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Investigation the Effect of L-Tryptophan on Growth and Chemical Composition of *Eucalyptus gomphocephala* Plants under Cadmium Stress

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ABSTRACT

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 clarifies the effect of amino acid L-Tryptophan (L-Trp) in reducing cadmium toxicity on *Eucalyptus gomphocephala* plants. The plants were cultivated in 30 cm plastic pots, 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 L-Trp which was added at two concentrations (100 and 200 ppm). It was noticed from the data that lowest mean value of all vegetative growth parameters (plant height, leaf area, root length, and dry weight for leaves, stems and roots), as well as pigments content (chlorophyll a, b and carotenoids) in leaves, carbohydrates content in all plant organs, and peroxidase isozymes were obtained from plants exposed to high concentration of Cd (200ppm), but the same Cd concentration led to increasing cadmium content in all whole plant, proline content and catalase isozyme in leave. It is noticed also that vegetative characteristics were significantly affected by L-Trp at the concentration of 100ppm alone or combined with cadmium at all concentration except root length and dry weight of roots in the first season were increased with L-Trp at 200ppm. Photosynthetic pigments, carbohydrate content in all plant organs and proline content increased in plants treated with L-Trp at 100ppm alone or combined with cadmium, but cadmium content in all plant organs increased in plants treated with L-trp at 200ppm alone or combined with cadmium at 100ppm. Isozymes peroxidase and catalase increased in plants treated with cadmium 50+ L-Trp 100 as compared to plants treated with cadmium only without L-Trp.

Keywords: *Eucalyptus gomphocephala*, Tuart, cadmium , heavy metal, L-Tryptophan, amino acid.

Introduction

Plants require different metals as micronutrients, these are part of soil. Biosphere has become polluted due to toxic metals (Saba *et al*., 2013). Among the hazardous metals, cadmium (Cd) is of particular concern because of its high toxicity to living organisms; Cd has no known biological function but shows high mobility in the soil leading to Cd accumulation by plants and thus in the entire food chain (Yang *et al*., 1996). Heavy metals are added to soil from the metal working industries, waste incinerators, urban traffic, cement factories, and as a by product of phosphate fertilizers (Pál *et al*., 2006). The main sources of Cd in the environment are industrial processes and phosphate fertilizers (Wagner, 1993). Cadmium is particularly a hazardous pollutant due to its high toxicity and great solubility in water (Gülser and Sönmez, 2012) and it is invariably concentrated in the organic surface horizons of soils (Alloway, 1995). The presence of heavy metals in excess amounts is a global problem, threatening the health of vegetation, wildlife and humans (Heckathorn, 2004).

Eucalyptus gomphocephala A. DC. family Myrtaceae, Native to the southwest of Western Australia. Its common name is "tuart". Tuart is a tall tree, reaching up to 42 m in height and having a diameter of over 2–3m, the trunk is short, often no more than half the tree's height, and is frequently forked. The tree is characterized by fibrous, pale – gray bark, with thick, shiny leaves, it has both a

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deep taproot and extensive surface roots, and it therefore exploits booth deep groundwater and surface water.

Material and Methods

This 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. Uniformal seedlings of *Eucalyptus gomphocephala* (10 - 15 leaves and 25 –30 cm length) were obtained from nursery of woody trees, Agriculture Research Centre.

Experiment procedure:

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 (0, 50, 100 and 200ppm), and supplied separately and/or with three different concentration of L-tryptophan (L-Trp) (0, 100 and 200ppm).

The experiment was a complete randomized block design. At the end of the experiment on November 15th in seasons 2014 and 2015, the following data was recorded: plant height, leaf area, root length and dry weight for leaves, stems and roots. Photosynthetic pigments content (chlorophyll a, b and carotenoids) was determined in fresh leaves according to Saric *et al*. (1967). Carbohydrates (%) was determined from the dry weight of leaves, stems and roots according to Dubois *et al.* (1956). Content of free proline was determined in fresh leaves according to the method described by Bates *et al*., (1973). Cadmium content (ppm) was measured in the suspension using Atomic Absorption Spectrophotometer (PerkinElmer 100 B), as described by Meuwly and Rauser (1992). Antioxidant Isozymes including Peroxidase isozyme (POD) according to Brown (1978) and Catalase isozyme (CAT) according to Woodbury *et al*. (1971).

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

Vegetative growth:

Results pertaining to the effect of cadmium at the different concentrations on plant height, leaf area root length, dry weight of leaves, stems and roots are presented in tables (2&3) the data showed that in both seasons increasing cadmium concentration in the soil from 0 to 200ppm caused significant reduction in all vegetative parameters of *Eucalyptus gomphocephala*. Our results are in agreement with those obtained by Shah *et al.* (2008) on *Dalbergia sisso*, Fan *et al.* (2011) on *Swietenia macrophylla*, Gogorcena *et al*. (2011) on *Quercus suber* and Pandey and Tripathi (2011) on *Albizia procera.* The reduction of vegetative parameters could be attributed to toxic Cd levels, which induced negative effects on some key metabolic processes coupled to growth in the plant (Van Assche *et al*., 1984). The impact of Cd uptake by living cells has been shown to be drastic, normally leading to cell death depending on metal dose and time-length of exposure (Vitória *et al*., 2001).

The presented data showed that the application of L-Trp treatment significantly increased vegetative growth parameters

The exogenous application of L-Trp showed an increase in values of all vegetative growth parameters and this increase was clearer in plants which were sprayed with L-Trp at 100ppm, in both seasons, except root length in both seasons and dry weight of root in the first season which were increased when the plants were treated with L-Trp at the concentration of 200ppm. These results similar to those obtained by Abou Dahab and Abd El-Aziz (2006) on *Philodendron erubescens*, Abd El-Aziz *et al*. (2009) on *Antirrhinum majus* and Abd El-Aziz *et al.* (2010) on *Thuja orientalis.* It may be due to L-Tryptophan being known to be a physiological precursor of auxins in higher plants (Zahir *et al*., 1999).

Regarding the effect of interaction between Cd levels and L-Trp treatments, the results indicated that, the *Eucalyptus gomphocephala* plants grown in soil polluted with Cd at concentrations (0, 50, 100 and 200ppm) and treated with L-Trp at the concentration of 100ppm gave the highest values of all vegetative growth parameters as compared to plants grown in soil polluted with Cd at the same concentrations but treated with L-Trp at the concentration of 200ppm except root length in both seasons and dry weight of root in the first season which increased when the plants were treated with L-Trp at the concentration of 200ppm

Table 2: Effect of cadmium and/or L-tryptophan on plant height, leaf area and root length (cm) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

Treatments		**Plant height**		**Leaf area**		**Root length**	
		1st	**2nd**	**1st**	**2nd**	**1st**	**2nd**
Cd0		148.08	154.76	23.70	26.67	59.48	63.83
Cd50		127.45	139.86	19.06	21.25	48.71	45.52
Cd100		116.92	126.17	14.84	15.21	35.71	36.22
Cd200		103.40	114.76	10.86	13.60	25.68	26.74
T0		113.46	119.09	14.44	16.84	33.74	33.45
T100		134.83	147.46	19.94	22.14	42.83	45.63
T200		123.59	135.11	16.97	18.57	50.62	50.16
Cd0+T0		131.11	132.41	20.59	24.90	48.77	51.23
Cd0+T100		162.67	170.65	26.52	29.91	61.67	68.57
Cd0+T200		150.45	161.22	24.00	25.21	68.00	71.68
Cd50+T0		116.35	124.67	16.00	18.81	39.68	36.69
Cd50+T100		140.00	157.19	23.00	24.68	50.30	47.63
Cd50+T200		126.00	137.72	18.18	20.25	56.16	52.24
Cd100+T0		110.87	111.50	12.12	13.50	26.43	24.25
Cd100+T100		124.97	142.00	17.12	17.16	34.69	38.72
Cd100+T200		114.92	125.00	15.27	14.98	46.00	45.70
Cd200+T0		95.50	107.78	9.04	10.15	20.07	21.62
Cd200+T100		111.69	120.00	13.10	16.79	24.65	27.59
Cd200+T200		103.00	116.50	10.44	13.85	32.33	31.00
LSD at (5%)	**Cd**	**1.76**	**2.33**	**2.22**	**2.17**	**3.25**	**2.18**
	T	**1.52**	**2.01**	**1.92**	**1.88**	**2.82**	**1.89**
	Cd X T	**2.48**	**3.28**	**3.15**	**3.08**	**4.60**	**3.09**

Chemical constituents:

Pigments and carbohydrates content:

Data shown in Figs (1-6) illustrate that the heavy metal Cd has a negative effect on pigments content in leaves (chlorophyll a, b and carotenoid (mg. g^{-1} F.W.) and carbohydrates (%) in all plant parts. The highest content of pigments and carbohydrates was obtained from plants grown in soil free of cadmium followed by plants exposed to Cd at concentration of 50ppm, the lowest content of pigments and carbohydrates were found in the plants exposed to Cd at concentration of 200ppm. These results were in agreement with the findings of several researchers, such as Tandon and Srivastava (2014) on sugar beet plant and Mohamed (2015) on *Tagetes erecta* plant.

Plants of *Eucalyptus gomphocephala* which were treated with L-Trp treatments gave the highest values of pigments content in leaves and carbohydrate content in whole plant as compared with untreated plants.

Table 3: Effect of cadmium and/or L-tryptophan on dry weight of leaves, stems and roots of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

Treatments		Leaves dry weight		Stems dry weight		Roots dry weight	
		1st	2nd	1st	2nd	1st	2nd
Cd0		19.33	24.78	23.59	27.13	14.19	17.01
Cd50		14.75	19.57	19.5	21.27	9.20	13.04
Cd100		10.33	15.14	15.14	16.10	6.95	8.59
Cd200		6.30	10.49	11.13	11.18	5.38	5.62
T0		8.26	12.29	11.36	14.42	5.5	7.03
T100		16.31	20.4	21.83	22.81	10.19	14.19
T200		13.47	19.79	18.83	19.54	11.1	11.97
Cd0+T0		13.51	18.61	17.53	22	6.95	10.93
Cd0+T100		23.49	28.33	27.65	30.94	16.76	21.66
Cd0+T200		21	27.39	25.58	28.45	18.85	18.44
Cd50+T0		9.35	13.4	10.99	15.71	5.87	7.85
Cd50+T100		19.66	22.79	25.99	25.52	10.77	17.59
Cd50+T200		15.23	22.51	21.52	22.58	10.97	13.67
Cd100+T0		5.77	8.87	9.5	10.93	4.83	5
Cd100+T100		14.42	19.95	19.56	20.71	7.76	10.68
Cd100+T200		10.8	16.59	16.35	16.67	8.25	10.08
Cd200+T0		4.4	8.28	7.42	9.05	4.36	4.32
Cd200+T100		7.66	10.52	14.11	14.05	5.47	6.85
Cd200+T200		6.85	12.67	11.86	10.45	6.31	5.68
LSD at (5%)	**Cd**	1.63	1.68	1.79	1.75	2.25	2.19
	T	1.41	1.45	1.55	1.51	1.82	1.89
	Cd X T	2.29	2.37	2.53	2.47	3.60	3.08

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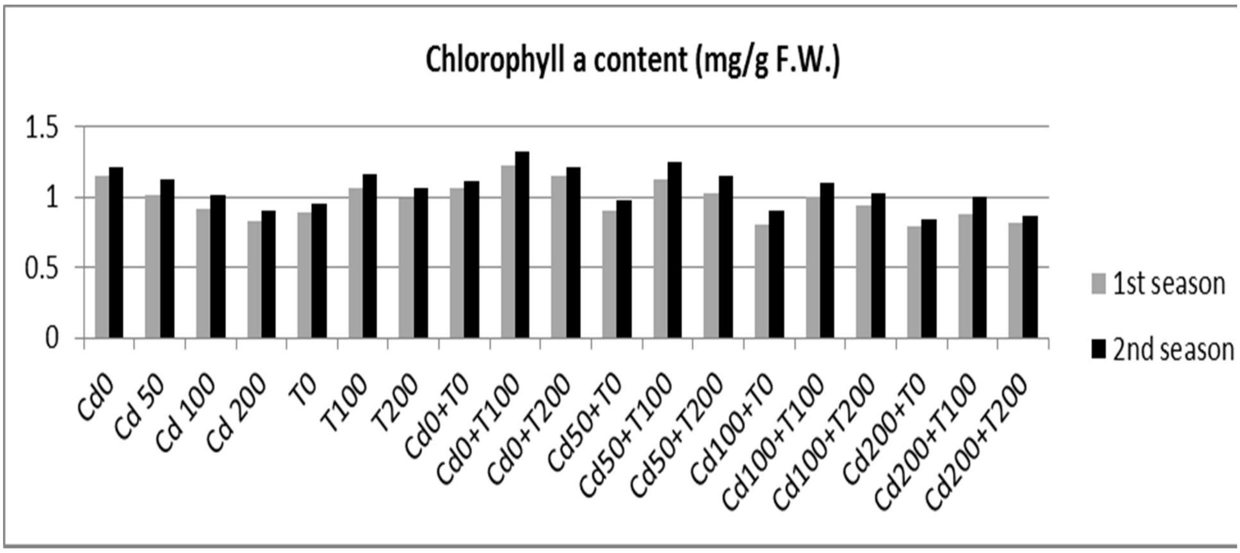

Fig. 1: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves chlorophyll a content (mg/g F.W.) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

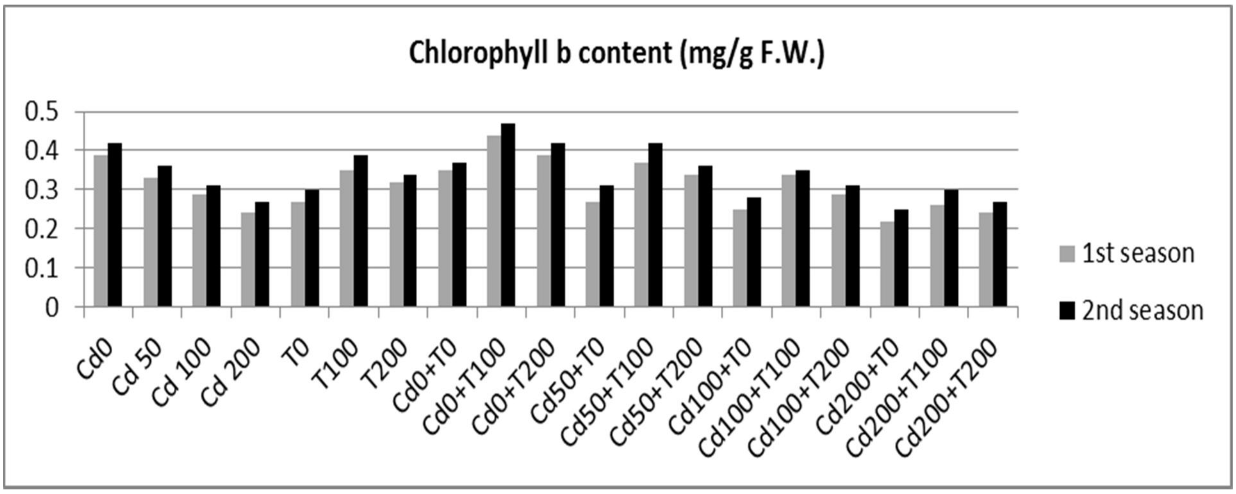

Fig. 2: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves chlorophyll b content (mg/g F.W.) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

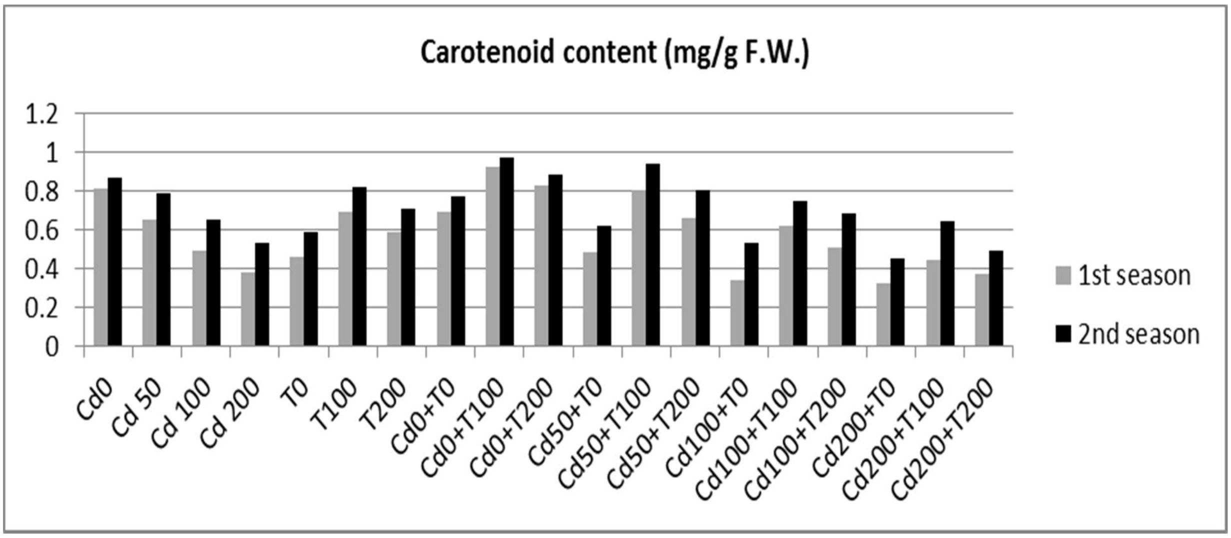

Fig. 3: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves carotenoids content (mg/g F.W.) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

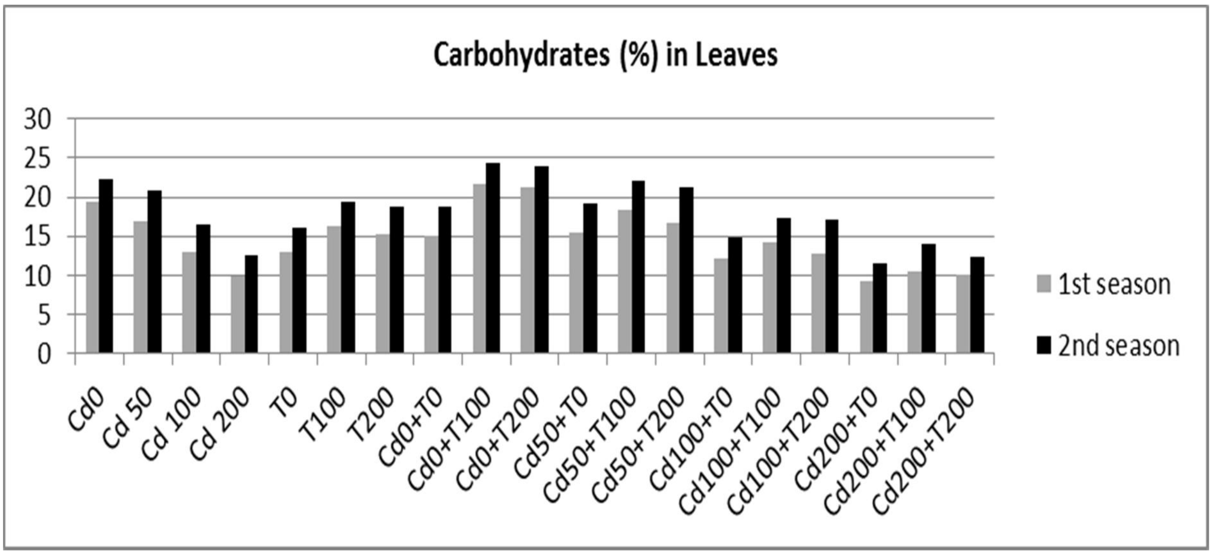

Fig. 4: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves carbohydrates (%) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons.

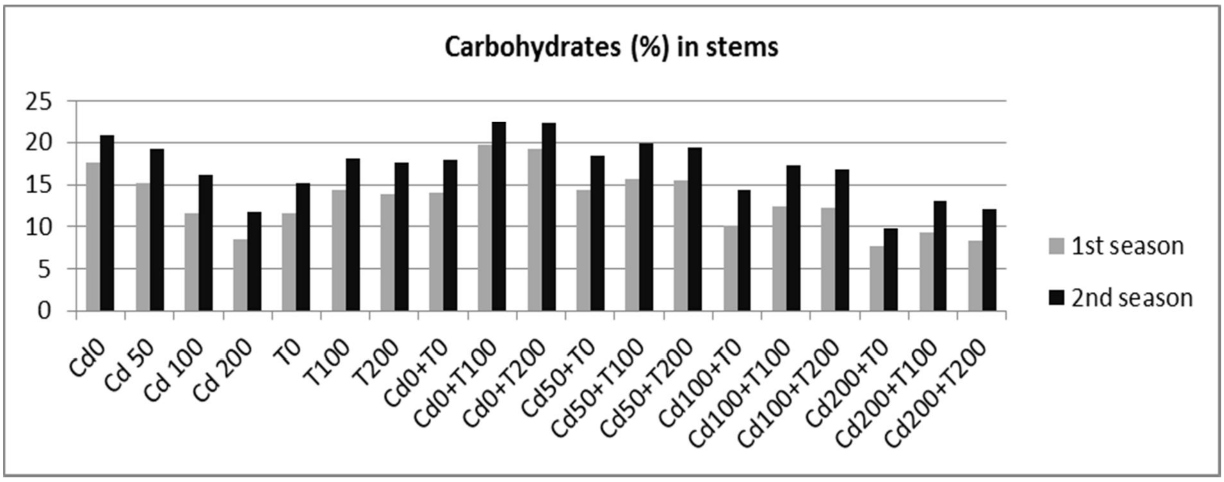

Fig. 5: Effect of cadmium (Cd) and/or L-Tryptophan (T) on stems carbohydrates (%) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

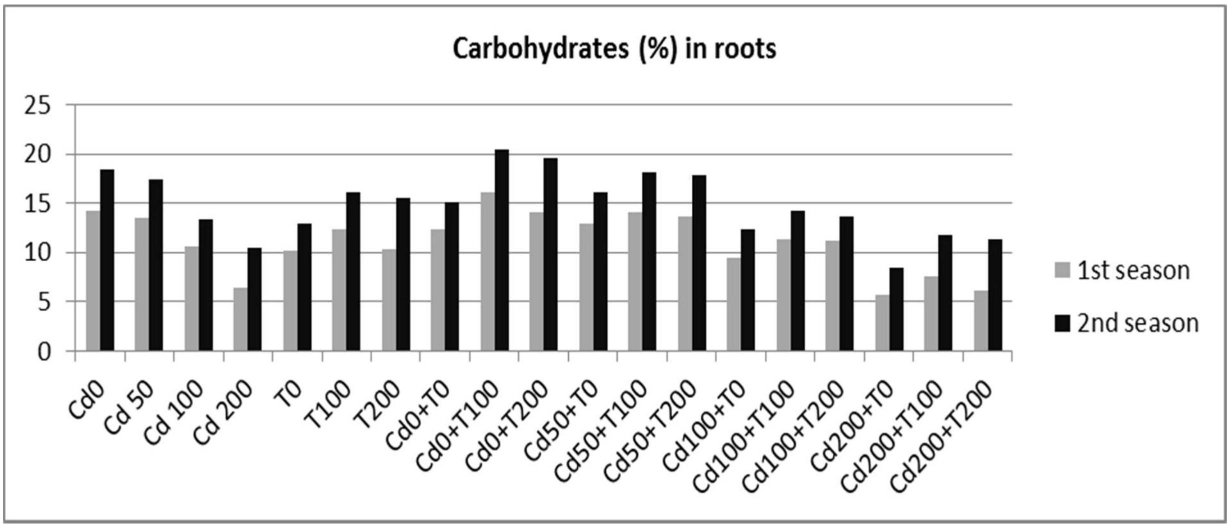

Fig. 6: Effect of cadmium (Cd) and/or L-Tryptophan (T) on roots carbohydrates (%) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

The highest value was obtained from plants treated with L-Trp at concentration 100ppm; these results are in agreement with Abou Dahab and Abd El-Aziz (2006) on *Philodendron erubescens* and Abd El-Aziz *et al.* (2009) on *Antirrhinum majus.*

Concerning the application of L-Trp at different concentrations on *Eucalyptus gomphocephala* plants grown under Cd stress conditions there was an improvement in pigments and carbohydrate content. It was noticed that the highest content of pigments and carbohydrate under each level of Cd obtained from plants treated with L-Trp at concentration 100ppm as compared with other L-Trp concentration under the same level of Cd.

Cadmium content

The data presented in Figs (7 – 9) showed that the Cd content in all parts of *Eucalyptus gomphocephala* plant was affected by Cd levels in the soil in both seasons. The highest Cd content was obtained from plants which received Cd at concentration 200ppm and decreased gradually by decreasing Cd level in the soil. The lowest content of Cd obtained from control plants.

All L-Trp treatments increased Cd content in all plant parts in both seasons. The treatment of L-Trp at concentration 100ppm gave the highest value of Cd content in all plant parts.

Regarding the effect of interaction of Cd and L-Trp on Cd content in all plant parts of *Eucalyptus gomphocephala* shown in Figs (7 – 9). The highest Cd content was obtained from plants treated with L-Trp at concentration of 100ppm under each concentration of Cd treatment, the highest value was obtained from plants received Cd at concentration 200ppm and sprayed with L-Trp 100ppm.

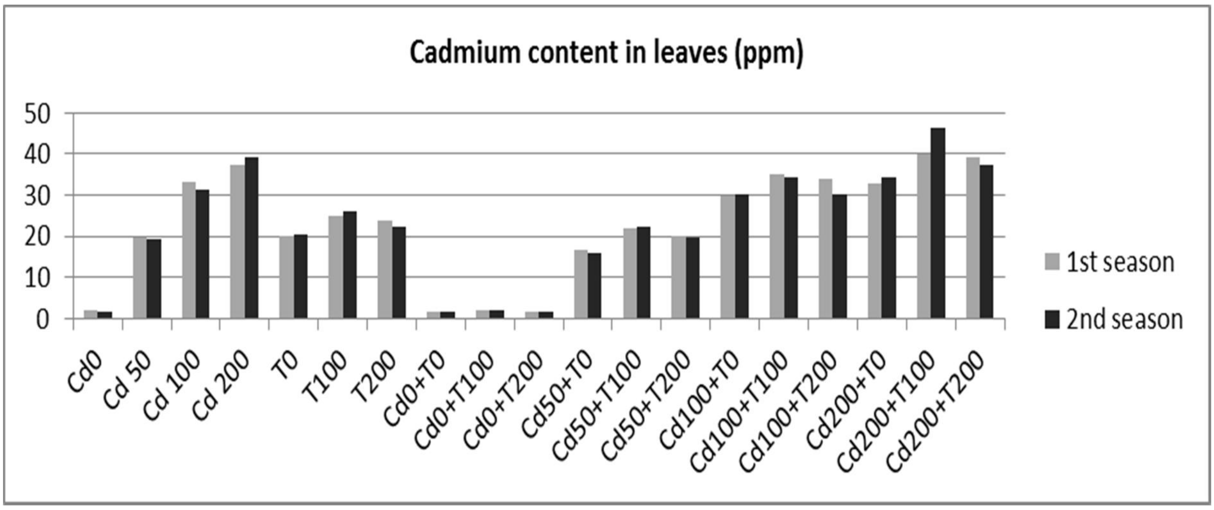

Fig. 7: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves cadmium concentration (ppm) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons.

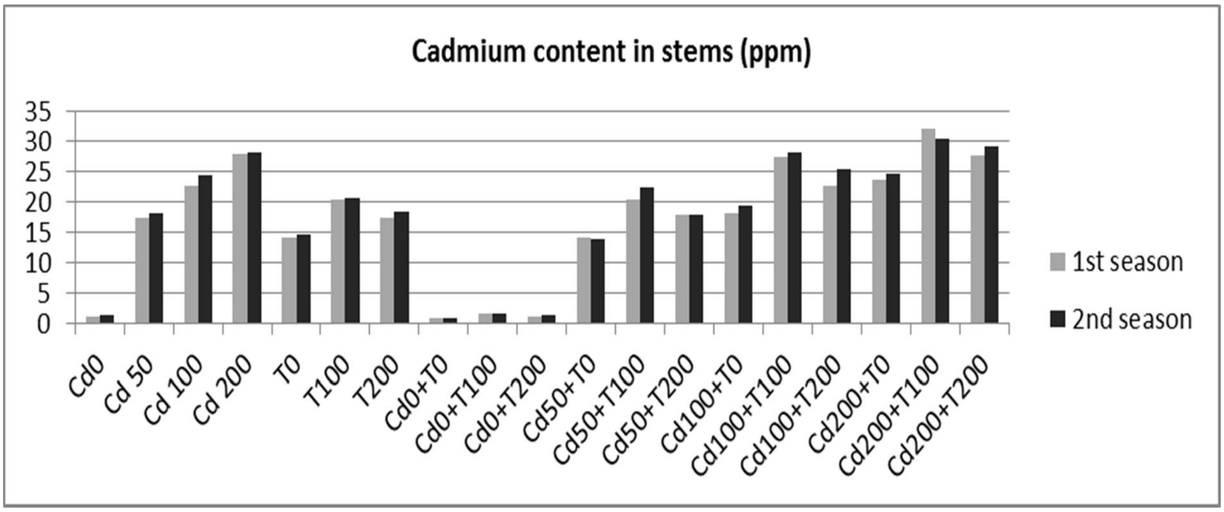

Fig. 8: Effect of cadmium (Cd) and/or L-Tryptophan (T) on stems cadmium concentration (ppm) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons.

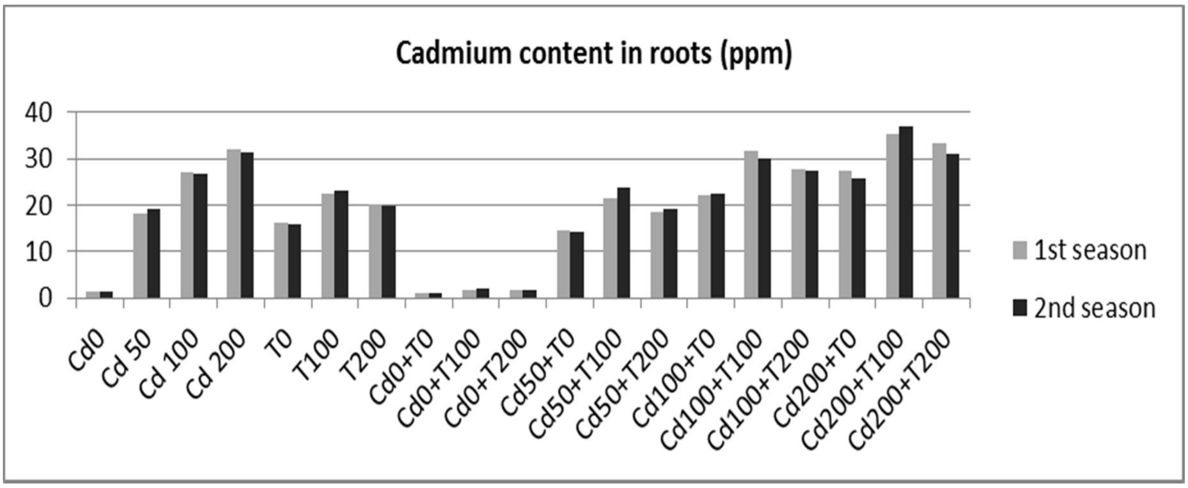

Fig. 9: Effect of cadmium (Cd) and/or L-Tryptophan (T) on roots cadmium concentration (ppm) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

Proline content:

The data presented in Fig (10) showed that proline content in leaves of *Eucalyptus gomphocephala* significantly increased by raising Cd level in the soil to 200ppm as compared with the untreated plants in both seasons. These results are in agreement with the findings of Abdul Qados (2015) on *Acacia saligna*, *Eucalyptus rostrata* and *Conocarpus erecta* and Dezhban *et al*. (2015) on *Robinia pseudoacacia*. It has been often suggested that proline accumulation may contribute to osmotic adjustment at the cellular level (Perez-Alfocea *et al.,* 1993) and stabilizes the structure of macromolecules and organelles. Proline also acts as a major reservoir of energy and nitrogen, which can be used in resuming the growth after the stress removal (Chandrashekar and Sandhyarani, 1996).

The data in Fig. (10) also showed that the proline content in leaves increased in plants treated with L-Trp at concentration 100ppm. While the lowest mean values of proline content in leaves in both seasons was obtained from control plants.

The results recorded in the two seasons also showed that using the different combinations of Cd and L-Trp treatments caused some differences in the proline content in leaves. The highest mean values were obtained from plants grown in soil polluted with Cd at 200ppm combined with L-Trp at 100ppm, followed by plants receiving Cd at the concentration of 100ppm combined with L-Trp at 100ppm. On the other hand, the plants grown in soil free of cadmium and not treated with L-Trp produced the lowest mean values of proline content in both seasons.

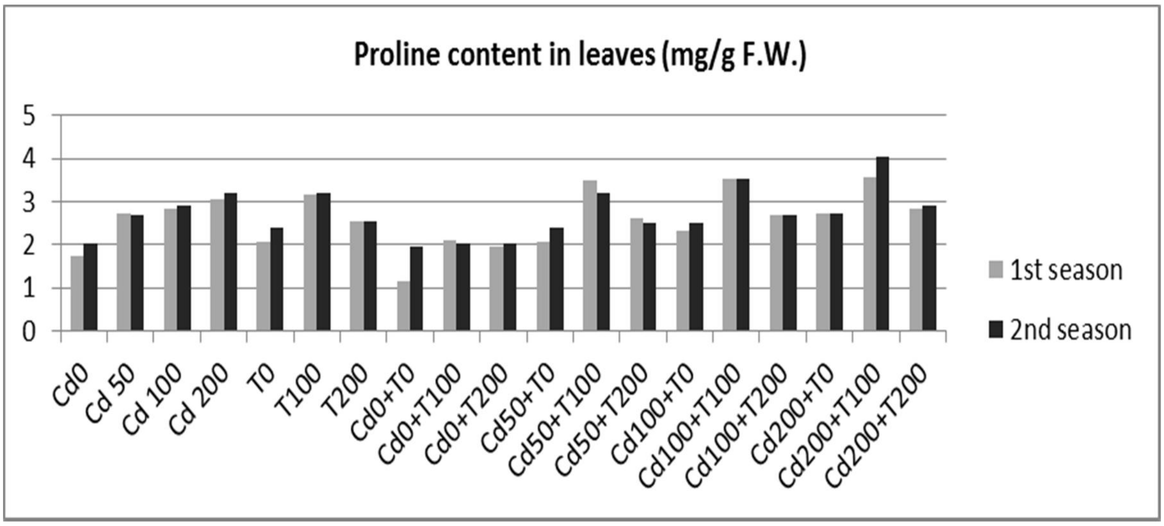

Fig. 10: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves cadmium concentration (ppm) of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

Antioxidant isozyme:

The results illustrated in Fig (11) and Table (4) indicate that the peroxidase isozyme in *Eucalyptus gomphhocephala* plants grown in soil contaminated with cadmium at concentrations (0 - 200ppm) were decreased with raising cadmium concentration in the soil, the lowest value of peroxidase isozyme was recorded at 200ppm. The plants which were grown in soil containing cadmium at 50ppm combined with L-Trp at 100ppm gave the highest value of peroxidase isozyme.

Regarding catalase isozyme, it was found as shown in Fig. (12) and Table (5) that catalase isozyme values increased by increasing cadmium stress condition in the soil. The stressed plants with cadmium at 50ppm and treated with L-Trp at 100ppm gave the highest value of catalase isozyme as compared to cadmium treatment without L-Trp and also control plants. These results were in agreement with the finding of Nickolic *et al.* (2008) on poplar plant and Haribabu and Sudha (2011) on Cleome gynandra.

Fig. 11: Effect of cadmium (Cd) and/or L-Tryptophan (T) peroxidase isozyme of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

Table 4: Ideogram analysis of peroxidase isozyme in *Eucalyptus gomphocephala* plants

R_f	Cd0	Cd50	Cd100	Cd200	Cd50+L-Trp100
0.254	—	—	—	—	+
0.319	+	+	+	+	+
0.424	+	+	+	+	+
0.470	+	+	+	—	++
0.514	+	+	—	—	+

(Cd): Cadmium (T): L-Tryptophan

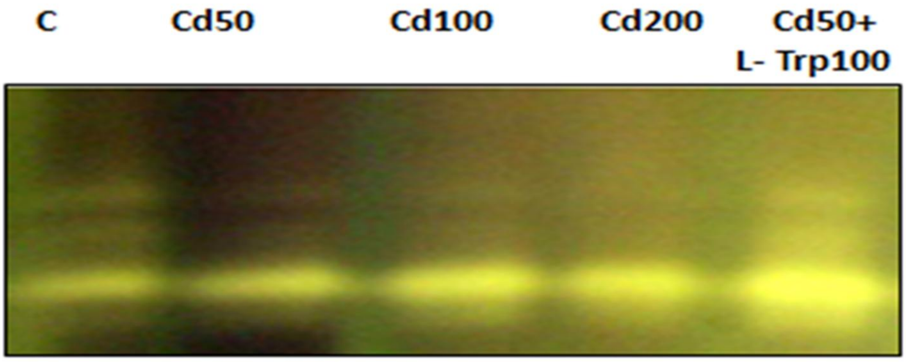

Fig. 12: Effect of cadmium (Cd) and/or L-Tryptophan (T) on catalase isozyme of *Eucalyptus gomphocephala* plants during 2014 and 2015 seasons

Table 5: Ideogram analysis of catalase isozyme in *Eucalyptus gomphocephala* plant

R_f	C	Cd50	Cd100	Cd200	Cd50+L-Trp100
0.756	+	++	+++	+++	++++

(Cd): Cadmium (T): L-Tryptophan

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