12 treatments. At the end of the experiment on November 15th in seasons 2014 and 2015, the following data was recorded: plant height, number of leaves/plant, leaves fresh and dry weight. Photosynthetic pigments (chlorophyll a, b and carotenoids) contents were determined in fresh leaves according to Saric et al. (1967). Total hydrolyzable sugar percentage was determined from the dry weight of leaves, stems and roots according to Dubois et al. (1956).

Statistical analysis

All previous data were subjected to statistical analysis by using least significant differences (L.S.D) at 5% level according to method described by Snedecor and Cochran (1980).

Table 1. Physical and chemical properties of the soil.

Soil sample	Coarse sand%			Fine sand%			Silt%		Clay%	
Sandy loam	49.23			11.05			21.00		18.72	
	$E.C._{(1:1)}$ (dS/m)	pH	Cd (mg/kg)	Anion (meq/l)			Cation (meq/l)			
				HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^{++}	K^+
	1.52	8.19	0.153	7.20	4.00	4.35	5.85	1.46	7.08	0.99

RESULTS AND DISCUSSION

Growth characteristics

Plant height

The results presented in Table (2) showed that the plant height of *E. gomphocephala* plants were reduced gradually by increasing the concentration of cadmium as compared to the control in both seasons. These results are in agreement with the findings of several researchers who reported that the plant height decreased by increasing cadmium concentration, such as *Ipomoea aquatica* (Bhaduri and Fulekar, 2012), sunflower (Moradkhani et al. 2012), *Prunus dulicis* (Elloumi et al. 2014), *Eucalyptus* spp. (Patil and Umadevi, 2012) and *Tagets erecta* (Eid et al. 2016). Meanwhile, plants treated with mycorrhiza fungi at 5 and 10g/pot stimulated significantly the plant height as compared to the control plants, and also enhanced the plant height of Cd-stressed plants as compared to the Cd-stressed plants without mycorrhiza treatment in both seasons. These results are in agreement with the findings of several researchers who reported that the mycorrhiza fungi improve growth under cadmium stress of *Coriandrum sativum* L. (Haneef et al. 2013) and *Triticum aestivum* (Kanwal et al. 2015).

Number of leaves

The results presented in Table (2) showed that increasing the concentration of cadmium inhibited the number of leaves for *Eucalyptus gomphocephala* plants as compared to the control in both seasons. These results are in agreement with findings of several researchers who reported that the number of leaves decreased with increasing Cd concentration *in Alstonia scolaris* L. (Hashish et al. 2016 b), sunflower (Moradhkani et al. 2012) and *Dalbergia sisso* (Shah et al. 2008). The application of mycorrhiza fungi at 5 and 10g/pot significantly increased the number of leaves as compared control plants, whereas, the plants of *E. gomphocephala* treated with mycorrhiza fungi in presence of cadmium increased the number of leaves as compared to Cd-stressed plants without mycorrhiza treatment in both seasons in both seasons.

Fresh and dry weight of leaves

The data presented in Table (3) showed that in both seasons, raising Cd at the rate from 0 to 200ppm caused significant reduction in the mean values of leaves fresh and dry weights of *E. gomphocephala* , the greatest value of leaves fresh and dry weight produced from plants grown in media without Cd, and the lowest value of leaves fresh and dry weight produced in plants that received Cd at concentration of 200 ppm.These results are in accordance with Moussa and El-Gamal (2010) on wheat plant. Application of mycorrhiza fungi caused an increase in leaves fresh and dry weights, compared with the control. Regarding the effect of interaction between Cd stress and the mycorrhiza addition treatment on leaves fresh and dry weight, it is noticed that mycrorrhiza fungi increased the mean value of leaves fresh and dry weight of *E. gomphocephala* plants which grown in soil contaminated with cadmium at the different rates. The cadmium results could be attributed to its toxicity which causes growth reduction. Cd toxicity has several effects; however, stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways are the most common of those effects. Various Cd concentrations used in this investigation had an effect on the growth of *E. gomphocephala*. *E. gomphocephala*'s growth reduction may have been due to the inhibition of elongation of cells which is because of the inhibition caused by Cd on the proton pump in charge of the process which is an inhibition than cannot reverse (Rahat et al. 2012).

In regards to the effect of mycorrhiza, these results are in agreement with those obtained by the investigators who report a beneficial effect of the inoculation of plants with mycorrhiza with regards to their growth. Growth enhancement appeared to be because of the better absorption of phosphorus by mycorrhizal plants (Hamel and Smith, 1991).

Chemical constituents.

Pigments content

We demonstrated that Cd-induced growth inhibition was accompanied by a significant decrease in the chlorophyll content. The data presented in Table (4) illustrated that in both seasons, raising Cd at the rate from 0 to 200 ppm caused a significant reduction in the photosynthetic pigment (chloroylla, b and carotenoids) in both seasons.

Table 2. Effect of cadmium and/or mycorrhiza (g/pot) on plant height (cm) and number of leaves of *Eucalyptus gomphocephala* plant during 2014 and 2015 seasons.

Cadmium treatment (ppm)	Plant height								Number of leaves/plant							
	1st season				2nd season				1st season				2nd season			
	Mycorrhiza				Mycorrhiza				Mycorrhiza				Mycorrhiza			
	0	5	10	Mean	0	5	10	Mean	0	5	10	Mean	0	5	10	Mean
0.0	131.11	146.23	158.50	145.28	132.41	154.67	165.78	150.95	147.67	164.33	174.00	162.00	159.67	173.67	182.67	172.00
50	116.35	120.67	137.64	124.89	124.67	134.20	148.33	135.73	135.00	143.00	159.67	145.89	127.33	154.00	165.67	149.00
100	110.87	113.33	119.34	114.51	111.50	122.22	129.25	120.99	117.00	130.00	146.33	131.11	110.50	140.33	148.67	133.17
200	95.50	98.14	105.00	99.55	107.78	113.55	119.12	113.48	109.50	115.33	138.33	121.05	104.67	116.00	133.33	118.00
Mean	113.46	119.59	130.12		119.09	131.16	140.62		127.29	138.17	154.58		125.54	146.00	157.59	
LSD (5%)	Cd	1.63				2.31				2.70				3.19		
	M	1.41				1.99				2.34				2.77		
	CdXM	2.312				3.260				3.821				4.521		

Table 3. Effect of cadmium and/or mycorrhiza (g/pot) on fresh and dry weight of leaves (g) of *Eucalyptus gomphocephala* plant during 2014 and 2015 seasons.

Cadmium treatment (ppm)	Fresh weight of leaves								Dry weight of leaves							
	1st season				2nd season				1st season				2nd season			
	Mycorrhiza				Mycorrhiza				Mycorrhiza				Mycorrhiza			
	0	5	10	Mean	0	5	10	Mean	0	5	10	Mean	0	5	10	Mean
0.0	34.35	45.06	52.11	**43.84**	47.20	58.18	61.03	**55.47**	13.51	18.29	21.77	**17.86**	18.61	23.73	25.03	**22.46**
50	24.41	30.45	43.1	**32.65**	34.98	45.97	52.92	**44.62**	9.35	11.88	17.34	**12.86**	13.40	17.98	21.06	**17.48**
100	15.19	23.08	33.31	**23.86**	23.35	39.01	37.75	**33.37**	5.77	8.84	13.00	**9.20**	8.87	15.17	14.59	**12.88**
200	11.61	12.41	27.13	**17.05**	21.90	22.24	30.54	**24.89**	4.40	4.71	10.49	**6.53**	8.28	8.44	11.64	**9.45**
Mean	**21.39**	**27.75**	**38.91**		**31.85**	**41.35**	**45.56**		**8.26**	**10.93**	**15.65**		**12.29**	**16.33**	**18.08**	
LSD (5%)	**Cd**	**2.83**				**2.51**				**1.47**				**1.81**		
	M	**2.45**				**2.17**				**1.27**				**1.57**		
	CdXM	**4.059**				**3.549**				**2.079**				**2.561**		

Table 4. Effect of cadmium and/or mycorrhiza (g/pot) on chlorophyll *a, b* and carotenoids (mg/gFW) of *Eucalyptus gomphocephala* plant during 2014 and 2015 seasons.

Cadmium treatment (ppm)	Chlorophyll *a*				Chlorophyll *b*				Carotenoids			
	Mycorrhiza (g/ pot)											
	0	**5**	**10**	**Mean**	**0**	**5**	**10**	**Mean**	**0**	**5**	**10**	**Mean**
	1st season											
0.0	1.06	1.17	1.2	1.14	0.35	0.40	0.42	0.39	0.69	0.84	0.87	0.80
50	0.91	1.09	1.10	1.03	0.27	0.35	0.36	0.33	0.48	0.73	0.76	0.66
100	0.81	0.95	0.98	0.91	0.25	0.31	0.33	0.30	0.34	0.55	0.58	0.49
200	0.79	0.83	0.86	0.83	0.22	0.24	0.26	0.24	0.32	0.40	0.43	0.38
Mean	**0.89**	**1.01**	**1.04**		**0.27**	**0.33**	**0.34**		**0.46**	**0.63**	**0.66**	
LSD (5%)	Cd	0.08				0.04				0.06		
	M	0.07				0.03				0.05		
	CdXM	0.116				0.50				0.091		
	2nd season											
0.0	1.12	1.24	1.28	**1.21**	0.37	0.43	0.44	**0.41**	0.77	0.91	0.96	**0.88**
50	0.98	1.18	1.20	**1.12**	0.31	0.39	0.41	**0.37**	0.62	0.83	0.86	**0.77**
100	0.90	1.05	1.08	**1.01**	0.28	0.32	0.34	**0.31**	0.53	0.71	0.73	**0.66**
200	0.84	0.93	0.95	**0.91**	0.25	0.28	0.29	**0.27**	0.45	0.55	0.58	**0.53**
Mean	**0.96**	**1.1**	**1.13**		**0.30**	**0.36**	**0.37**		**0.59**	**0.75**	**0.78**	
LSD (5%)	**Cd**	**0.10**				**0.03**				**0.04**		
	M	**0.08**				**0.03**				**0.03**		
	CdXM	**0.143**				**0.048**				**0.051**		

Table 5. Effect of cadmium and/or mycorrhiza (g/pot) on total hydrolyzable carbohydrate (%) in leaves, stem and root of *Eucalyptus gomphocephala* plant during 2014 and 2015 seasons.

Cadmium treatment (ppm)	Leaves				Stem				Root			
	Mycorrhiza (g/ pot)											
	0	5	10	Mean	0	5	10	Mean	0	5	10	Mean
	1st season											
0.0	15.03	20.00	22.91	**19.31**	13.95	18.44	20.30	**17.56**	12.41	15.41	17.63	**15.15**
50	15.52	15.98	18.68	**16.73**	14.32	15.23	17.05	**15.53**	12.89	13.22	15.25	**13.79**
100	12.10	12.35	14.46	**12.97**	10.10	10.69	13.04	**11.28**	9.49	9.98	11.95	**10.47**
200	9.27	9.81	11.41	**10.16**	7.65	8.00	9.64	**8.43**	5.77	6.04	8.23	**6.68**
Mean	**12.98**	**14.54**	**16.87**		**11.51**	**13.09**	**15.01**		**10.14**	**11.16**	**13.27**	
LSD (5%)	Cd	1.74				1.84				1.93		
	M	1.51				1.59				1.67		
	CdXM	2.461				2.599				2.724		
	2nd season											
0.0	18.70	23.50	25.81	**22.67**	17.88	21.80	22.98	**20.89**	15.11	18.94	20.81	**18.29**
50	19.17	19.38	22.83	**20.46**	18.42	18.90	21.20	**19.51**	16.05	17.31	18.42	**17.26**
100	14.85	16.67	18.21	**16.58**	14.42	15.69	17.69	**15.93**	12.42	13.17	14.85	**13.48**
200	11.52	12.04	14.13	**12.56**	9.87	11.6	13.90	**11.79**	8.40	10.22	12.23	**10.28**
Mean	**16.06**	**17.90**	**20.25**		**15.15**	**17.00**	**18.94**		**12.99**	**14.91**	**16.58**	
LSD (5%)	Cd	1.66				1.82				2.62		
	M	1.44				1.58				2.27		
	CdXM	2.350				2.577				3.700		

Cd-induced inhibition of photosynthesis has also been attributed to an inhibition of the activity of key enzymes of the Calvin cycle and the photosynthetic electron transport chain (Nwugo and Huerta, 2008), and inhibition occurred at the uptake level or in translocation of nutrients. Cadmium may interfere with the nutrient uptake altering the plasma membrane permeability, leakage of nutrients through plasma membrane (Obata and Umebayashi, 1997) and affect the element-transport processes across the membrane (Gussarson and Jensen, 1992). Our results were corroborated with the findings of some researchers in plants of *Dalbergia sissoo* (Shah et al. 2008), wheat (Moussa and El-Gamal, 2010) and *Brassica juncea* (John et al. 2009). Also, Cd-stressed plants treated with mycorrhiza fungi increased significantly the photosynthetic pigment (chloroyll a, b and carotenoids) in both seasons as compared to the control plants. Most investigators believe that mycorrhiza symbiosis raises the number of photosynthesis and enhances the storage and export rates of photosynthesis at the same time (Auge, 2001).

The growth inhibition produced by Cd could be at least partially due to the effect of this heavy metal on the photosynthesis rate (Metwally et al*.* 2003). Cd stress decreased the rate of CO_2 assimilation, photosynthetic efficiency and a degradation of chlorophyll as well as an inhibition of their biosynthesis, which could result in disturbances in the electron transport rates of PS 1 and PS 2, leading to the generation of oxygen free radicals (Moussa 2004).

Total hydrolysable carbohydrate (%)

The data presented in Table (5) revealed that, total hydrolyzable **carbohydrate** percentage increased at low concentrations of Cd and decreased at high concentration of Cd (John et al. 2008) on *Lemna polyrrhiza* L and (Hashish et al.2016) on *Dalbergia sissoo.* The total hydrolyzable carbohydrate percentage in each organ of *E. gomphocephala* plant increased with increasing mycorrhiza rate separated or with Cd treatments as compared to Cd-stressed plants and the control plants at the two seasons. The results are in agreement with (Datta and Kulkarni, 2014) on *Acacia arabica* and (Khalil and El-Noemani, 2015) on *Origanum vulgare*.

CONCLUSION

It is concluded that, the results of the present investigation clearly manifested that the addition of the mycorrhiza fungi (10 g/pot), to *Eucalyptus gomphocephala* plants grown in soil contaminated by cadmium up to 200 ppm, boosted plants to overcome or even reduce cadmium toxicity and thus obtained relatively better growth, better quality and yield, as well as better chemical composition.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest".

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AUTHOR CONTRIBUTIONS

MAD and AAMM designed and carried out the experiment. SME carried out the laboratory analysis. NGA and MHM conducted the data analysis and wrote the manuscript. NTS reviewed the manuscript. All authors read and approved the final version.

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