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Antioxidant Isoenzymes, Chemical Constituents and Growth Parameters of Cadmium-Stressed *Dimorphotheca ecklonis* Plant and Affected by Humic Acid

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Abstract

This experiment was conducted in the greenhouse of the National Research Centre during the two seasons of 2019/2020 and 2020/2021 to study the ability of *Dimorphotheca ecklonis* plants to grow in the soil contaminated with cadmium (Cd) at different concentrations (0, 25, 50 and 100 mg. kg^{-1} soil), the humic acid was used at concentrations (0, 200 and 400 mg. l^{-1}) to improve the plants growth. The plants grown in soil contaminated with Cd at 25mg. kg^{-1} soil gave the highest mean values of all vegetative growth parameters, photosynthetic pigments and total sugar content; whereas, the plants grown in soil free of cadmium gave the highest mean values for RWC% and all inflorescences parameters in both seasons. The treatment Cd 100 mg. kg^{-1} increased the leaves content of total phenol, total proline, MDA content and cadmium content in both shoot and root in both seasons. The plants that received humic acid (HA) at concentration 200 mg. l^{-1} showed significant increase for all vegetative growth parameters, RWC%, all inflorescences parameters, photosynthetic pigments,total sugar content and total phenol content in both seasons, while; proline and MDA content were increased at control HA treatments, Cd content in shoot and root were increased by HA 400mg. l^{-1}. Regarding the interaction treatments, the plants treated with Cd at 25 mg. kg^{-1} and HA at 200 mg. l^{-1} increased all vegetative growth parameters, photosynthetic pigments and total sugar content in both seasons, while the treatment of Cd at 0mg. kg^{-1} and HA at 200 mg. l^{-1} significantly increased RWC% and inflorescences parameters, the highest value of total phenol content were produced by treatment Cd at 100 mg. kg^{-1} and HA at 400 mg. l^{-1}, proline content and MDA by treatment Cd at 100 mg. kg^{-1} and HA at 0 mg. l^{-1} and Cd content in shoot and root by treatment Cd at 100 mg. kg^{-1} and HA at 400 mg. l^{-1}. Polyphenol oxidase and peroxidase isoenzymes showed overexpression in treatment Cd at 100 mg. kg^{-1} and HA at 400 mg. l^{-1} in the second season.

Keywords: *Dimorphotheca ecklonis*; cadmium; heavy metal; humic acid; lipid peroxidation; isoenzyme activity

1. Introduction

Dimorphotheca genus is native to Africa and Australia, it is considered one of family *Astraceae* members [1]. *Dimorphotheca ecklonis* is an annual dwarf shrub that grows to be 25 to 50 centimeters tall. The leaves are elliptical, glandular, partially succulent, narrow obovate, and either entire or serrated. The heads are on stems that are 15 to 20 centimeters long and have a diameter of 5 to 8 centimeters. The bracts are glandular and 13 to 16 millimeters long. The rays are white on top and reddish blue on the bottom. The disc is either dark blue or purple in colour. The surface of the fruit is of wrinkled texture [2]. It is widely used as an ornamental plant in pots, borders and balcony gardens. *Dimorphotheca ecklonis* must be planted in full sun in order for the flowers to open to their full potential, although they will tolerate some shade during the day. They can be mass-planted as a groundcover, as a border to a shrubbery, or to line pathways.

Heavy metal stress leads to loss in a regular productivity and induces hazardous health effects. Heavy metals have largest availability in soil and aquatic ecosystems and to a relatively smaller proportion in atmosphere as particular or vapors [3]. Among heavy metals, cadmium (Cd) is a potentially important environmental pollutant. Cadmium is a toxic trace pollutant for humans, animals and plants entering the environment mainly from industrial processes and phosphate fertilizers and then may be transferred to food chain [4]. Cadmium is phytotoxic

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as it can interfere with photosynthetic and respiratory activities, mineral nutrition, enzymatic activities, membrane functions, and hormone balance [5].

Soil organic contents are one of the most important parts that they directly affect the soil fertility and textures with their complex and heterogenous structures although they occupy a minor percentage of the soil weight. Humic acids are an important soil component that can improve nutrient availability and impact on their important chemical, biological and physical properties of soils [6]. Humic acids are very structurally large macromolecular complexes with a brown-black appearance. They were soluble in water pH greater than 2, and generally heterogeneous in structure and consist mainly of carbon, oxygen, hydrogen, nitrogen and occasionally sulphur and phosphorus [7]. They are described as assemblies of aromatic and aliphatic residues with carboxylic, phenolic, carbonyl, quinone, ketone and amine groups. Humic substances can form different types of complex with metals depending on the metal involved and its concentration [8]. It was mentioned that humic substances have the ability to raise the growth of plants that grow under different stress conditions [9] The aim of this study is to investigate the ability of *Dimorphotheca ecklonis* to grow in cadmium-contaminated soils in presence of humic acid as growth enhancer.

2. Materials and Methods

2.1. Location and plant material

The Experiment was conducted during the two seasons of 2019/2020 and 2020/2021 in the greenhouse of the National research centre, Dokki, Giza, and the seeds were obtained from the Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

2.2. Experiment procedure

The seeds were sown in plastic trays on the 1st of October; the germination started a week after the planting. The early seedlings were transplanted on the 1st of November when they started having 4 to 5 real leaves in 15 cm pots, and then were transplanted again at the end of November after growing to almost 15 cm high in 30 cm plastic pots filled with the mixture soil composed of clay and sand (1:1) the physical and chemical analysis were shown in Table (1) which were determined according to Jackson [10]. Cadmium was mixed in the soil during last transplanting at concentrations of 0, 25, 50, and 100 mg. kg-1 soil respectively. After one week of transplanting, humic acid was added with 0, 200, and 400 mg. l-1 concentrations as a soil drench and these treatments were repeated again after a month. Agricultural treatments such as fertilization, removing weeds, were applied as usual during the growing season.

The plants were harvested at the end of May to measure the vegetative parameters, inflorescence parameters and chemical analysis

2.3. Data recorded

2.3.1. Growth parameters

Plant height (cm), stem diameter (cm), number of leaves/ plant, leaf area (cm^2), number of branches/ plant, root length (cm), shoot and root fresh and dry weights (g/ plant).

2.3.2. Inflorescence parameters

Number of inflorescences/ plant, inflorescence diameter (cm), inflorescence fresh and dry weights (g/plant).

2.3.3. Relative water content (RWC %)

Relative water content (RWC %) was determined according Turner [11] as follow: the fresh weight for leaf disc (1 cm diameter) for 3 leaves for each plant was recorded, then floated them in distilled water for 24h at 4°C and then weighted another time (turgid weight). The leaves discs were oven dried till constant weight. RWC% was calculated using formula:

RWC%= ((fresh weight – dry weight)/ (turgid weight – dry weight))*100

Table 1 physical and chemical properties of the soil

Soil sample	Coarse sand%				Fine sand%			Silt%		Clay%	
Sandy loam	61.44				9.36			12		17.20	
	E.C.(1:1) (dS/m)	pH	Cd (mg/kg)	O.M. (%)	Anion (meq/l)			Cation (meq/l)			
					$HCO3^-$	Cl^-	$SO4^-$	Ca^{++}	Mg^{++}	Na^{++}	K^+
	0.48	8.1	2.6	1.36	6.88	3.25	2.47	6.00	1.82	2.79	0.78

2.3.4. Chemical analysis

Photosynthetic pigments (mg. g^{-1} F.W.) were determined according to Saric *et al*. [12], Total sugars (mg. g^{-1} F.W.) were determined according to Dubois *et al*. [13], Total phenols (mg. g^{-1} F.W.) were determined according to Quettier *et al.* [14], Lipid peroxidation (µmol. g^{-1}F.W.) was expressed as malondialdehyde (MDA) content and was determined according to Buege and Aust [15], Proline content (µg. g^{-1}F.W) was determined according to Bates et al. [16]. All the previous chemical analysis were carried out in fresh leaves samples. Cadmium content (ppm) in shoot and root according to Meuwly and Rauser [17] in the solution of digested dry shoot and root by sulfuric and perchloric acids [18].

Antioxidant Isoenzymes Electrophoresis: native polyacrylamide gel electrophoresis (Native-PAGE) isoenzyme electrophoresis was performed to identify isoenzyme differences between control and treatment in the second season. Polyphenol oxidase isenzozymes PPO (E.C. 1.10.3.1) in leaves (100 mg fresh weight) samples were estimated as described by [19, 20]. Peroxidase isoenzmes POD (E.C. 1.11.1.7) in leaves sample were assessed by the procedure defined by Barceló et al. [21].

2.4. Experiment Layout and statistical analysis

The layout of the experiment was a factorial experiment in complete block design, with 12 treatments (4 cadmium (Cd) concentrations X 3 humic acid (HA) concentrations), with 3 replicates for each season. All the 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 [22].

3. Results

3.3. Growth parameters

Data presented in Tables (2 & 3) showed that in both seasons, soil application of cadmium at 25 mg. kg^{-1} soil significantly increased all growth parameters (plant height, stem diameter, No. of leaves and branches, leaf area, root length, fresh and dry weight of shoot and root). The increments were (32%, 28.8%, 42.7%, 62.6%, 61.0%, 23.7 %, 28.9%, 47.7%, 117.6% and 122.9%), respectively in the first season compared with cadmium at high concentration 100 mg. kg^{-1}soil, while in the second season, the increments were (31.6%, 24.1%, 38.4%, 68.9%, 70.7%, 39.5%, 28.2%, 43.2%, 83.8% and 92.5%), respectively, compared with Cd at 100 mg. kg^{-1} soil.

Increasing the concentration of cadmium from 25 to 100 mg. kg^{-1}soil in both seasons significantly decreases all growth parameters of *Dimorphotheca ecklonis*. It is generally accepted that trace amounts of Cd stimulate plant growth, while high amounts of Cd can cause plant injury.

Concerning the effect of humic acid on vegetative growth, data in Tables (2 & 3) showed that the application of humic acid at 200 mg. l^{-1} significantly increased all growth parameters of *Dimorphotheca ecklonis* (plant height, stem diameter, No. of leaves and branches, leaf area, root length, fresh and dry weight of shoot and root) compared with control plant. The increments were (15.0%, 20.0%, 22.2%, 49.5%, 23.6%, 44.3%, 21.1%, 36.0% and 37.4%), respectively, compared with control plants of *Dimorphotheca ecklonis* in the first season, while in the second season, the increment were (18.4%, 20.0%, 15.5%, 42.7%, 21.5%, 32.3%, 16.9%, 25.6%, 30.7% and 33.2%), respectively, compared with control of *Dimorphotheca ecklonis***.**

Regarding the effect of interaction between Cd and humic acid addition treatment on all growth parameters data in Tables (2&3) noticed that humic acid at 200 mg. l^{-1} increased the mean value of all growth parameters of *Dimorphotheca ecklonis* plants, which grown in soil contaminated with cadmium at 25 mg. kg^{-1} soil, in both seasons.

3.4. Inflorescence parameters

The results in Table (4) illustrated that the plant grown in an unpolluted soil with Cd had the highest significant number of inflorescence, inflorescences diameter, inflorescence fresh and dry weight in the both seasons of *Dimorphotheca ecklonis* plants.

Increasing the concentration of Cd significantly decreased the value of these parameters of inflorescence compared with plants grown in unpolluted soil in both seasons.

Data presented in Table (4) showed that soil application of humic acid at 200 mg. l^{-1} significantly increased the values of inflorescence characters in both seasons compared with control plants of *Dimorphotheca ecklonis* plants. The increments were (41.4%, 24.0%, 17.5 and 20.6%), respectively,

Table 2. Effect of cadmium (Cd) and humic acid (HA) on plant height, stem diameter, No. of leaves, No. of branches, leaf area and root length of *Dimorphotheca ecklonis* during 2019/202 and 2020/2021 seasons

Treatments		Plant height (cm)		Stem diameter (cm)		No. of leaves/ plant		No. of branches/ plant		Leaf area (cm^2)		Root length (cm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2st season	1nd season	2st season	1nd season	2st season
Cd0		33.60	34.71	0.61	0.65	337.44	339.78	12.00	10.56	2.79	2.85	26.73	27.44
Cd25		38.44	41.35	0.67	0.72	402.45	391.00	14.28	13.33	3.19	3.26	30.93	32.73
Cd50		35.33	35.90	0.65	0.68	358.00	353.11	12.89	11.67	2.76	2.35	29.15	29.06
Cd100		29.12	31.42	0.52	0.58	282.00	282.45	8.78	7.89	1.98	1.91	25.00	23.46
HA0		31.88	33.02	0.55	0.60	309.08	316.84	9.92	9.00	2.42	2.33	22.69	23.98
HA200		36.67	39.09	0.66	0.72	377.75	366.00	14.83	12.84	2.99	2.83	32.75	31.73
HA400		33.78	35.43	0.63	0.65	348.08	344.92	11.21	10.75	2.63	2.62	28.42	28.81
Cd0+HA0		31.00	31.83	0.53	0.58	284.00	320.67	10.00	9.00	2.43	2.51	20.53	22.82
Cd0+HA200		37.70	38.40	0.66	0.72	377.00	352.00	14.67	12.00	3.22	3.28	32.67	30.83
Cd0+HA400		32.10	33.90	0.64	0.64	351.33	346.67	11.33	10.67	2.74	2.77	27.00	28.67
Cd25+HA0		35.77	38.73	0.61	0.68	372.67	372.67	12.00	11.00	2.84	2.90	26.37	29.77
Cd25+HA200		41.03	44.40	0.72	0.77	425.00	412.33	17.33	15.67	3.57	3.66	34.00	35.00
Cd25+HA400		38.53	40.93	0.69	0.71	409.67	388.00	13.5	13.33	3.17	3.21	32.41	33.42
Cd50+HA0		33.83	32.73	0.57	0.63	323.33	332.00	10.67	9.67	2.67	2.34	23.87	24.00
Cd50+HA200		37.43	39.53	0.70	0.74	386.33	369.00	16.33	14.00	2.90	2.07	33.00	32.67
Cd50+HA400		34.73	35.43	0.67	0.68	364.33	358.33	11.67	11.33	2.71	2.64	30.59	30.52
Cd100+HA0		26.93	28.77	0.49	0.52	256.33	242.00	7.00	6.33	1.77	1.57	20.00	19.34
Cd100+HA200		30.67	34.03	0.56	0.65	322.67	330.67	11.00	9.67	2.27	2.30	31.33	28.41
Cd100+HA400		29.77	31.47	0.51	0.58	267.00	286.67	8.33	7.67	1.91	1.85	23.67	22.62
LSD at 5%	**Cd**	1.54	1.60	0.03	0.03	5.61	6.43	1.72	1.68	0.05	0.05	1.74	1.81
	HA	1.33	1.38	0.03	0.02	4.86	5.56	1.49	1.45	0.04	0.04	1.51	1.63
	Cd*HA	2.66	2.77	0.05	0.05	9.73	11.13	2.99	2.91	0.25	0.18	3.02	3.22

Table 3 Effect of cadmium (Cd) and humic acid (HA) on shoot and root fresh and dry weights of Dimorphotheca ecklonis during 2019/2020 and 2020/2021 seasons

Treatments		Shoot F.W. (g/plant)		Root F.W. (g/plant)		Shoot D.W. (g/plant)		Root D.W. (g/plant)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Cd0		127.82	136.48	5.77	5.88	25.54	31.88	3.95	3.97
Cd25		143.30	151.79	7.05	7.35	30.86	37.45	4.86	5.10
Cd50		135.17	142.19	6.25	6.72	28.00	34.10	4.31	4.60
Cd100		111.21	118.36	3.24	4.00	20.90	26.15	2.18	2.65
HA0		116.71	126.44	4.72	5.22	22.27	28.84	3.21	3.52
HA200		141.32	147.9	6.42	6.82	30.33	36.21	4.41	4.69
HA400		130.10	142.99	5.61	3.92	26.37	32.14	3.86	4.03
Cd0+HA0		115.17	124.37	4.75	5.23	21.54	27.61	3.22	3.50
Cd0+HA200		139.71	146.62	6.72	6.42	29.62	35.63	4.66	4.37
Cd0+HA400		128.57	138.44	5.85	5.98	25.46	32.39	3.98	4.05
Cd25+HA0		132.59	141.50	6.17	6.62	26.65	33.96	4.23	4.55
Cd25+HA200		153.55	162.32	7.85	8.02	34.55	41.23	5.34	5.61
Cd25+HA400		143.76	149.79	7.14	7.42	31.34	37.15	5.01	5.13
Cd50+HA0		123.33	132.82	5.53	5.89	23.56	30.55	3.76	3.96
Cd50+HA200		145.76	153.02	7.00	7.72	32.21	38.40	4.88	5.37
Cd50+HA400		136.41	140.75	6.23	6.54	28.24	33.36	4.30	4.46
Cd100+HA0		95.73	107.08	2.41	3.15	17.33	23.24	1.61	2.07
Cd100+HA200		126.24	129.64	4.11	5.12	24.94	29.56	2.77	3.41
Cd100+HA400		111.67	117.12	3.20	3.74	20.43	25.65	2.15	2.47
LSD at 5%	**Cd**	2.59	4.67	0.52	0.53	2.32	2.21	0.52	0.34
	HA	2.24	4.04	0.45	0.46	2.01	1.91	0.45	0.29
	Cd*HA	4.48	8.07	0.90	0.92	4.01	3.83	0.90	0.58

compared with control plant in the first season, while in the second season, the increments were (53.6%, 18.6%, 18.2% and 21.1%), respectively, comparedwith control plant of *Dimorphotheca ecklonis*.

Regarding the effect of interaction between Cd and humic acid on inflorescence parameters, it is noticed that plants grown in soil free of Cd treatments with humic acid at 200 ppm gave the highest value of inflorescences parameters in both seasons of *Dimorphotheca ecklonis* plants.

3.5. Relative water content (RWC %)

The data in Table (4) showed that increasing the concentration of Cd reduced the RWC % from unpolluted soil to high concentration of Cd in both seasons of *Dimorphotheca ecklonis*.

It was noticed from data tabulated in Table (4) that the application of humic acid at 200 ppm significantly increased RWC %. The increment percentage were (7.9% and 9.19%) in both seasons, respectively, compared with the control plants.

The interaction between the soil free of Cd and humic acid at 200 mg. l^{-1} gave a significantly increase the relative water content in both season of *Dimorphotheca ecklonis* plants.

Table 4 Effect of cadmium (Cd) and humic acid (HA) on RWC%, No. of Inflorescences/ plant, Inflorescence diameter, Inflorescence fresh and dry weights of Dimorphotheca ecklonis during 2019/2020 and 2020/2021 seasons

Treatments		RWC%		No. of Inflorescences/ plant		Inflorescence diameter (cm)		Inflorescence F.W. (g/plant)		Inflorescence D.W. (g/plant)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Cd0		82.67	82.11	11.11	10.11	3.45	3.73	1.94	2.00	0.90	0.93
Cd25		74.96	74.13	10.45	8.44	3.10	3.49	1.78	1.84	0.81	0.83
Cd50		70.28	68.64	6.56	6.78	2.84	3.07	1.66	1.72	0.73	0.76
Cd100		66.92	64.20	5.00	4.11	2.29	2.27	1.35	1.44	0.58	0.62
HA0		70.76	69.21	6.84	5.75	2.63	2.85	1.54	1.59	0.68	0.71
HA200		76.32	75.57	9.67	8.83	3.26	3.38	1.81	1.88	0.82	0.86
HA400		74.04	72.03	8.33	7.50	2.87	3.19	1.70	1.76	0.77	0.80
Cd0+HA0		79.61	80.12	9.33	8.67	3.27	3.35	1.74	1.82	0.79	0.83
Cd0+HA200		85.17	83.72	12.67	11.33	3.74	4.02	2.1	2.15	0.99	1.01
Cd0+HA400		83.22	82.49	11.33	10.33	3.35	3.81	1.97	2.03	0.92	0.95
Cd25+HA0		71.58	70.24	8.67	6.00	2.82	3.17	1.67	1.70	0.74	0.75
Cd25+HA200		77.90	78.69	11.67	10.00	3.45	3.73	1.86	1.93	0.86	0.89
Cd25+HA400		75.41	73.45	11.00	9.33	3.03	3.57	1.81	1.88	0.83	0.86
Cd50+HA0		67.49	65.08	5.67	5.33	2.53	2.87	1.60	1.62	0.70	0.71
Cd50+HA200		72.61	72.19	7.33	8.33	3.11	3.25	1.73	1.82	0.77	0.82
Cd50+HA400		70.74	68.65	6.67	6.67	2.89	3.09	1.64	1.71	0.72	0.76
Cd100+HA0		64.37	61.40	3.67	3.00	1.90	2.02	1.13	1.25	0.48	0.54
Cd100+HA200		69.58	67.67	7.00	5.67	2.75	2.50	1.55	1.64	0.67	0.72
Cd100+HA400		66.80	63.52	4.33	3.67	2.22	2.28	1.37	1.42	0.59	0.61
LSD at 5%	**Cd**	2.49	2.55	1.67	1.40	0.21	0.20	0.17	0.11	0.04	0.05
	HA	2.16	2.21	1.45	1.22	0.18	0.18	0.14	0.09	0.04	0.04
	Cd*HA	4.31	4.41	2.90	4.11	0.36	0.35	0.29	0.18	0.07	0.08

3.6. Chemical constituents

3.6.1. osynthetic pigments and total sugar

The results presented in Table (5) showed that the highest content of photosynthetic pigments (Chl a, b and carotenoids) and total sugar were obtained from plants applied soil with Cd at 25 mg. kg^{-1} soil in the first and second seasons of *Dimorphotheca ecklonis* plants.

Concerning the effect of humic acid on photosynthetic pigments and total sugar, data resulted from plant chemical analysis presented in Table (5) showed that, the humic acid application at 200 mg. l^{1} gave the highest Chl a (5.21), Chl b (1.76), carotenoids (2.46) mg. g^{-1} F.W. and total sugar (19.32

mg. g^{-1} F.W) in the first season, respectively, while in the second season, the values were (5.96, 2.12, 3.59 and 23.92 mg. g^{-1} F.W.), respectively, of *Dimorphotheca ecklonis* plants.

The interaction between soil application of Cd at 25 mg. kg^{-1} soil and humic acid at 200 mg. l^{-1} gave the highest value of photosynthetic pigments and total sugar in both seasons of *Dimorphotheca ecklonis* plants.

3.6.2. Total phenols

The data in Table (5) showed that the applying of Cd at 100 mg. kg^{-1} soil on total phenols of *Dimorphotheca ecklonis* plants significantly increased the total phenol content giving values 11.57 and 12.69 mg. g^{-1} F.W. in both seasons, respectively. Addition of humic acid at 200 mg. l^{-1} to the soil significantly increased of total phenols content giving values 9.59 and 9.32 mg. g^{-1} F.W. in both seasons, respectively, while the interaction between Cd at 100 mg/kg soil and humic acid at 400 mg. l^{-1} significantly increased total phenols in both seasons giving 12.08 and 14.07mg. g^{-1} F.W., respectively.

Table 5 Effect of cadmium (Cd) and humic acid (HA) on photosynthetic pigments (chlorophyll a, b and carotenoids), total sugars and total phenol contents of Dimorphotheca ecklonis during 2019/2020 and 2020/2021 seasons

Treatments		Chlorophyll a (mg. g^{-1}F.W.)		Chlorophyll b (mg. g^{-1}F.W.)		Carotenoids (mg. g^{-1}F.W.)		Total sugars (mg. g^{-1}F.W.)		Total phenol (mg. g^{-1}F.W.)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Cd0		3.84	4.86	1.35	1.57	1.87	2.98	17.87	20.68	6.50	6.23
Cd25		5.92	6.59	1.94	2.11	2.81	3.85	22.13	23.85	7.87	7.23
Cd50		4.64	5.47	1.56	1.86	2.38	3.53	17.37	18.91	9.85	9.65
Cd100		3.17	3.27	1.14	1.29	1.52	1.78	12.27	15.77	11.57	12.69
HA0		4.13	4.77	1.44	1.58	2.27	2.91	15.55	14.36	8.25	8.30
HA200		5.21	5.96	1.76	2.12	2.46	3.59	19.32	23.92	9,59	9.32
HA400		3.84	4.42	1.30	1.43	1.72	2.62	17.37	21.13	9.01	9.24
Cd0+HA0		3.81	4.52	1.31	1.51	1.86	2.74	16.34	15.65	6.06	5.67
Cd0+HA200		4.42	5.89	1.51	1.74	2.13	3.68	19.02	24.71	7.15	6.82
Cd0+HA400		3.28	4.17	1.24	1.47	1.63	2.52	18.26	21.69	6.30	6.21
Cd25+HA0		4.79	6.12	1.67	1.82	2.79	3.73	20.62	18.49	7.22	6.53
Cd25+HA200		7.23	7.72	2.56	2.87	3.51	4.45	24.23	27.82	8.76	7.92
Cd25+HA400		5.73	5.93	1.59	1.65	2.14	3.38	21.55	25.24	7.62	7.24
Cd50+HA0		4.29	5.22	1.51	1.77	2.72	3.33	15.41	13.07	9.00	9.18
Cd50+HA200		5.43	6.32	1.69	2.26	2.42	4.08	19.27	23.80	10.54	10.34
Cd50+HA400		4.19	4.88	1.49	1.55	2.00	3.19	17.44	19.85	10.02	9.44
Cd100+HA0		3.63	3.22	1.25	1.21	1.70	1.82	9.82	10.21	10.72	11.80
Cd100+HA200		3.74	3.92	1.30	1.59	1.76	2.15	14.76	19.36	11.92	12.20
Cd100+HA400		2.14	2.68	0.87	1.06	1.09	1.37	12.23	17.73	12.08	14.07
LSD	**Cd**	0.12	0.20	0.06	0.11	0.06	0.14	1.33	1.07	0.57	0.23
at 5%	**HA**	0.10	0.17	0.05	0.09	0.05	0.12	1.15	0.92	0.49	0.20
	Cd*HA	0.21	0.34	0.09	0.18	0.11	0.24	2.29	1.85	0.98	0.41

3.6.3. Lipid peroxidation (MDA) and proline

The data in Table (6) illustrated that the MDA and proline content of the *Dimorphotheca ecklonis* plants significantly increased when the plants treated with Cd concentration at 100 mg. kg^{-1} soil in both seasons, the increments were (141.9% and 164.6%) and (110.7% and 110.6%), respectively, compared with the control plants, in the first and second seasons of *Dimorphotheca ecklonis* plants. The plants that didn't receive humic acid treatment gave high significant of (MDA) and proline content compared with other concentration of humic acid in both seasons.

The interaction between Cd at high concentration (100 mg. kg^{-1}) with control plants of humic acid significantly increased (MDA) and proline content in both seasons compared with other treatments and control plants.

3.6.4. Cadmium (Cd) content in shoots and roots

Data in Table (6) indicated that Cd content in shoots and roots of *Dimorphotheca ecklonis* plants significantly increased with increasing Cd concentration at 100 mg. kg^{-1} soil in both seasons, the increments were (211.4% and 201.0%) in shoots and (151.3% and 172.2%) in roots, respectively, in the first and second seasons compared with control plants, while, the lowest values of Cd content in shoot and root were obtained from plants grown in unpolluted soil with Cd in both seasons.

The addition of humic acid at 400 mg. l^{-1} significantly increased Cd content in shoots and roots compared with other concentration and control plants in both seasons.

For the interaction between the soil application of Cd and addition of humic acid concentration, data presented in Table (6) showed that the highest mean values were (37.66 and 38.26 ppm) in shoots and (20.56 and 24.85 ppm), in roots in the first and second seasons, respectively, were obtained from plants with application of Cd at 100 mg. kg^{-1} soil and humic acid at 400 mg. l^{-1}, while the lowest mean values were obtained from control plants.

Table 6 Effect of cadmium (Cd) and humic acid (HA) on lipid peroxidation (MDA), proline content, cadmium content in shoots and roots of Dimorphotheca ecklonis during 2019/2020 and 2020/2021 seasons

Treatments		MDA (μmol. g^{-1}F.W.)		Proline content (μg. g^{-1}F.W)		Cd content in shoots (ppm)		Cd content in roots (ppm)	
		1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Cd0		1.17	1.13	145.96	149.89	1.42	1.51	1.12	1.11
Cd25		1.75	1.68	219.34	227.84	17.88	18.90	13.72	15.11
Cd50		2.26	2.20	248.82	252.33	24.43	25.64	16.51	17.54
Cd100		2.83	2.99	307.50	315.69	31.44	31.86	18.06	20.22
HA0		2.33	2.25	271.86	266.12	15.39	16.47	10.19	10.04
HA200		1.74	1.72	185.95	198.18	18.71	19.13	12.14	13.42
HA400		1.93	2.03	233.41	245.02	22.28	22.83	14.74	17.03
Cd0+HA0		1.07	1.10	147.53	151.34	1.07	1.12	0.96	1.02
Cd0+HA200		1.19	1.12	143.49	148.02	1.42	1.49	1.14	1.11
Cd0+HA400		1.25	1.17	146.87	150.30	1.78	1.91	1.27	1.20
Cd25+HA0		2.19	1.98	264.49	252.27	15.81	16.22	10.43	11.06
Cd25+HA200		1.45	1.34	183.35	197.15	17.24	19.05	13.22	14.82
Cd25+HA400		1.61	1.72	210.18	234.11	20.59	21.44	17.51	19.46
Cd50+HA0		2.67	2.56	297.80	279.62	19.47	21.57	13.86	12.26
Cd50+HA200		1.96	1.85	196.43	218.46	24.72	25.64	16.07	17.74
Cd50+HA400		2.14	2.20	252.22	258.92	29.09	29.71	19.60	22.61
Cd100+HA0		3.40	3.37	377.61	381.25	25.21	26.98	15.50	15.80
Cd100+HA200		2.36	2.55	220.53	229.08	31.45	30.33	18.11	20.01
Cd100+HA400		2.72	3.04	324.37	336.74	37.66	38.26	20.56	24.85
LSD at 5%	**Cd**	0.05	0.05	1.07	1.75	0.78	0.58	0.61	0.31
	HA	0.04	0.04	0.92	1.25	0.68	0.50	0.53	0.27
	Cd*HA	0.08	0.09	1.85	3.04	1.36	1.01	1.06	0.54

3.6.5. Antioxidant isoenzymes

3.6.5.1. Polyphenol Oxidase (PPO)

The Figures (1&2) and Table (7) revealed that the PPO isoenzymes of *Dimorphotheca ecklonis* plant leaves treatments showed five PPO isoenzymes at R_F (0.313, 0.420, 0.511, 0.629 and 0.776), PPO isoenzymes, five well-resolved bands were detected in treatments Cd at 100 mg. kg^{-1} soil + HA at 400 mg. l^{-1} that got 2 high density bands, 2 faint bands, while the last one was moderated, followed by treatment Cd at 100 mg. kg^{-1} soil+ HA at 200 mg. l^{-1} that gave 2 highly density bands and 2 moderated bands, then treatment (Cd at 100 mg. kg^{-1} soil +HA at 0mg. l^{-1}) that showed 3 moderate bands. These results showed that *Dimorphotheca ecklonis* plants grown in soil contaminated with Cd at 100 mg. kg^{-1} and application of HA at 400 mg. l^{-1} had the highest activity of PPO isoenzymes, followed by the plants grown in soil contaminated with Cd at 100 mg. kg^{-1} soil and received HA at 200 mg. l^{-1} then the treatment Cd at 100 mg. kg^{-1} without HA treatment as compared to control plant that showed the lowest activity.

Table 7 Isomers of polyphenol oxidase enzyme (+/-) and their retention factor (RF) of effect of cadmium (Cd) and/or humic acid (HA) on polyphenol oxidase isoenzyme of Dimorphotica ecklonis seedlings

R_F	Control	Cd0+ HA200	Cd0+ HA400	Cd25+ HA0	Cd25+ HA200	Cd25+ HA400	Cd50+ HA0	Cd50+ HA200	Cd50+ HA400	Cd100+ HA0	Cd100+ HA200	Cd100+ HA400
0.313	+	++	+	++	++	++	++	++	+++	+++	+++	+++
0.420	+	+	++	++	+	+	++	++	++	++	+++	+++

0.511	-	-	-	-	+	+	-	-	-	-	-	+
0.629	-	-	-	-	+	+	+	+	-	++	++	+
0.776	-	-	-	-	+	+	-	+	-	++	++	++

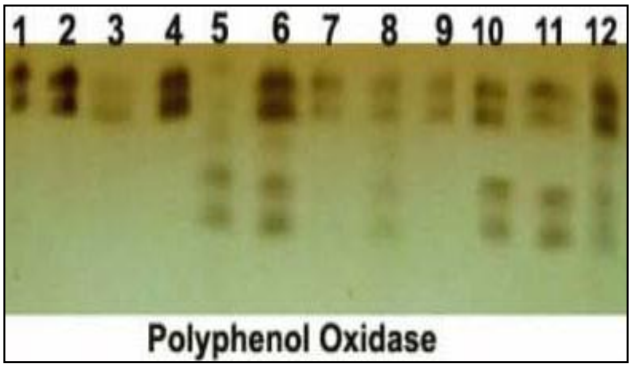

Fig. 1. Effect of cadmium (Cd) and/or humic acid (HA) on polyphenol oxidase isoenzymes of *Dimorphothica ecklonis* plant (1: control, 2: Cd0+HA200, 3: Cd0+HA400, 4: Cd25+HA0, 5: Cd25+HA200, 6: Cd25+HA400, 7: Cd50+HA0, 8: Cd50+HA200, 9: Cd50+HA400, 10: Cd100+HA0, 11: Cd100+HA200 and 12: Cd100+HA400)

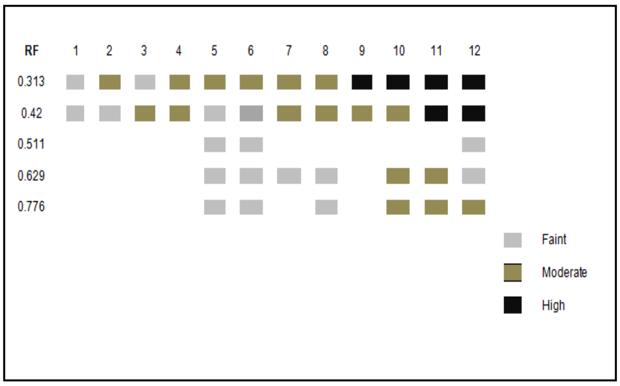

Fig. 2. Ideogram analysis of effect of cadmium (Cd) and/or humic acid (HA) on polyphenol oxidase isoenzymes of *Dimorphothica ecklonis* seedlings (1: control, 2: Cd0+HA200, 3: Cd0+HA400, 4: Cd25+HA0, 5: Cd25+HA200, 6: Cd25+HA400, 7: Cd50+HA0, 8: Cd50+HA200, 9: Cd50+HA400, 10: Cd100+HA0, 11: Cd100+HA200 and 12: Cd100+HA400)

3.6.5.2. Peroxidase (POD)

Native PAGE in Figures (3&4) and Table (8) showed four POD isoenzymes at R_F (0.346, 0.787, 0.862, and 0.916). Treatment Cd at 100 mg. kg^{-1} soil + HA at 400 mg. l^{-1} showed highly overexpressed POD which recorded four bands that were highly dense at the first band and faint at the other three bands, followed by treatments Cd at 100 mg. kg^{-1} soil +HA at 200 mg. l^{-1} and Cd at 50 mg. kg^{-1} soil + HA at 400 mg. l^{-1} which recorded three bands with the same expression. The mentioned results illustrated that *Dimorphotheca ecklonis* plants grown in soil contaminated with Cd at 100 mg. kg^{-1} and treated with humic acid at 400 mg. l^{-1} produced the highest activity of the peroxidase isoenzyme followed by the plants grown in soil contaminated with Cd at 100 mg. kg^{-1} and treated with humic acid at 200 mg. l^{-1} as compared to other treatments and control plants.

Table 8 Isomers of peroxidase enzyme (+/-) and their retention factor (RF) of effect of cadmium (Cd) and/or humic acid (HA) on peroxidase isoenzyme of Dimorphothica ecklonis seedlings

R_F	Control	Cd0+ HA200	Cd0+ HA400	Cd25+ HA0	Cd25+ HA200	Cd25+ HA400	Cd50+ HA0	Cd50+ HA200	Cd50+ HA400	Cd100 + HA0	Cd100 + HA200	Cd100 + HA400
0.346	+	++	++	++	++	++	+++	+++	+++	+++	+++	+++
0.787	+	+	+	+	+	+	+	+	++	+	++	+
0.862	-	-	+	-	+	+	-	+	+	+	+	+
0.916	-	-	-	+		+	-	-	-	-	-	+

4. Discussion

Cadmium is one of the most toxic metals, negatively affecting all biological processes in plants, including leaf chlorosis, photosynthesis, root growth, absorption, and transport of nutrients. (Gabrijel *et al.*, 2009 [23]; Orrono and lavado, 2009 [24] and Karbassi and Pazoki, 2015[25]

This study revealed that at high heavy metal concentrations, all vegetative growth parameters were significantly decreased. The results presented were in agreement with other earlier reports on other plants [(Piotrowska *et al.*, 2010 [26] on *Wolffia arrhiza*, Tiryakioglu *et al.*, 2006 [27] on barley *Hardum vulgare*, Bah *et al.*, 2011 [28] on *Typha angustifolia*, El-Shanhorey and Emam, 2016

[29] on *Jatropha curcas* plants, El-Shanhorey and Barakt, 2020 [30] on *Salvia splendens* plant, El-Shanhorey and El-Sayed, 2017[31] and Benavides *et al*., 2005 [32] found that Cd has been shown to cause many morphological, physiological and biochemical changes in plants, such as growth inhibition and water imbalance.

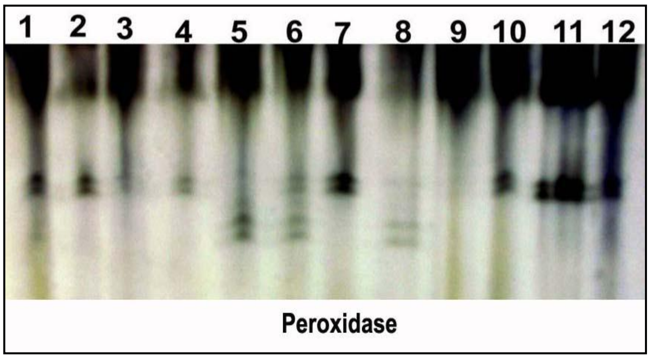

Fig. 3. Effect of cadmium (Cd) and/or humic acid (HA) on peroxidase isoenzyme of *Dimorphothica ecklonis* seedlings (1: control, 2: Cd0+HA200, 3: Cd0+HA400, 4: Cd25+HA0, 5: Cd25+HA200, 6: Cd25+HA400, 7: Cd50+HA0, 8: Cd50+HA200, 9: Cd50+HA400, 10: Cd100+HA0, 11: Cd100+HA200 and 12: Cd100+HA400)

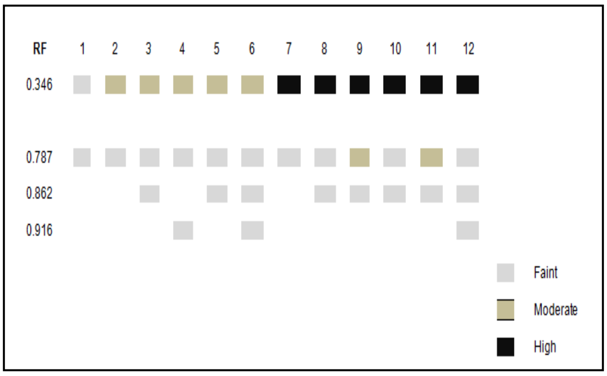

Fig. 4. Ideogram analysis of effect of cadmium (Cd) and/or humic acid (HA) on peroxidase isoenzyme of *Dimorphothica ecklonis* seedlings (1: control, 2: Cd0+HA200, 3: Cd0+HA400, 4: Cd25+HA0, 5: Cd25+HA200, 6: Cd25+HA400, 7: Cd50+HA0, 8: Cd50+HA200, 9: Cd50+HA400, 10: Cd100+HA0, 11: Cd100+HA200 and 12: Cd100+HA400)

Cadmium at higher doses was found to decrease all the morphological characteristics; this decrement in growth could be attributed to the suppression of the cell's elongation growth rate due to an irreversible inhibition performed by Cd on the proton pump responsible for the process. (Aidid and Okamote, 1993[33].The inhibition of root growth in Cd-treated plants may be due to the reduction of water uptake, as well as a reduction in both new cell formation and cell elongation of the root (Raziuddin *et al*., 2011[34]).

Cadmium stress significantly reduces the percentage of relative water content (RWC%) in leaves, which is consistent with the findings of Hassanein *et al*., 2016 [35] on *Triticum aestivum*. The decrement in leaf conductance and relative water content could be due to a directly induced stomatal closure (Perfus-Barbeocb *et al*., 2002 [36]), which could be caused by a disruption in hormonal balance, which would reduce water transport and RWC%.

Our findings are consistent with those of El-Shanhorey and Barakat, 2020 [30], who found that in both seasons of *Salvia splendens*, plants that were not polluted with Cd had the highest inflorescences parameters, while plants that were irrigated with the highest Cd concentration had the lowest values of parameters

ıd stomatal conductance have all been measured in response to cadmium heavy metal Chen *et al*., 2012 [37]. Piotrowska *et al*., 2010 [26] reported that the content of chlorophylls and carotenoids was critical in the energy manifestation of green plants. Any significant alteration of their contents may have had a significant impact on the plant's overall metabolism. Cadmium at high concentrations resulted in a significant reduction in chlorophyll biosynthesis or a breakdown of pigments and their precursors. Cadmium may replace the central Mg from chlorophyll molecules, reducing plant photosynthetic light harvesting ability (Agrawal and Mishra, 2009[38]). This results agreement with (El-Shanhorey and El-Sayed, 2017 [31] on *Senecio cineraria* plants,

Proline is an important substance has an important role as osmotic protective that plays a necessary role in maintaining the normal function of cells, protecting the structure of cell membranes, heavy metal stress impacts the water balance in plants and induces a large increase in proline, which is involved in the osmotic regulation of cells (Rady, M. M. and Hemida, 2015[39]), Dhir *et al*., 2004 [40] found that proline accumulation in shoots of *Brassica juncea* and *Triticumae stivum* increased in response to Cd toxicity. Zengin and Munzuraylue, 2006 [41] on sun flower and El-Shanhorey and Barakat, 2020 (30) on *Saliva splendens* plants reported similar results of increasing proline content by Cd.

Our findings agree with those of John *et al*., 2008 [42] on Lemon and Kuntal *et al*., 2017 [43] on *Tagets erecta* L. plants, who discovered that soluble sugar content increased at lower Cd concentrations this increase may be due to Cd reducing water transfer to the leaves, and the accumulation of sugars in plants that were exposed to cadmium may provide an adaptive mechanism maintaining a favourable osmotic potential under stress condition of cadmium (Verma and Dubey 2001[44]). On the other hand, the total sugar content decreased by increasing Cd concentration, it could be caused by decreased synthesis or a diversion of metabolites to other synthesis processes (Aldoobie and Beltagi, 2013[45]).

The former studies found that cadmium increased the production of plant total phenols in Brassica rape (Thiruvengadam and Chung, 2015[46]), Gursoy *et al*., 2009[47] on seven *Morchella* sp., and Mohd *et al*., 2017 [48] on *Orthosiphon stamineus*. phenolic compounds can act as metal chelators and on the other hand phenolics can directly scavenge molecular species of active oxygen (Michalak, 2006 [49]) Our results indicated that the combination of Cd and HA can increase the production of total phenols.

Malondialdehyde (MDA) is the first product of membrane lipid peroxidation and accumulates when plants are repeatedly exposed to oxidative stresses. As a result, MDA concentration is commonly regarded as a general indicator of lipid peroxidation and stress level. (Chaoui *et al*., 1997 [50]). The MDA content in leaves of *Dimorphothica ecklonis* increased with Cd stress. These findings are consistent with the observation of Metwally *et al*., 2003 [51] on barley, Li *et al*., 2013[52] on *Hibiscus cannabinus* plants and Chen *et al*., 2007[53] on rice seedlings.

The lowest mean Cd content in shoot and root in the first and second seasons were found in the unpolluted plants, whereas the highest content in shoots and roots were found in plants irrigated with the highest concentration of Cd (El-Shanhorey and Barakat, 2020 [30]) on *Salvia splendens* plants.

Regarding the effect of Humic acid on growth, Sergiev *et al*., 2012 [54] indicated that humic substances have the ability to increase the growth of various plant species that grow under different stress conditions. The use of humic acid in contaminated soil increased metal bioavailability and mobility. (Pizzeghello *et al*., 2013[55] and Topcuoglu, 2012 [56].

Foliar application of humic acid had a significant stimulatory effect on growth parameters of *Chrysanthum indica* plants(Mazhar *et al*., 2012 [57]) the results agree with our results which may be due to the role of humic acid as a nutrient supply which increases soil fertility and the availability of nutrients as reported by David *et al*., 1994[58].

Our results are in agreement with Mazhar *et al*., 2012 [57] and Abd El-Al *et al*., 2005 [59] who found that humic acid increases in flowering parameters, these results may be due to the role of humic acid as a source of nutrients and increasing the soil fertility which consequently increased the growth of flowers.

Humic acid increased the soluble sugars; this result could be due to the role of humic acid in repairing plant chlorosis and thus increasing photosynthesis density (Chen and Avaid, 1990[60]).These results were in accordance with those by Zaghloul *et al*., 2009[61].

Proline content was significantly reduced by increasing the level of humic acid which may be due to the role of humic acid in increasing the soil content of organic matter which reduces the harmful effect of stress (Erik *et al*., 2000[62]).

Cd in the plant increased with increasing humic acid and heavy metal concentration, probably due to the formation of chelates from humic acid. Humic compounds can effectively bind to certain elements.

Metal absorption by plants occurs simultaneously with nutrient uptake from soil water. Although high metal concentrations can be toxic to plants, humic acid can bind to such toxic metals in soil water. (Khaled and Fawy, 2011[63]). Application of HA can ameliorate plant stress on tobacco, Corn and Oat, (Mylonas and Mc Cants, 1980 [64]).

This suggests that humic acid may play a role in mitigating the negative effects of cadmium application on the *Orthosiphon stamineus* plant (Mohd *et al*., 2017 [48]). The application of HA in plants under Cd toxicity can reduce total chlorophyll content and photosynthesis; this result may be due to Cd causing an irreversible dissociation of the large and small subunits of ribulose-1,5-bisphosphate carboxylase (RuBPCase), leading to total inhibition of the enzyme and thus reducing plant photosynthesis (Stiborova 1988 [65]) and Malik *et al*., (1992 (66)). According to Mohd *et al*., 2017[48]), the combination of cadmium and humic acid can increase the production of total phenolics in *Dimorphotheca ecklonis.*

Many reports suggested that Cd may stimulate the production of ROS in the mitochondrial electron transfer chain (Heyno *et al*., 2008[67]). A variety of proteins function as scavengers for these ROS including antioxidant isoenzyme. The induction of these isoenzymes is considered to play an important role in the cellular defence against oxidative stress, caused by toxic metal exposure (ElBeltagi *et al*., 2010[68]).

Our results indicated that polyphenol oxidase (PPO) and peroxidase (POD) isoform activities gradually increase with increasing of the Cd concentration. The changes of PPO activity might participate in the defence mechanism in plants against cadmium toxicity (Constabel and Barbehenn, 2008 [69]). The mechanism of peroxidase action in plants exposed to elevated concentrations of heavy metals has not been completely elucidated, nor has the evaluation of the phytotoxicity of metal contaminated soils been performed completely either, PPO and POD activity may play a role in plants' defensive mechanisms against cadmium poisoning and POD activity may be used as a potential biomarker for sublethal metal toxicity in examined plant species (Dunwell *et al*., 2008 [70]).

Our results are in agreement with El-Sayed *et al*., 2019 [71] on *Eucalyptus gomphocephala* plants indicate that peroxidase isozyme expression was decreased with raising cadmium concentration in the soil, (Zheng *et al*., 2010 [72]) on *Glycyrrhiza uralensis* seedlings POD and PPO activity increased gradually with increasing cadmium concentrations.

Thipyapong *et al*., 2007 [73] revealed that PPO and POD relate closely with 'the callus reaction', meanwhile, they also have some relationships with the synthesis of cell compounds containing phenol groups such as lignin, which also agree with Kováčik and Klejdus, 2008[74] and Saffar *et al.*. 2009[75] they found that PPO activity in some plant species was observed under heavy metal stress, and showed a significant increase compared to the control, (Ruiz *et al.*, 1999[76]) raveled that induction of PPO activity might be due to its role in phenolic compound synthesis, which plays an important role in detoxification of heavy metals in plants .

5. Conclusion

From this study, it can be concluded that *Dimorphotheca ecklonis* showed superiority in vegetative growth characteristics and some chemical characteristics including (photosynthetic pigments and total sugars content) when grown in soil contaminated with cadmium at a concentration of 25 mg. kg^{-1} soil. While the values of inflorescence characteristics and RWC% gradually decreased with increasing cadmium concentration, while the values of the rest of the chemical components increased, where the highest activity was at the highest concentration of cadmium 100 mg. kg^{-1} soil. Humic acid treatment at concentration 200 mg. l^{-1} showed an improvement in all vegetative, flowering and chemical components of the plant. Regarding the interaction treatments, it was found that plants grown in soil contaminated with cadmium at a concentration of 25 mg. kg^{-1} soil and sprayed with humic acid at 200 mg. l^{-1} gave the best values in the aforementioned plant characteristics.

Conflicts of interest

There are no conflicts to declare.

6. References

[1] Bailey, L.H. and Bailey, E.Z.. Hortus third: A concise dictionary of plants cultivated in the United States and Canada. New York: Macmillan(1976).

[2] Munz, P.A. 1968. Supplement to a California Flora. University of California. Press , Berkele. 1-224.

[3] Agami R.A. and Mohamed G.F., Exogenous treatment with indole-3-aceticacid and salicylic acid alleviates cadmium toxicity in wheat seedlings. *Ecotoxicol. Environ. Saf.*, **94**, 164-171(2013).

[4] Jianguo L., Kunquan L., Jiakuan X., Jiansheng L., Xiaolong L., Jianchang Y. and Qingsen Z., Interaction of Cd and five mineral nutrients for uptake and accumulation in different rice cultivars and genotypes. *Field Crop Res* **83**, 271–281(2003).

[5] Chien H.F. and Kao C.H., Accumulation of ammonium in rice leaves in response to excess cadmium. *Plant Sci*., **156**,111–115 (2000).

[6] Hussein Kh. and Hassan A.F., Effect of Different Levels of Humic Acids on the Nutrient Content, Plant Growth, and Soil Properties under Conditions of Salinity. *Soil & Water Res*., **6** (1), 21–29 (2011).

[7] Li Y., Fang F. and Wei J., Humic Acid Fertilizer Improved Soil Properties and Soil Microbial Diversity of Continuous Cropping Peanut: A Three-Year Experiment. *Scientific Reports*, **9** (12014), 1-9 (2019).

[8] Zhang Y., Yang X., Zhang S., Tian Y., Guo W., Wang J., The influence of humic acids on the accumulation of lead (Pb) and cadmium (Cd) in tobacco leaves grown in different soils. *Journal of Soil Science and Plant Nutrition*, **13**(1), 43-53 (2013).

[9] Yildirim E., Ekinci M., Turan M., Ağar G., Dursun A., Kul R., Alim Z. and Argin S., Humic + Fulvic acid mitigated Cd adverse effects on plant growth,

physiology and biochemical properties of garden cress. *Scientific Reports*, **11**(8040), 1-8 (2021)

[10] Jackson M.L., Soil Chemical Analysis. Prentice-Hall, Inc. Limited, New York. p. 125-179 (1973).

[11] Turner N.C., Techniques and experimental approaches for the measurements of plant water status. *Plant and Soil*, **58**, 339 – 366(1981).

[12] Saric, M., Katrori R., Curic R., Cupina T. and Gric I., Chlorophyll determination. Univerzitet U. Noveon Sadu Praktikum iz Fiziologize Biljaka-beograd, Hauena Anjiga, pp: 215 (1967).

[13] Dubois M., Gilles K.A., Hamilton J.K., Rebers P.A. and Smith F.F., Colorimetric method for the determination of sugars and related substances. *Anal. Chem*., **28**, 350-356(1956).

[14] Quettier D.C., Gressier B., Vasseur J., Dine T., Brunet C., Luyckx M.C., Cayin J.C., Bailleul F., Trotin F., Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. *J. Ethnopharmacol*., **72**, 35-42 (2000).

[15] Buege, J.A. and Aust, S.D., Microsomal lipid peroxidation. *Meth. Enzymol.*, **52**, 302–310(1972).

[16] Bates L.S., Waldren R.P. and Teare I.D., Rapid determination of free proline for water-stress studies. *Plant and Soi*l, **39** (1), 205–207(1973).

[17] Meuwly P. and Rauser W.E., Alteration of thiol pools in roots and shoots of maize seedlings exposed to cadmium: adaptation and developing cost. *J. Plant Physiol*., **99**, 8-15(1992).

[18] Black C.A., Evans D.D., White J.L., Ensminger L.E. and Clark F.E., Methods of Soil Analysis. Part 2: Chemical and Microbiological Properties. Madison, USA: American Society of Agronomy, 1572p(1965).

[19] Bradford M.M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254(1976).

[20] Thipyapong P., Hunt M.D., Steffens J.C., Systemic wound induction of potato (*Solanum tuberosum*) polyphenol oxidase. *Phytochemistry*, **40**, 673–676(1995).

[21] Barceló A.R., Muñoz R. and Sabater F., Lupin peroxidases. I. Isolation and characterization of cell wall-bound isoperoxidase activity. *Physiol. Plant.*, **71**, 448–454(1987).

[22] Snedecor C.W. and Cochran W.G. Statistical Methods.6th ed. lowaStat Univ., Press, Ames.lowa, USA., 953p(1980)..

[23] Gabrijel O., Davor R., Zed R., Marija R. and Monika Z., Cadmium accumulation by muskmelon under stress in contaminated organic soil. *Sci. Total Environ*., **407**, 2175- 2182 (2009).

[24] Orrono, D. and Lavado, R., Heavy metal accumulation in *Pelargonium hortorum*: Effects on growth and development. *¥OTON: Revista Int. De Botanica Expert Int.*, **78**, 75-84(2009).

[25] Karbassi A.R. and Pazoki M., Environmental qualitative
assessment of rivers sediments. *Global J. Environ. Sci.
Manage.*, **1**(2), 109-116(2015).

[26] Piotrowska A., Bajguz A., Godlewska-Zylkiewicz B. and Zambrzycka E., Changes in growth, biochemical components, and antioxidant activity in aquatic plant *Wolffiaarrhiza* (Lemnaceae) exposed to cadmium and lead. *Arch. Environ. Contam.Toxicol*., **58**, 594-604(2010).

[27] Tiryakioglu M., Eker S., Ozkutlu F., Husted S. and Cakmak I., Antioxidant defense system and cadmium uptake in barley genotypes differing in cadmium tolerance. *J. Trace Elem. Med. Biol.,* **20**, 181-189(2006).

[28] Bah A.M., Dai H., Zhao J. Sun H., Cao F., Zhang G. and Wu F., Effects of cadmium, chromium and lead on growth, metal uptake and antioxidative capacity in *Typha angustifolia*. *Biol. Trace Elem. Res*., **142**, 77-92(2011).

[29] El-Shanhorey N.A. and Emam K.A., Response of *Jatropha curcas* plants to foliar applied ascorbic acid for decreasing the harmful effect of cadmium pollution in the irrigation water. *Alex. J. Agric. Sci.*, **61**(3), 221-235(2016).

[30] El-Shanhorey N.A. and Barakat A.A. Effect of lead and cadmium in irrigation water and foliar applied malic acid on vegetative growth, flowering and chemical composition of *Salvia splendens* plants (b) effect of cadmium. *Scientific j. Flowers & ornamental plants*, **7**(4), 483-499(2020).

[31] El-Shanhorey N.A. and EL-Sayed S.G., The use of *Senecio cineraria* plants sprayed with citric acid for cadmium pollution phytoremediation. *Alexandria Science Exchange Journal*, **38**(2), 343-355(2017).

[32] Benavides M.P., Gallego S.M. and Tomaro M.L., Cadmium toxicity in plants. *Braz. J. Plant Physiol*., **17**, 21-34(2005).

[33] Aidid S.B. and Okamoto H., Responses of elongation growthrate, turgor pressure and cell wall extensibility of stemcells of Impatiens balsamina to lead, cadmium and zinc. *Biometals*, **6**, 245-249(1993).

[34] Raziuddin F., Ghulam H., Akmal M., Shah S.S., Mohammad F., Shafi M., Bakht J. and Zhou W., Effects of cadmium and salinity on growth and

photosynthesis parameters of brassica species. *Pak. J. Bot*., **43**, 333-340(2011).

[35] Hassanein R.A., Abd El-kader A.F. and Faramawy H.M., Alleviation of Cadmium Toxicity in *Triticum aestivum* Using the Coagulant Defatted *Moringa oleifera* and *Moringa peregrine* Seeds Powder. *Egypt. J. Bot*., **56**(3), 573-594(2016).

[36] Perfus-Barbeoch L., Leonhardt N., Vavasseur A. and Forestier C., Heavy metal toxicity: cadmium permeates through calcium channels and disturbs the plant water status. *Plant J*., **32**, 539-548(2002).

[37] Chen L., Han Y., Jiang H., Korpelainen H. and Li C., Nitrogen nutrient status induces sexual differences in responses to cadmium in *Populus yunnanensis*. *J. Exp. Bot*., **62**, 5037-5050(2012).

[38] Agrawal S.B. and Mishra S., Effects of supplemental ultraviolet-B and cadmium on growth, antioxidants and yield of *Pisum sativum* L. *Ecotoxicol. Environ. Saf*.,**72**,610-618(2009).

[39] Rady, M. M. and Hemida, K. A. Modulation of cadmium toxicity and enhancing cadmium-tolerance in wheat seedlings by exogenous application of polyamines. *Ecotoxicol Environ. Saf*., **119**, 178–185 (2015).

[40] Dhir B., Sharmila P. and Saradhi P.P., Hydrophytes lack potential toexhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. *Aquatic Toxicol*, **66**,141-147(2004).

[41] Zengin F.K. and Munzuroglu O., Toxic effects of cadmium (Cd^{++}) on metabolism of sunflower (*Helianthus annuus*L.) seedlings. *Acta Agricul. Scand., Sect. B, Plant Soil Sci.,* **56**, 224-229(2006).

[42] John R.P., Ahmad P., Gadgil K., Sharma S., Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L. *Plant Soil Environ*., **54**(6):262-270(2008).

[43] Kuntal S., Mankad A.U. and Reddy M.N. Cadmium accumulation and its effects on growth and biochemical parameters in *Tagetes erecta* L. *Journal of Pharmacognosy and Phytochemistry*, **6**(3), 111-115(2017).

[44] Verma S. and Dubey R.S., Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. *Biologia Plantarium*, **44** (1),117-123 (2001)

[45] Aldoobie N.F. and Beltagi M.S., Physiological, biochemical and molecular responses of common bean (*Phaseolus vulgaris* L.) plants to heavy metals stress. African Journal of Biotechnology, **12**(29), 4614-4622 (2013).

[46] Thiruvengadam M. and Chung I.M., Selenium, putrescine, and cadmium influence health promoting phytochemicals and molecular level effects on turnip (*Brassica rapa* ssp. rapa). *Food Chem*., **173**, 185-193(2015).

[47] Gursoy N., Sarikurkcu C., Cengiz M., Solak M.H. Antioxidant activities, metal contents, total phenolics and flavonoids of seven *Morchella* species. *Food ChemToxicol*., **47**, 2381-388(2009)

[48] Mohd H.I., Ismail A., Omar H. and Zain N.M., Application effects of cadmium and humic acid on the growth, chlorophyll fluorescence, leaf gasexchange and secondary metabolites in misaikucing (*Orthosiphon stamineus*) benth. *Annual Research & Review in Biology*, **18**(3), 1-14(2017).

[49] Michalak A., Phenolic Compounds and Their Antioxidant Activity in Plants Growing under *Heavy Metal Stress. Polish J. of Environ. Stud.* **15**, 4, 523-530 (2006).

[50] Chaoui A., Mazhoudi S., Ghorbal M.H. and Ferjani E.E.L., Cadmium and zinc induction of lipid peroxidation and effects on antioxidant enzyme activities in bean (*Phaseolus vulgaris* L.). *Plant Science*, **127**, 139-147(1997).

[51] Metwally A., Finkemeier I., Georgi M., Dietz K.J. Salicylic acid alleviates the cadmium toxicity in barley seedlings. *Plant Physiology*, **132**, 272-281(2003).

[52] Li F., Qi J., Zhang G., Lin L., Fang P., Tao A. and Xu J., Effect of Cadmium Stress on the Growth, Antioxidative Enzymes and Lipid Peroxidation in Two Kenaf (*Hibiscus cannabinus* L.) Plant Seedlings. *Journal of Integrative Agriculture*, **12**(4), 610-620(2013).

[53] Chen J., Zhu C., Lin D. and Sun Z.X., The effects of Cd on lipid peroxidation, hydrogen peroxide content and antioxidant enzyme activities in Cd-sensitive mutant rice seedlings. *Can. J. Plant Sci.*, **87**, 49-57(2007).

[54] Sergiev I., Todorova D., Moskova I.,Georgieva N., Nikolova A., Simova S., Polizoev D., Alexieva V., Protective effect of humic acids against heavy metal stress in triticale. *ComptesRendus Acad. Bulgare Sci. Tome*., **66**(1), 53–59 (2012).

[55] Pizzeghello D., Francioso O., Ertani A., Muscolo A. and Nardi S., Isopentenyladenosine and cytokinin-like activity of different humic substances. *J. Geochem. Ex.*, **129**, 70-75(2013).

[56] Topcuoglu, B., The influence of humic acids on the metal bioavailability and phytoextraction efficiency in long term sludge applied soil. Conference on International Research on Food Security, Natural Resource Management and Rural Development. Tropentag, Gottingen, Germany (2012).

[57] Mazhar A.A.M., Shedeed Sh.I., Abdel-Aziz N.G., Mahgoub M.H., Growth, flowering and chemical constituents of *Chrysanthemum indicum* L.

plant in response to different levels of humic acid and salinity. *J. Appl. Sci. Res.*, **8**(7), 3697-3706(2012).

[58] David P.P., Nelson P.V. and Sanders D.C., A humic acid improves growth of tomato seedling in solution culture. *J. Plant Nutr.*, **17**(1), 173-184(1994).

[59] Abd El-Al F., Shafeek M.R., Ahmed A.A. and Shaheen A.M., Response of growth and yield of onion plants to potassium fertilizer and humic acid. *J. Agric. Sci. Mansoura Univ*., **30**(1): 441-452(2005).

[60] Chen Y. and Avaid T., Effect of humic substances on plant growth. Pp. 161-186. In: American Society of Agronomy and Soil Science Society of America (eds.). Humic substances in soil and crop science; selected readings. American Society of Agronom, Madison, WI. (1990).

[61] Zaghloul, S.M., El-Quesni, E.M.F. and Mazhar A.A.M., Influence of potassium humate on growth and chemical constituents of *Thuja orientalis* L. seedlings. *Ozean Journal of Applied Science*, **2**(1): 73-78 (2009).

[62] Erik B.G., Feibert G.C. and Saundres D. L., Evaluation of humic acid and other non ventional fertilizer additions for onion production.Malheur Experiment satiation Oregon State Univeristy. Ontario, OR(2000).

[63] Khaled H. and Fawy A.H., Effect of different levels of humic acids on the nutrient content, plant growth, and soil properties under conditions of salinity. *Soil & Water Res*., **6**(1), 21–29(2011).

[64] Mylonas V.A. and McCants C.B., Effects of humic and fulvic acids on growth of tobacco. I. Root initiation and elongation. *Plant and Soil*, **54** (3), 485–490(1980).

[65] Stiborova M., Cd^{2+} ions effect on the quaternary structure of ribulose-1,5- bisphosphate carboxylase from barley leaves. *Biochemie und Physiologie der Pflanzen.*,**183**, 371–378(1988).

[66] Malik D., Sheoran S. and Singh P., Carbon metabolism in leaves of cadmium treated wheat seedlings. *Plant Physiol Biochem*., **30**, 223–229(1992).

[67] Heyno E., Klose C. and Krieger-Liszkay A., Origin of cadmium-induced reactive oxygen species production: mitochondrial electron transfer versus plasma membrane NADPH oxidase. *New Phytol*., **179**, 687–699(2008).

[68] El-Beltagi H.S., Mohamed A.A. and Rashed M.M., Response of antioxidative enzymes to cadmium stress in leaves and roots of radish (*Raphanus sativus* L.). *Not. Sci. Biol.*, **2**, 76–82(2010).

[69] Constabel C.P. and Barbehenn R., Defensive roles of polyphenol oxidase in plants," in Induced Plant Resistance to Herbivory, ed. A. Schaller (Dordrecht: Springer Science and Business Media B.V.), 253–269(2008). doi: 10.1007/978-1-4020-8182-8_12

[70] Dunwell J.M., Gibbings J.G., Mahmood T. and Saqlan N.S.M., Germin and germin-like proteins: evolution, structure and function. *Crit. Rev. Plant Sci.*, **27**, 342–375(2008). doi: 10.1080/07352680802333938

[71] El-Sayed S.M., Mazhar A.A.M., Mahgoub M.H., Abd El-Aziz N.G., Darwish M.A. and Shanan N.T., Investigation the Effect of L-Tryptophan on Growth and Chemical Composition of *Eucalyptus gomphocephala* Plants under Cadmium Stress. *Middle East Journal of Agriculture Research*, **8**(1), 106-116(2019).

[72] Zheng G., Lv H.P., Gao S. and Wang S.R., Effects of cadmium on growth and antioxidant responses in *Glycyrrhiza uralensis* seedlings. *Plant Soil Environ*., **56**, (11): 508-515(2010)

[73] Thipyapong P., Stout M.J. and Attajarusit J., Functional analysis of polyphenol oxidases by antisense/sense technology. *Molecules*, **12**, 1569–1595(2007).

[74] Kováčik J. and Klejdus B., Dynamics of phenolic acids and lignin accumulation in metal-treated *Matricaria chamomilla* roots. *Plant Cell Reports*, **27**, 605–615(2008).

[75] Saffar A., Bagherieh N.M.B. and Mianabadi M., Activity of antioxidant enzymes in response to cadmium in *Arabidopsis thaliana*. *Journal of Biological Sciences*, **9**(1), 44-50(2009).

[76] Ruiz J.M., García P.C., Rivero R.M. and Romero L. Response of phenolic metabolism to the application of carbendazim plus boron in tobacco. *Physiologia Plantarum*, **106**: 151–157(1999).