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Response of *Khaya senegalensis* plants to growth improvement by L-Tryptophan under cadmium stress condition

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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 *Khaya senegalensis* 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). The results showed that *K. senegalensis* plants exposed to cadmium gave lowest value of all vegetative growth (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. On the other hand the cadmium content in all plant organs, proline content and catalase isozyme in leaves increased by increasing cadmium concentration in the soil. In addition, it is noticed that vegetative characteristics were significantly affected by L-Trp at the concentration of 200ppm alone or combined with cadmium at all concentration except dry weight of leaves in the first season were increased with L-Trp at 100ppm. 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 concentration in all plant organs increased in plants treated with L-trp at 200ppm alone or combined with cadmium at 200ppm. Isozymes peroxidase and catalase increased in plants treated with cadmium 50+ L-Trp 200 as compared to plants treated with cadmium only without L-Trp.

Keywords: *Khaya senegalensis*, African mahogany, cadmium chloride, heavy metal, L-Tryptophan, amino acid.

Introduction

Khaya senegalensis (Desr.) A. Juss, Common name "dry-zone mahogany, khaya wood, African mahogany", family Meliaceae. Native to Africa. Is a deciduous evergreen tree, 15-30 m high, up to 1 m in diameter, buttresses are not prominent or absent; bark dark grey, with small, thin, reddish-tinged scales; slash dark pink to bright crimson, exuding a red sap.

The pollution of soil and water by heavy metals is due to industrial, agricultural activities and urbanization, and has become a serious problem with great environmental impacts (Carneiro *et al*., 2002). Cadmium is a heavy metal toxic for humans, animals and plants. It is a widespread trace pollutant with a long biological half-life (Wagner, 1993). This metal enters agricultural soils mainly from industrial processes, phosphatic fertilizers and atmospheric deposition, and is then transferred to the food chain.

The addition of a suitable amount of auxin under cadmium stress reduced the damage of plants due to cadmium to a certain level (Mingming *et al*., 2010). Sanjaya *et al*. (2008) reported that the exogenous application of L-tryptophan (L-Trp) improves the cadmium tolerance ability of Arabidopsis and tomato plants.

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Materials 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 *khaya seneglensis* (7–10 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 concentrations 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 were recorded: plant height, leaf area, root length and dry weight for leaves, stems and roots. Photosynthetic pigments (chlorophyll a, b and carotenoids) contents were 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). 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:

Cadmium is nonessential element for plant growth and it is a pollutant for major concern due to its strong mobility in the biosphere. The results in our study show in Tables (2 & 3) indicated that plants which were grown in soil contaminated with cadmium significantly decreased the vegetative growth parameters (plant height, leaf area, root length, dry weight of leaves, stems and roots) of *Khaya senegalensis* by increasing cadmium concentration in the soil in both seasons. The highest reduction obtained from plants exposed to cadmium concentration 200ppm for all parameters in the two seasons. These negative effects of cadmium were increased by increasing cadmium levels in the soil; it may be due to the fact that cadmium is easily absorbed by the plant roots. This metal is toxic for the plant and interferes with many cellular actions by formation of the compound complexes with the secondary groups of organic compounds such as proteins and, thus, prevents the necessary cellular activities (Metwally *et al*., 2003). Cadmium ion stops cell division in the meristemic region and ceases the cells growth in the growth zone (Nazarian *et al*., 2016).

Table 2: Effect of cadmium and/or L-Tryptophan (ppm) on plant height (cm), leaf area (cm) and root length (cm) of *Khaya senegalensis* plant during 2014 and 2015 seasons.

Treatments	Plant height		Leaf area		Root length	
	1st season	2nd season	1st season	2nd season	1st season	2nd season
Cd 0	89.59	101.52	93.56	101.84	50.21	52.36
Cd 50	79.59	93.06	84.3	92.21	44.76	46.47
Cd 100	74.65	87.85	73.73	76.29	39.4	41.83
Cd 200	65.99	87.93	70.45	71.59	33.2	36.49
T 0	71.26	84.66	67.24	81.98	33.97	33.76
T 100	77.90	90.72	81.12	84.58	42.52	45.47
T 200	83.19	95.65	93.2	89.62	49.18	53.64
Cd 0+T 0	83.35	94.65	71.46	100.04	38.42	41.33
Cd 0+T 100	97.42	103.37	100.65	101.11	46.20	52.4
Cd 0+ T 200	88.00	106.55	108.58	104.39	66.00	63.35
Cd 50+T 0	75.11	89.45	69.36	88.65	37.21	34.00
Cd 50+T100	76.00	92.02	78.35	92.06	46.00	46.7
Cd 50+T200	87.65	97.72	105.2	95.92	51.07	58.71
Cd 100+T 0	65.58	84.53	66.66	72.34	32.67	30.00
Cd 100+T100	74.03	88.75	74.35	75.92	42.00	45.61
Cd 100+T200	84.33	90.27	80.17	80.62	43.53	49.88
Cd 200+T 0	61.00	70.00	61.49	66.90	27.60	29.71
Cd 200+T100	64.16	78.75	71.13	70.34	35.88	37.17
Cd 200+T200	72.81	88.05	78.84	77.54	36.12	42.60
LSD at (5%) Cd	**2.46**	**2.75**	**2.67**	**1.76**	**2.58**	**2.22**
T	**2.13**	**2.38**	**2.31**	**2.04**	**2.23**	**1.92**
Cd X T	**3.47**	**3.88**	**3.77**	**2.88**	**2.05**	**3.14**

Table 3: Effect of cadmium and/or L-Tryptophan (ppm) on dry weight of leaves, stems and roots of *Khaya senegalensis* plant during 2014 and 2015 seasons.

Treatments	Leaves dry weight		Stems dry weight		Roots dry weight	
	1st season	**2nd season**	**1st season**	**2nd season**	**1st season**	**2nd season**
Cd 0	17.37	24.36	18.15	22.49	19.11	18.90
Cd 50	14.08	19.32	13.62	18.12	14.15	15.43
Cd 100	10.28	14.16	10.46	13.23	8.71	11.84
Cd 200	7.49	9.71	7.45	8.86	6.25	7.79
T 0	9.82	12.94	9.89	11.87	8.88	11.07
T 100	14.02	17.60	13.10	16.80	12.91	13.65
T 200	13.08	20.14	14.35	18.37	14.38	15.75
Cd 0+T 0	13.88	19.19	14.88	17.64	14.03	14.78
Cd 0+T 100	20.23	25.94	19.27	24.55	21.23	19.22
Cd 0+ T 200	18.01	27.96	20.31	25.30	22.08	22.71
Cd 50+T 0	11.21	15.63	10.72	12.74	10.19	12.14
Cd 50+T100	16.14	20.42	13.78	20.60	15.05	15.98
Cd 50+T200	14.89	21.91	16.37	21.02	17.22	18.16
Cd 100+T 0	7.46	9.33	7.90	9.91	5.91	10.54
Cd 100+T100	12.77	14.28	11.15	13.82	9.29	11.78
Cd 100+T200	10.6	18.88	12.32	15.96	10.93	13.19
Cd 200+T 0	6.71	7.60	6.04	7.19	5.38	6.82
Cd 200+T100	6.95	9.74	8.20	8.21	6.07	7.6
Cd 200+T200	8.83	11.80	8.39	11.18	7.30	8.94
LSD at 5% Cd	0.97	1.06	1.15	1.11	1.22	1.14
T	0.83	0.92	0.99	0.96	1.05	0.98
Cd X T	1.37	1.49	1.62	1.56	1.72	1.61

Also, Larsson *et al*. (1998) reported a decline in biomass production due to disruptions in the processes of respiration, photosynthesis, and nitrogen metabolism owing to the toxic concentrations of cadmium.

Similar results have been reported by Miras-Moreno *et al*., (2014) on *Silene vulgaris* who mentioned that the shoot height and root length were decreased at the highest concentration of Cd (120µM), and Mohamed (2015) who reported that different cadmium concentrations decreased plant height, stem diameter and number of leaves of *Tagetes erecta* plants.

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 200ppm, in the two seasons, except in the first season application of 100ppm L-Trp significantly increased dry weight of leaves.

Regarding the effect of interaction between Cd levels and L-Trp, the results showed that in Tables (2 & 3) the plants treated with L-Trp at 100ppm combined with no cadmium treatment significantly increased plant height and dry weight of leaves in the first season, while application of L-Trp at 200ppm combined with no cadmium treatment significantly increased the other growth parameters in the two seasons including plant height and dry weight of leaves in the second season.

Chemical constituents:

Pigments and carbohydrates content:

In this study we found that Cd-induced growth inhibition was associated by a significant decrease in the chlorophyll a, b and carotenoid content and carbohydrates. The data presented in Figs (1-6) illustrated that in both seasons, raising Cd from 0 to 200 ppm caused a significant reduction in the photosynthetic pigment (chlorophyll a, b and carotenoids) in leaves and carbohydrates content in plant organs (leaves, stems and roots).

These results were in agreement with the findings of several researchers who reported that Cd stress decreased pigments content in leaves and carbohydrate content in whole plant, such as Bhardwaj *et al.* (2009) on *Phaseolus vulgaris* L., Mazid *et al.* (2010) on mung bean plant, Tandon and Srivastava (2014) on sugar beet plant and Mohamed (2015) on *Tagetes erecta* plant.

Heavy metals induced changes in chlorophyll contents which may be ascribed to the decreased Fe contents in leaves or an impairment of the ability of roots for transport of Fe. These could be a sequence of Cd induced changes Mg and Fe contents in leaf (Soundari *et al*., 2015). (Battacharyya and Choudhari, 1994) reported that the inhibition of chlorophyll biosynthesis at protochlorophyllide stage was due to the interference of the enzyme protochlorophyllide reductase.

Khaya senegalensis plants which were treated with L-Trp treatments gave the highest values of pigments content in leaves and carbohydrate content in all plant organs as compared with control plants. 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.*

The results in Figs (1-6) indicated that the plants of *Khaya senegalensis* grown in soil free of cadmium and sprayed with L-Trp at 100ppm gave the highest content of the leaves pigments and carbohydrate content in whole plant as compared with other interaction treatments.

The decrease in total sugar content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive center of ribulosebisphosphate carboxylase (Stiborová *et al.* 1987).

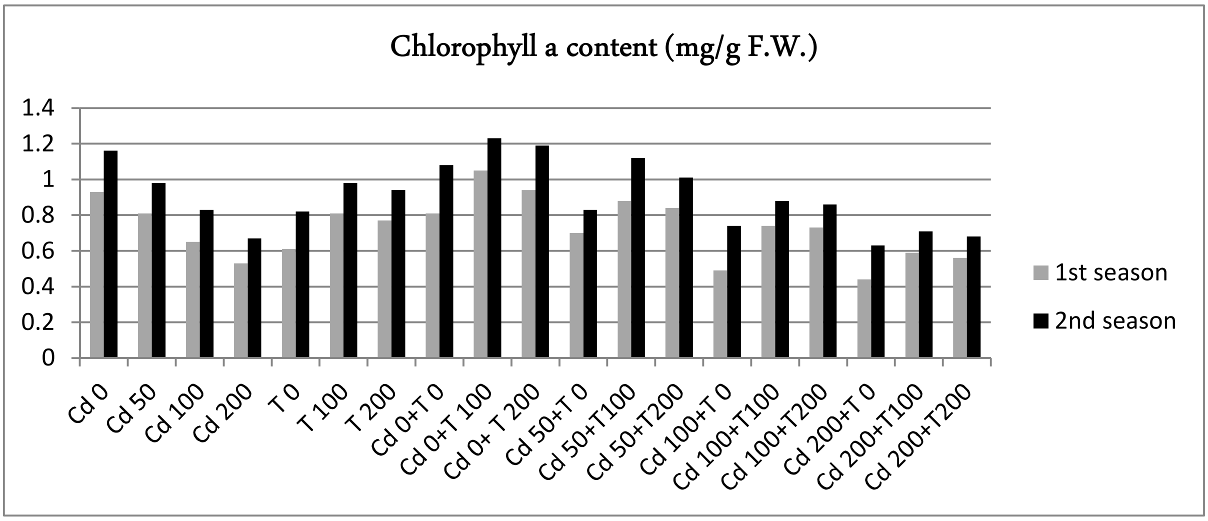

Fig. 1: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves chlorophll a content (mg/g F.W.) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

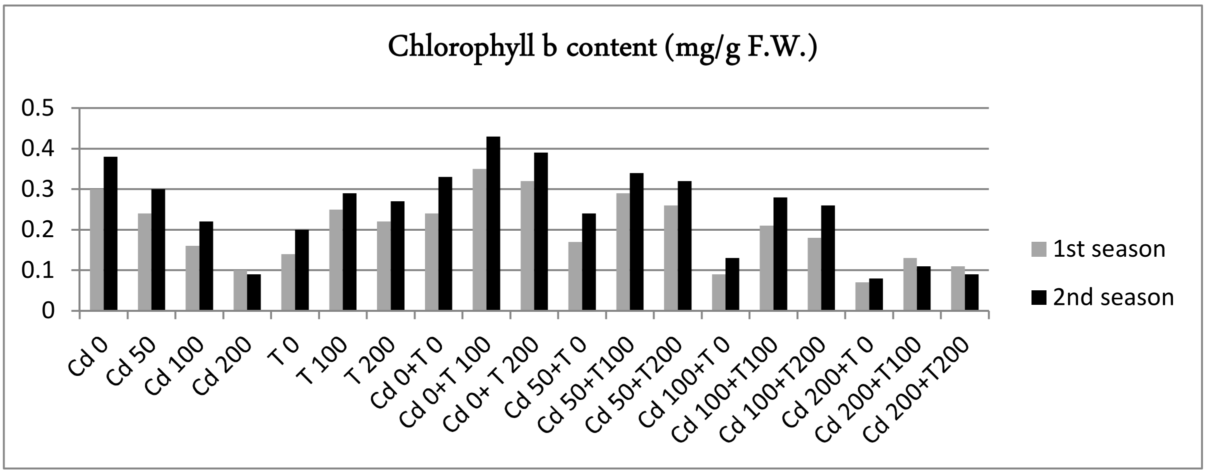

Fig. 2: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves chlorophll b content (mg/g F.W.) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

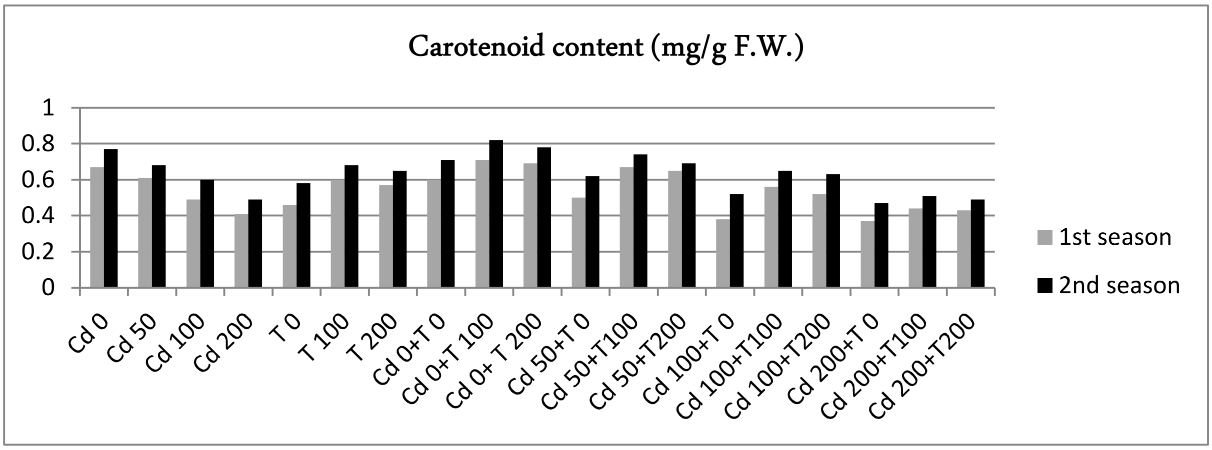

Fig. 3: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves carotenoids content (mg/g F.W.) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

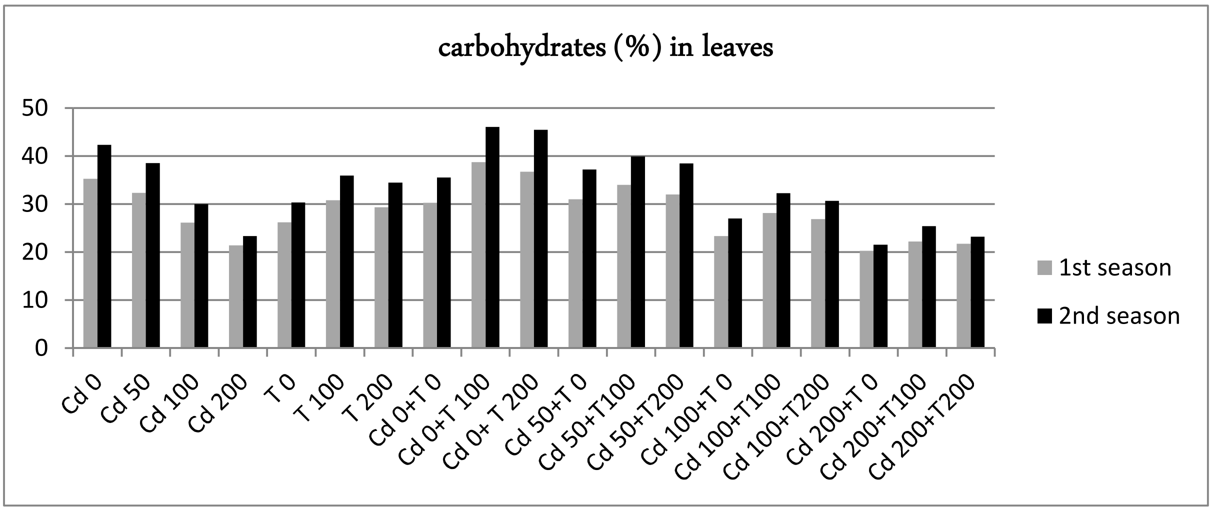

Fig. 4: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves carbohydrates content (%) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

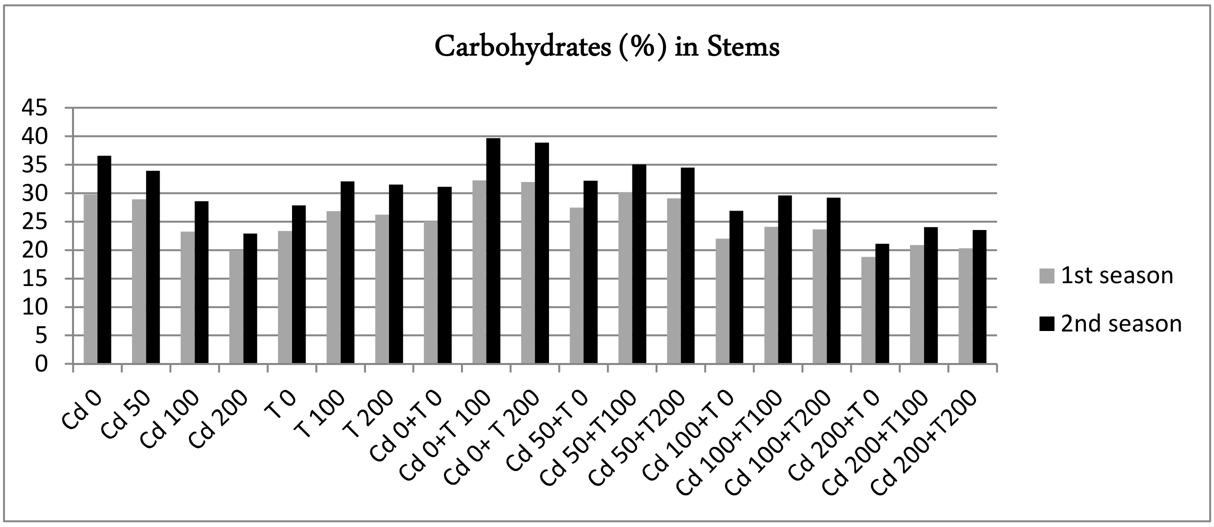

Fig. 5: Effect of cadmium (Cd) and/or L-Tryptophan (T) on stems carbohydrates content (%) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

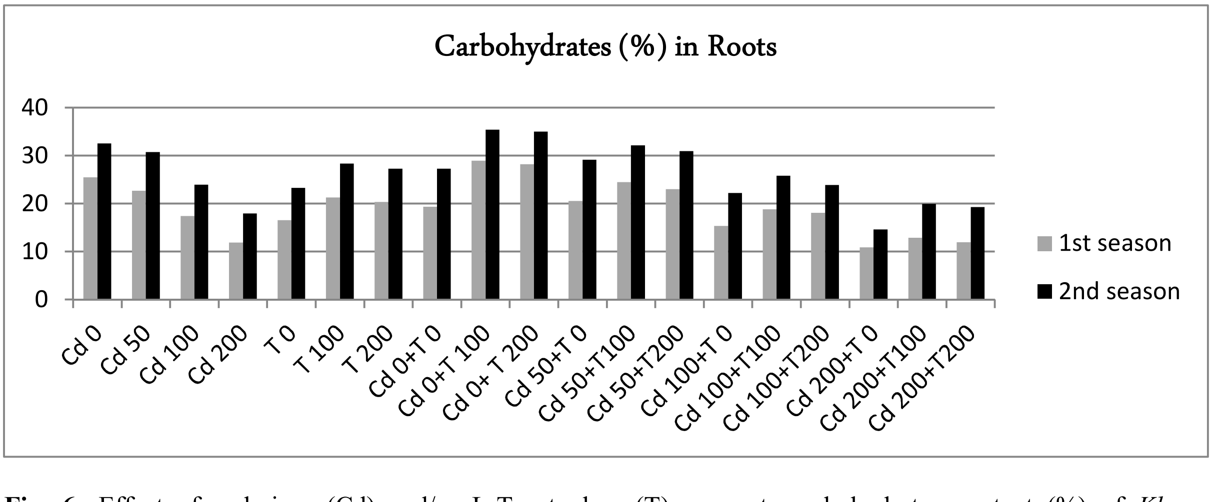

Fig. 6: Effect of cadmium (Cd) and/or L-Tryptophan (T) on roots carbohydrates content (%) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

Cadmium content (ppm):

The data in Figs (7-9) showed that *Khaya senegalemsis* plants exposed to Cd at the concentration of 200ppm significantly increased Cd content in each organ as compared to the other treatments. On the other hand, plants that did not receive any Cd (control) significantly decreased Cd content, compared with other concentration. It is noticed that the cadmium accumulated in roots> stems > leaves in both seasons.

The application of L-Trp also increased the content of cadmium, the maximum concentration of cadmium (200) in all parts of *Khaya senegalensis* plant compared with the control plants in both seasons.

The interaction between the Cd and L-Trp treatments resulted in considerable differences on the Cd content in all organs of plants receiving the different treatment combinations. The highest accumulation was noticed in plants grown in soil contaminated with cadmium at the concentration of 200ppm combined with L-Trp at 200ppm in both seasons. On the other hand, the lowest mean values in both seasons were obtained from plants did not receive any treatment.

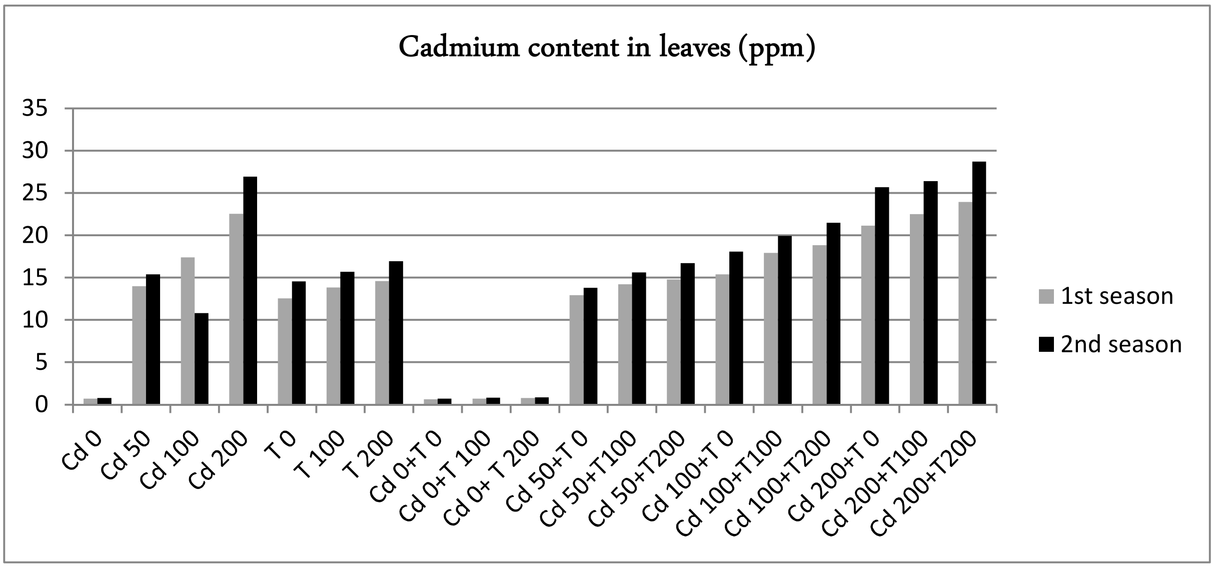

Fig. 7: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves cadmium concentration (ppm) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

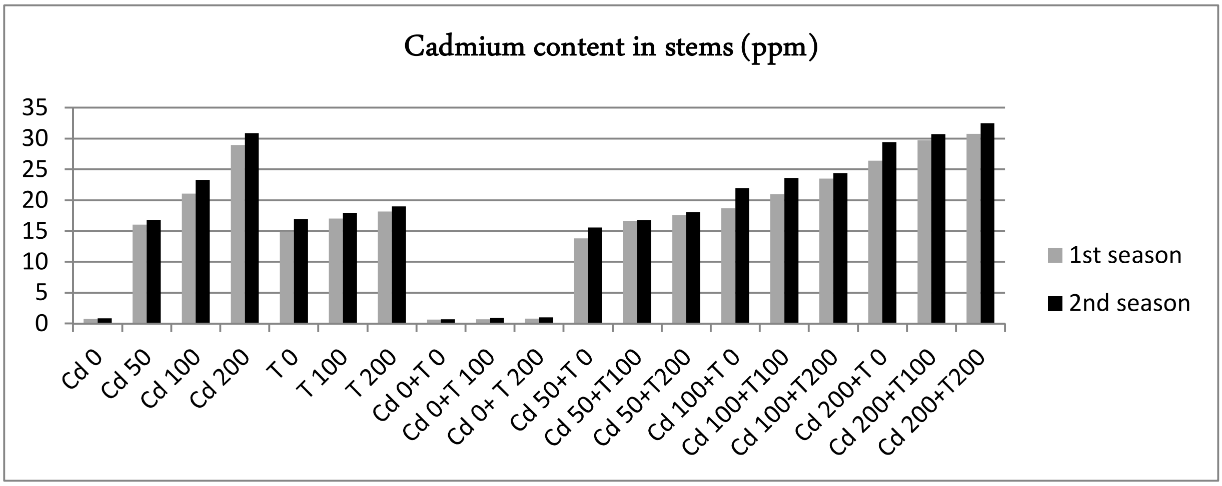

Fig. 8: Effect of cadmium (Cd) and/or L-Tryptophan (T) on stems cadmium concentration (ppm) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

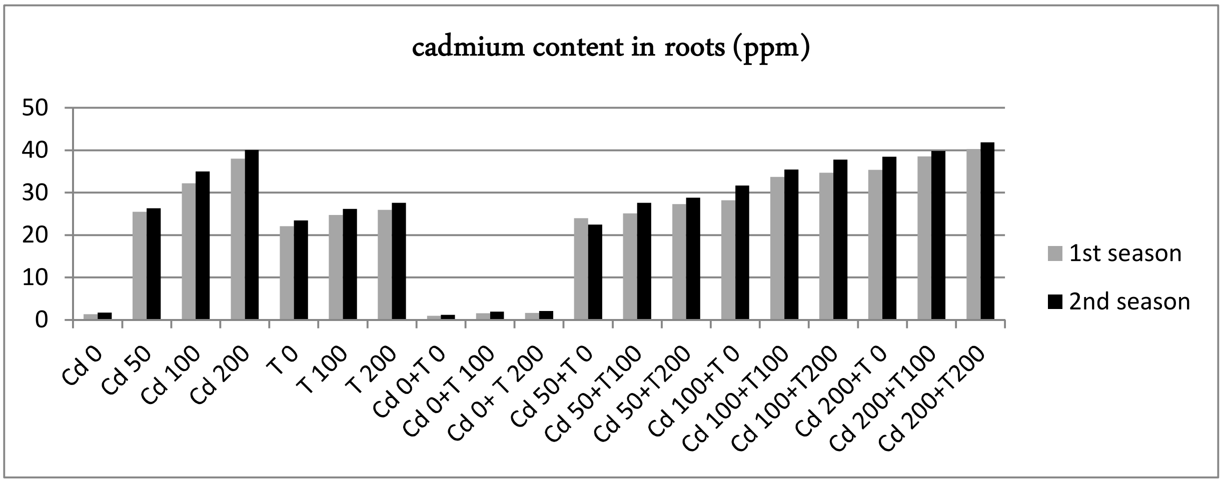

Fig. 9: Effect of cadmium (Cd) and/or L-Tryptophan (T) on roots cadmium concentration (ppm) of *Khaya senegalensis* plants during 2014 and 2015 seasons

Proline content (mg/g F.W.):

The data in Fig (10) showed that raising the Cd level in the soil to 200ppm significantly increased the proline content in leaves of *K. senegalensis* as compared with the control in both seasons. These results are in agreement with the findings of Abdul Qados (2015) on *Acacia saligna*, *Eucalyptus rostrata* and *Conocarpus erecta*, Ali *et al*. (2015) on flax plant 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 (Chandrashekhar and Sandhyarani, 1996) after the stress removal.

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 were 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.

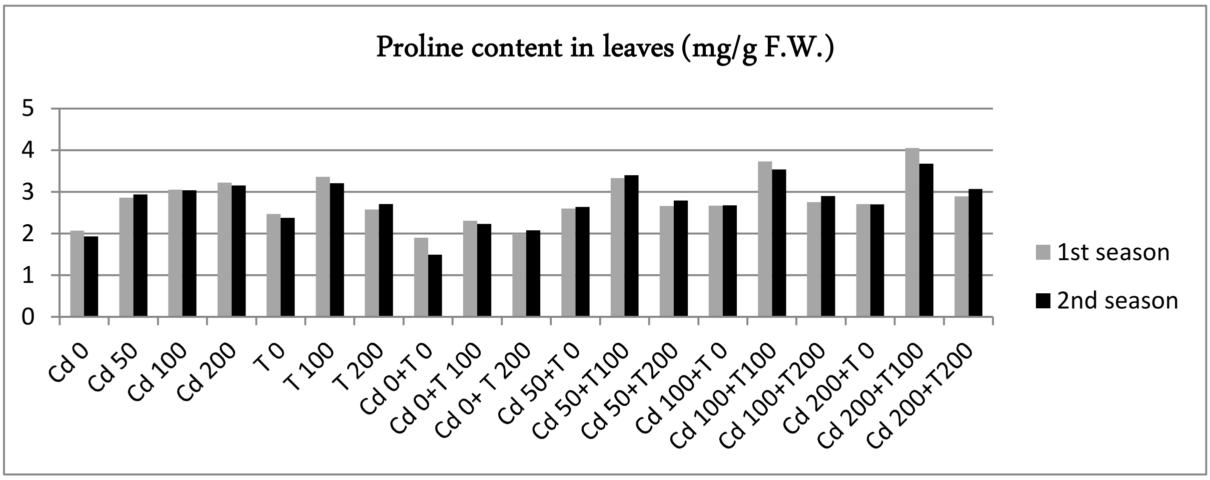

Fig. 10: Effect of cadmium (Cd) and/or L-Tryptophan (T) on leaves cadmium concentration (ppm) of *Khaya senegalensis* plants during 2014 and 2015 seasons.

Antioxidant isozyme:

The results showed in Fig (11) and Table (4) revealed that the peroxidase isozyme in *K. senegalensis* 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 200ppm gave the highest value of peroxidase isozyme.

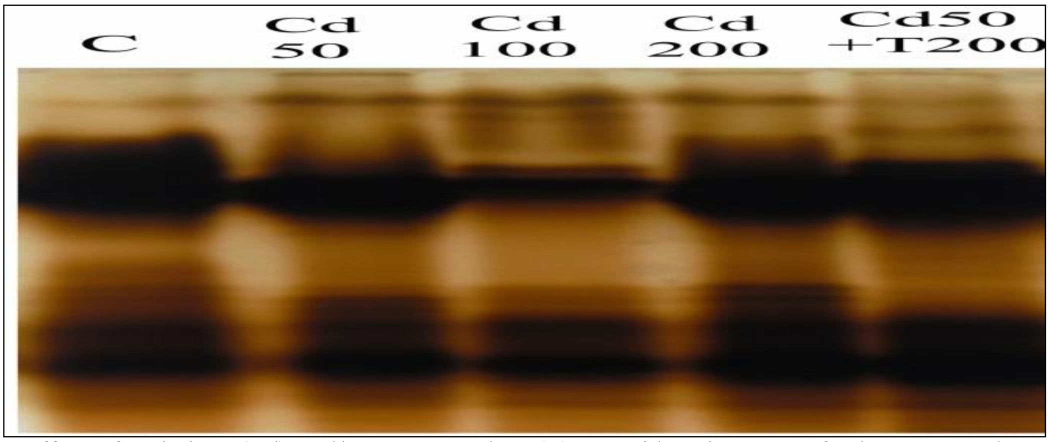

Fig. 11: Effect of cadmium (Cd) and/or L-Tryptophan (T) peroxidase isozyme of *Khaya senegalensis* plants during 2014 and 2015 seasons.

Table 4. Ideogram analysis of peroxidase isozyme in *Khaya senegalensis*

R_f	C	Cd 50	Cd 100	Cd 200	Cd 50+ L-Trp 200
0.090	+	+	+	+	+
0.158	—	—	—	—	+
0.319	++	++	++	+	++
0.624	++	+	+	—	++
0.706	+	+	+	+	++
0.823	—	—	—	—	—
0.935	++	++	+	+	++

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

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 200ppm 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*.

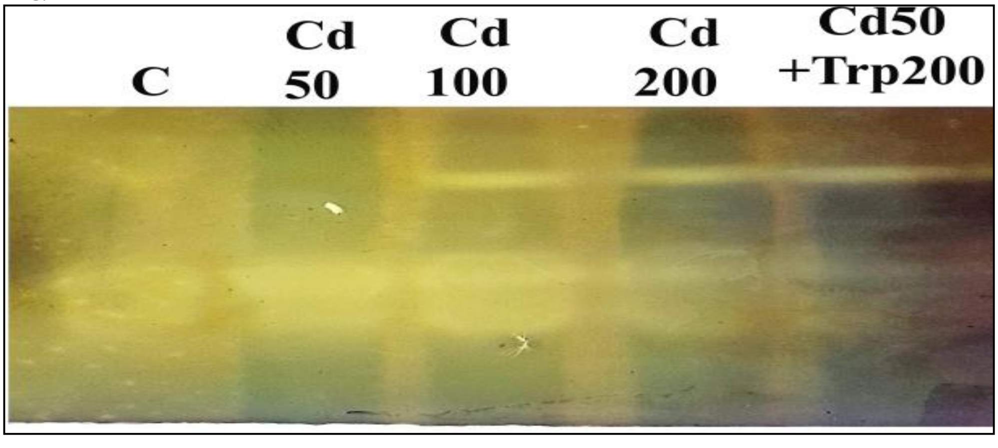

Fig. 12: Effect of cadmium (Cd) and/or L-Tryptophan (T) on catalase isozyme of *Khaya senegalensis* plants during 2014 and 2015 seasons.

Table 5: Ideogram analysis of catalase isozyme in *Khaya senegalensis*

R_f	C	Cd 50	Cd 100	Cd 200	Cd 50+ L-Trp 200	Cd 50+ M 10
0.529	—	–	+	+	++	++
0.664	+	++	+	++	++	+++

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

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