Impact of turmeric and carrot extracts on morphological, chemical composition and isozymes patterns of *Azadirachta indica* seedlings under water deficiency conditions

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Background
Azadirachta indica trees are of great importance because of the high-quality wood they produce, which is used in a wide range of industries, and their production of insect repellent oils.
Objective
Due to these great benefits that have drawn attention to them, it was necessary to find environmentally friendly solutions to improve the ability of this plant species to grow in the event of exposure to conditions of water shortage.
Materials and methods
The plants were sprayed with Carrot extract (C) at rates of 50 and 100 ml/ l and/or Turmeric extract (T) at a rate of 20 and 40 ml/l under irrigation intervals every 4 and 8 days. Total chlorophyll content, total sugars content (mg/g F.W.), flavonoids content (mg/g F.W.), total phenols (mg/g F.W.) and total indoles (mg/100 g F.W.) were determined. Also, Peroxidase isozymes (POD) and Polyphenol oxidase isozymes (PPO) were determined.
Results and conclusion
The obtained results confirmed that increasing irrigation periods (8 days) had a negative effect on most vegetative traits except for all root characteristics, and also all chemical properties except the total indoles content and lipid peroxidation, it was also observed that the enzymatic activity of peroxidase isozymes (POD) and polyphenol oxidase isozymes (PPO) compared to plants that were watered every 4 days. The most of growth parameters, chemical composition and the activity of POD in plants irrigated every four days were improved with C treatment at 100 ml/l, while the activity of PPO increased with 100 ml/l C+40 ml/l T. On the other hand, all growth parameters, chemical compositions and POD in plants irrigated every 8 days were increased with 100 ml/l C +20 ml/l T except lipid peroxidation which gave the highest value in control plants, whereas PPO increased with C treatment 50 ml/l+T 40 ml/l.

Keywords:
Azadirachta indica, carrot extract, drought stress, lipid peroxidation, Native-PAGE, neem, turmeric extract

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Introduction

Neem (*Azadirachta indica*) is a tree belonging to the family Meliaceae, is a tropical evergreen related to mahogany [1]. Native to east India and Burma, it grows in much of Southeast Asia and West Africa [2]; trees will reach up to 30 m tall with limbs reaching half as wide. The shiny dark green pinnately compound leaves are up to 30 cm long. Each leaf has 10–12 serrated leaflets that are 7 cm long by 2.5 cm wide. It grows where rainfall is as little, and thrives in areas that experience extreme heat of up to 48°C [3]. The wood is of good quality and has long been used as firewood. Its oil is burned in lamps throughout India. As well as packing boxes. Because its repellent properties help protect the contents from pests. The trunk of the tree is also often used to make posts for buildings or fences, as the wood is termite-resistant [4].

Water stress has a significant impact on plant nutrition, respiration, stomata function and transpiration and seed germination [5–7] Plant responses to water scarcity are often studied at the level of selected physiological parameters, such as water potential, relative water content, stomatal response, photosynthesis, and osmotic adaptation, which have been shown to be good indicators of drought in several studies [8–10]. However, the tolerance conditions in which plants grow from the moment of planting might affect their morphology and physiological response.

In plants, carotenoids which are precursors of vitamin A, play important role as antioxidants in protecting cells from the damaging effects of free radicals and the assembly of photo systems, in light harvesting and in photo protection and as antioxidants in protecting cells from the damaging effects of free radicals [11]. Carrot roots (*Daucus carota* L.) are rich in fibre, minerals, calcium, amino acids, vitamin C, minerals, glucose, and fructose [12,13]. Carrot is a source of carotene, a precursor of vitamin A, which plays a main role in protecting human against cancer and antiaging [14]

Turmeric (*Curcuma spp*.) is a rhizomatous perennial herb in the Zingiberaceae family. It performs a wide range of pharmacological functions, including antioxidant, antibacterial, anticarcinogenic, antifertility, antidiabetic, and antifungal functions [15]. Dry turmeric has a carbohydrate content of 69.43%, a protein content of 6.3%, an oil content of 5.1%, a mineral content of 3.5%, and other elements of 3.5% [16]. This study aims to investigate the effect of spraying carrot and turmeric extracts with different concentrations on morphological, chemical composition and isozymes expression of (neem) *Azadirachta indica* (neem) seedlings under water deficiency conditions.

Materials and methods

The experiment was carried out at Horticulture Research Institute, Agriculture Research Center, Giza, Egypt. During two seasons 2020 and 2021 and the chemical analyses were implemented in the National Research Centre. Seedlings of *Azadirachta indica* 30–40 cm in height were obtained from the nursery of Timber and Forestry Research Department, Horticulture Research Institute. The seedlings were planted on the 1st of March in plastic pots 30 cm (one plant/pot),) filled with 9 kg soil mixture (sand and clay 1:1 by volume) the physical and chemical analysis were shown in Table 1, which was determined according to Jackson [17].

All transplanted seedlings were received water regularly every 4 days for 3 weeks and divided into two groups, the first one irrigated every 4 days and the second one irrigated every 8 days. Both of the groups were treated with the following treatments: control, T 20 ml/l, T 40 ml/l, C 50 ml/l, C 100 ml/l, C 50 ml/l+T 20 ml/l, C 50 ml/l+T 40 ml/l, C100 ml/l+T 20 ml/l and C 100 ml/l+T 40 ml/l.

Carrot and turmeric extracts preparation

Turmeric extract

Dried rhizomes of *C. longa* were purchased from the herbal shop (Giza, Egypt). Dried rhizomes were extracted according to techniques described by Sedky *et al*. [18]. Dried powder (100 g) was soaked in 500 ml 70% ethyl alcohol, and kept in tightly sealed vessels at room temperature for 3 weeks, this mixture was filtered. The extraction of the residue was repeated three times in the same manner until a clear colorless supernatant extraction liquid was obtained. The extracted liquid was filtered and concentrated using rotary evaporator under reduced pressure at 50°C until the solvent was completely removed.

Carrot extract

Carrot roots were extracted according to the techniques described by Abbas and Akladious [19]. Orange carrot roots obtain from the local Markets in Egypt. About 100 g of the washed fresh carrot roots were weighed. It was sliced into tiny pieces and soaked in 160 ml sterile distilled water and 160 ml of ethanol. These were then transferred into separate flask and shaken for 1 hour and filtered with Whatman No. 1 filter paper and the filtrate was adjusted to pH 7.0 with 1 N Na OH. The chemical analysis of turmeric and carrot extracts is presented in Table 2.

Table 1 The physical and chemical properties of the soil mixture

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

Table 2 The chemical properties of orange carrot roots and turmeric extracts

Vitamin C (mg/100 g F.W.)	Total Phenolic (mg/100 g F.W)	Antioxidant % (200 µg. ml-1)	Total Flavonoids (mg/100 g F.W)	Total carotenoids (mg/g F.W)
Carrot (*Daucus carota*) extract				
7.1±2.3	17.5±2.8	60.7±3.5	6.06±1.02	3.94±0.88
Turmeric (Curcuma longa) extract				
1.7±0.17	30.27±0.33	16.55±1.01	14.14±0.23	2.67±0.55

The following vegetative data were recorded. Plant height (cm), Leaf area (three fully expanded leaves were collected and their leaf area (cm^2) was measured by using portable leaf area meter (Model: YMJ-A 20110122-1), stem diameter (cm^2), number of leaves/ plants, number of branches/ plant, fresh and dry weights of leaves, stems and roots (g/plant).

The following chemical analyses were determined. Total chlorophyll content was determined by using TYS-A chlorophyll meter portable according to Yadava [20] total sugars content (mg/ g F.W.) according to Dubois *et al.* [21], flavonoids content (mg/ g F.W.) according to Quettier *et al.* [22], total phenols (mg/ g F.W.) according to Swain and Hillis [23], total indoles (mg/ 100 g F.W.) according to Larsen *et al.* [24].

Antioxidant isozymes expression

Native Poly Acryalmide Gel Electrophoreses (Native-PAGE) was performed to identify isozymes differences between control and treatments in the second season. 1. Peroxidase isozymes (POD) (E.C. 1.11.1.7) in leaf samples were assessed by the procedure defined by Barceló *et al.* [25]. 2. Polyphenol oxidase isozymes (PPO) (E.C. 1.10.3.1) in leaf (100 mg fresh weight) samples were estimated as described by Thipyapong *et al.* [26]. The relative distance (Rf-value) of the bands

Table 3 Effect of irrigation treatment (A) and botanical extracts [carrot (C) and turmeric (T)] (B) on plant height (cm), leaf area (cm^2) and stem diameter (cm) of *A.indica* seedlings

	Irrigation treatments (A)					
	1st Season			2nd Season		
	Plant height (cm)					
Plant extracts (B)	4 days	8 days	Mean	4 days	8 days	Mean
Control	125.37	97.87	111.63	130.67	93.37	112.02
T 20	139.13	101.00	120.06	144.33	109.00	126.67
T 40	132.83	113.38	123.11	137.25	116.50	126.88
C 50	171.33	138.50	154.92	176.12	140.67	158.40
C 100	163.27	142.83	153.05	170.33	146.38	158.36
C 50+T20	150.17	129.50	139.84	154.00	133.00	143.50
C 50+T 40	147.00	120.67	133.84	151.12	120.50	135.81
C 100+T 20	155.45	156.50	155.98	157.00	161.33	159.17
C 100+T 40	122.00	90.00	106.00	127.67	102.00	114.84
Mean	145.17	121.14		149.83	124.75	
LSD at 5%	A: 1.17	B: 2.48	A*B: 3.50	A: 2.37	B: 5.02	A*B: 7.10
	Leaf area (cm^2)					
Control	330.83	286.38	308.61	317.63	268.02	292.83
T 20	375.94	343.94	359.94	360.07	325.74	342.91
T 40	336.25	313.02	324.64	320.10	286.06	303.08
C 50	390.00	361.82	375.91	376.22	344.74	360.48
C 100	394.53	369.02	381.78	385.30	351.62	368.46
C 50+T20	381.32	324.74	353.03	362.62	293.10	327.86
C 50+T 40	352.92	291.27	322.10	331.08	274.24	302.66
C 100+T 20	384.10	370.12	377.11	371.82	354.79	363.31
C 100+T 40	297.63	277.49	287.56	282.00	261.15	271.58
Mean	360.40	326.40		345.20	306.60	
LSD at 5%	A: 2.10	B: 4.46	A*B: 6.31	A: 2.76	B: 5.85	A*B: 8.27
	Stem diameter (cm)					
Control	1.24	1.11	1.18	1.22	1.07	1.15
T 20	1.29	1.25	1.27	1.30	1.25	1.28
T 40	1.33	1.14	1.24	1.38	1.12	1.25
C 50	1.52	1.29	1.41	1.57	1.27	1.42
C 100	1.59	1.31	1.45	1.63	1.33	1.48
C 50+T20	1.37	1.22	1.30	1.42	1.19	1.31
C 50+T 40	1.42	1.17	1.30	1.51	1.14	1.33
C 100+T 20	1.47	1.37	1.42	1.55	1.46	1.51
C 100+T 40	1.22	1.11	1.16	1.17	1.09	1.13
Mean	1.38	1.22		1.42	1.21	
LSD at 5%	A: 0.03	B: 0.06	A*B: 0.08	A: 0.02	B: 0.04	A*B: 0.06

on the gel was calculated as described by (Manganaris and Alston, [27] using rf = 1.0, distance to the fastest band, and Rf = 0.0, the starting point.

Experiment layout and statistical analysis

The experimental layout was set in factorial experiment in complete block design with two irrigation intervals and sprayed with 9 rates of carrot and/ or turmeric extracts to give 18 treatments with 3 replicates for each season. The obtained results were subjected to statistical analysis by using least significant differences (LSD) at 5% level according to method described by Snedecor and Cochran [28].

Results and discussion

Vegetative growth

The results recorded in Tables 3–6 during the two seasons showed that significant decrease was detected in all above-ground vegetative including plant height, stem diameter, leaf area, leaves number/plant, number of branches/ plants, leaves and stems (fresh and dry weights) as a result of increasing intervals between irrigation. The highest values for all these parameters were obtained due to the irrigation every 4 days which gave (145.17 and 149.83 cm) plant height, (360.40 and 345.20 cm^2) leaf area, (1.38 and 1.42 cm) stem diameter, (50.48 and 52.56) number of

Table 4 Effect of irrigation treatment (A) and botanical extracts [carrot (C) and turmeric (T)] (B) on number of leaves plant, number of branches/ plant and root length (cm) of *A. indica* seedlings

	Irrigation treatments (A)					
	1st Season			2nd Season		
	Number of leaves/ plants					
Plant extracts (B)	4 days	8 days	Mean	4 days	8 days	Mean
Control	39.00	32.67	35.84	36.67	34.00	35.34
T 20	51.33	44.67	48.00	54.00	45.00	49.50
T 40	43.67	37.33	40.50	41.00	35.33	38.17
C 50	58.67	47.00	52.84	66.67	47.67	57.17
C 100	67.67	48.33	58.00	73.33	51.00	62.17
C 50+T20	55.33	39.33	47.33	58.33	38.67	48.50
C 50+T 40	46.67	34.33	40.50	45.67	34.33	40.00
C 100+T 20	56.00	51.00	53.50	62.67	52.67	57.67
C 100+T 40	36.00	27.67	31.84	34.67	31.33	33.00
Mean	50.48	40.29		52.56	41.11	
LSD at 5%	A: 2.59	B: 5.50	A*B: 7.78	A: 2.08	B: 4.41	A*B: 6.24
	Number of branches/ plants					
Control	2.33	0.00	1.17	3.00	1.33	2.17
T 20	3.00	1.33	2.17	4.33	2.00	3.17
T 40	2.67	1.33	2.00	4.33	1.67	3.00
C 50	5.00	2.67	3.84	7.00	4.33	5.67
C 100	5.33	3.33	4.33	7.67	5.67	6.67
C 50+T20	3.67	2.33	3.00	6.00	3.67	4.84
C 50+T 40	3.33	1.67	2.50	6.00	2.00	4.00
C 100+T 20	4.67	3.67	4.17	6.33	6.00	6.17
C 100+T 40	1.67	1.00	1.34	2.00	1.67	1.84
Mean	3.52	1.93		5.18	3.15	
LSD at 5%	A: 0.51	B: 1.08	A*B: 1.53	A: 0.63	B: 1.33	A*B: 1.88
	Root length (cm)					
Control	40.00	44.17	42.09	39.90	46.00	42.95
T 20	31.42	38.50	34.96	32.67	36.33	34.50
T 40	26.48	41.00	33.74	25.48	42.39	33.94
C 50	44.90	51.04	47.97	48.30	55.17	51.74
C 100	52.00	55.80	53.90	55.33	58.50	56.92
C 50+T20	47.21	61.20	54.21	51.00	63.67	57.34
C 50+T 40	29.00	31.39	30.20	28.80	31.33	30.07
C 100+T 20	59.30	64.81	62.06	62.67	69.20	65.94
C 100+T 40	38.32	46.00	42.16	35.33	50.32	42.83
Mean	40.96	48.21		42.16	50.32	
LSD at 5%	A: 2.30	B: 4.87	A*B: 6.89	A: 1.91	B: 4.06	A*B: 5.74

leaves/plant, (3.52 and 5.18) number of branches/ plant, (75.43 and 79.37 g/ plant) leaves fresh weight, (126.40 and 129.10 g/ plant) stem fresh weight, (29.16 and 30.64 g/ plant) leaves dry weight and (61.27 and 62.62 g/ plant) stem dry weight respectively, in the 1st and 2nd season. Water stress could restrict internode elongation and leaf expansion through inhibiting cell expansion [29,30]. Also, drought causes losses water content which reduces turgor pressure in cell, thereby inhibiting enlargement and division of cell causing reduction of plant growth and dry matter accumulation [31]. Water stress is inevitably associated with increased oxidative stress due to enhanced accumulation of ROS, particularly O_2 and H_2O_2 in chloroplasts, mitochondria and peroxisomes. As a result, the induction of antioxidant enzyme activities is a general adaptation strategy that plants use to overcome oxidative stresses [10,32]. According to Kapor [33] growth is one of the physiological most sensitive to drought due to the cellular turgor, which in turn affects the cellular expansion, restricting the growth and development of plants.

The reduction in the growth might be due to a decrease in cell elongation caused by the inhibiting impact of water deficit on growth stimulating hormones which, in turn, lead to reduce in cell turgor, volume, and eventually growth [34].

Table 5 Effect of irrigation treatment (A) and botanical extracts [carrot (C) and turmeric (T)] (B) on leaves, stems and roots fresh weight (g/ plant) of *A. indica* seedlings

	Irrigation treatments (A)					
	1st Season			2nd Season		
	Leaves fresh weight (g/ plant)					
Plant extracts (B)	4 days	8 days	Mean	4 days	8 days	Mean
Control	58.70	39.92	49.31	61.02	43.76	52.39
T 20	80.22	65.42	72.82	86.29	68.31	77.30
T 40	60.47	46.06	53.27	64.52	52.37	58.45
C 50	94.84	70.49	82.67	97.38	73.88	85.63
C 100	98.32	73.47	85.90	102.65	77.08	89.87
C 50+T20	85.24	52.33	68.79	90.08	58.89	74.49
C 50+T 40	68.04	40.45	54.25	69.72	47.59	58.66
C 100+T 20	91.07	77.89	84.48	93.44	82.60	88.02
C 100+T 40	42.00	35.91	38.96	49.26	39.03	44.15
Mean	75.43	55.77		79.37	60.39	
LSD at 5%	A: 1.66	B: 3.52	A*B: 4.97	A: 1.96	B: 4.16	A*B: 5.88
	Stems fresh weight (g/ plant)					
Control	108.21	72.45	90.33	105.06	76.99	91.03
T 20	119.41	87.33	103.37	121.89	90.95	106.42
T 40	112.75	81.73	97.24	114.44	86.12	100.28
C 50	146.34	115.28	130.81	150.24	118.74	134.49
C 100	151.75	121.71	136.73	154.62	124.33	139.48
C 50+T20	133.84	110.70	122.27	141.71	110.29	126.00
C 50+T 40	125.22	95.28	110.25	129.69	93.48	111.59
C 100+T 20	138.41	130.12	134.27	145.38	135.65	140.52
C 100+T 40	101.28	76.68	88.98	98.67	82.25	90.46
Mean	126.40	99.03		129.10	102.10	
LSD at 5%	A: 3.01	B: 6.38	A*B: 9.02	A: 2.17	B: 4.60	A*B: 6.51
	Roots fresh weight (g/ plant)					
Control	41.89	48.82	45.36	45.33	51.94	48.64
T 20	30.74	39.18	34.96	37.38	42.47	39.93
T 40	23.65	43.39	33.52	25.47	47.00	36.24
C 50	49.50	57.33	53.42	54.26	66.29	60.28
C 100	63.38	69.26	66.32	70.74	75.50	73.12
C 50+T20	53.43	76.47	64.95	61.33	82.38	71.86
C 50+T 40	26.46	27.94	27.20	29.18	33.67	31.43
C 100+T 20	71.28	85.00	78.14	77.00	88.67	82.84
C 100+T 40	34.64	52.29	43.47	40.33	58.00	49.17
Mean	43.89	55.52		49.00	60.66	
LSD at 5%	A: 2.49	B: 5.28	A*B: 7.47	A: 1.76	B: 3.74	A*B: 5.28

Unlike all the prementioned vegetative growth parameters, root length and fresh and dry weights showed an opposite trend as they gradually decreased with the irrigation levels, which were sloping upward. With a higher root system, the lower water supply causes the root system to penetrate deeper and extend wider in the soil. Researchers are looking for moisture in the lower root system [35]. This result was on line with those reported by Hanafy [36], El-Sanatawy *et al.* [37], Elsayed *et al.* [38], and El-Sayed *et al.* [39].

As regard, the data in the same Tables illustrated those botanical extracts (carrot [C] and Turmeric [T]) had different effects on all-vegetative growth parameters. Carrot and turmeric treatments in all concentrations together or alone, have increased the values of all aforementioned except number of branches/plant and root length in the 1st season, and plant height and root fresh weight in the second season by treated seedlings with C at 100 ml/l+T at 40 ml/l and C at 100 ml/l+T at 20 ml/l gave the highest values of plant height, root length and fresh and dry weights of root in both seasons and stem diameter and (fresh and dry weights of stems) in the 2nd season only. While C at 100 ml/l reflected the highest values of number of leaves/plants, number of branches/ plant and fresh and dry weight of leaves in the two seasons and stem diameter and fresh and dry weights of stems in the 1st season.

Table 6 Effect of irrigation treatment (A) and botanical extracts [carrot (C) and turmeric (T)] (B) on leaves, stems and roots dry weight (g/ plant) of *A. indica* seedlings

	Irrigation treatments (A)					
	1st Season			2nd Season		
	Leaves dry weight (g/ plant)					
Plant extracts (B)	4 days	8 days	Mean	4 days	8 days	Mean
Control	21.67	14.06	17.87	22.50	15.42	18.96
T 20	31.15	24.54	27.85	33.46	25.61	29.54
T 40	22.59	16.67	19.63	24.10	18.95	21.53
C 50	37.67	26.80	32.24	38.66	28.77	33.72
C 100	39.29	28.07	33.68	40.95	29.45	35.20
C 50+T20	33.34	19.16	26.25	35.23	21.56	28.40
C 50+T 40	25.70	14.38	20.04	26.33	16.91	21.62
C 100+T 20	35.95	30.01	32.98	36.88	31.78	34.33
C 100+T 40	15.05	12.59	13.82	17.65	13.69	15.67
Mean	29.16	20.70		30.64	22.46	
LSD at 5%	A: 2.38	B: 5.05	A*B: 7.59	A: 1.73	B: 3.68	A*B: 5.20
	Stems dry weight (g/ plant)					
Control	50.80	32.00	41.40	49.33	33.93	41.63
T 20	57.35	40.31	48.83	58.51	41.98	50.25
T 40	53.23	37.02	45.13	54.11	39.18	46.65
C 50	72.64	55.14	63.89	74.46	56.70	65.58
C 100	75.72	58.76	67.24	77.20	59.94	68.57
C 50+T20	65.49	52.11	58.80	69.25	51.91	60.58
C 50+T 40	60.67	44.31	52.49	62.83	43.47	53.15
C 100+T 20	68.25	63.39	65.82	71.67	66.02	68.85
C 100+T 40	47.31	34.15	40.73	46.18	36.63	41.41
Mean	61.27	46.35		62.62	47.75	
LSD at 5%	A: 2.50	B: 5.31	A*B: 7.50	A: 1.79	B: 3.80	A*B: 5.37
	Roots dry weight (g/ plant)					
Control	20.40	24.03	22.22	22.08	25.61	23.85
T20	14.60	18.96	16.78	17.76	20.55	19.16
T40	10.91	21.31	16.11	11.73	23.08	17.41
C50	24.46	28.74	26.60	26.79	33.18	29.99
C100	31.89	35.07	33.48	35.78	38.32	37.05
C 50+T20	26.75	39.40	33.08	30.65	42.43	36.54
C 50+T 40	12.27	13.08	12.68	13.53	15.87	14.70
C 100+T 20	36.47	43.98	40.23	39.29	45.99	42.64
C 100+T 40	16.59	26.01	21.30	19.39	28.86	24.13
Mean	21.59	27.84		24.11	30.43	
LSD at 5%	A: 1.87	B: 3.96	A*B: 5.60	A: 1.62	B: 3.44	A*B: 4.86

The previous results may be due to turmeric Curcuma longa contains 2–9% curcuminoids, which contain 60% curcumin, curcumin oxidation yield vanillin. The essential role of turmeric extracts in stimulating cells division, the biosynthesis of organic compounds and the resistance of plants to all stresses Zia *et al.* [40]. These results are in agreement with Mahmoud and Ahmed [41]. The HPLC analysis of carrot root revealed that its extract contains a high concentration of vitamin A as beta carotene, protein, carbohydrates, fat, vitamins B1, B2, B6, C, D, and E, all of which are antioxidants that protect cells from free radicals [42]. The results were in agreement with Paine *et al.* [43], Abbas and Akladiousa [19], and Abdel Latef *et al.* [44].

Concerning the effect of interaction, the high values of leaf area (cm^2), stem diameter (cm), number of leaves/ plant, number of branches/ plant and fresh and dry weights of leaves and stems (g/ plant) were obtained by carrot extract at 100 ml/l and irrigated every 4 days, which gave (394.53, 1.59, 67.67, 5.33, 98.32, 151.75, 39.29, and 75.72) in the 1st season, respectively, (385.30, 1.63, 73.33, 7.67, 102.65, 154.62, 40.95, and 77.20) in the second season, respectively.

In this respect, C at 100 ml/l+T at 20 ml/l gave the highest values of root length and fresh and dry weight of roots when irrigated every 8 days which showed (64.81 and 69.20 cm) root length, (85.00 and 88.67 g/ plant) root fresh weight and (43.98 and 45.99 g/ plant) root dry weight, in the first and second seasons, respectively.

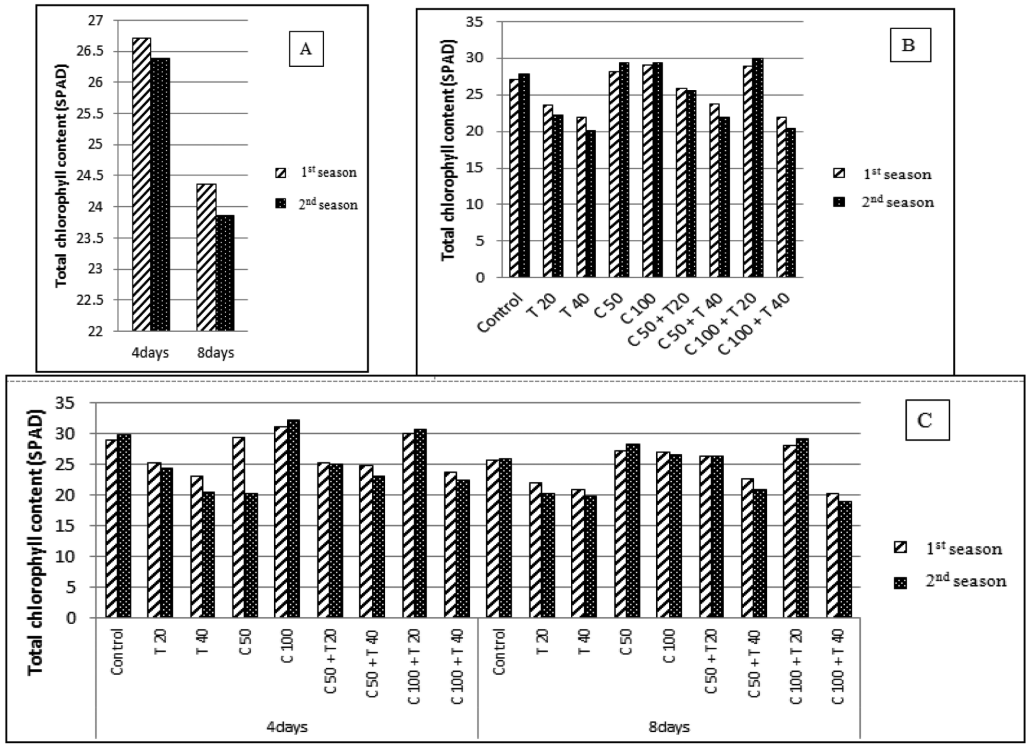
Chemical constituents

Total chlorophyll (SPAD) and total sugars content (mg g^{-1} F.W)

The results presented in Figs. 1 and 2 indicate that the highest contents of total chlorophyll were (26.70 and 26.38) and total sugars content (7.84 and 7.43 mg g^{-1} F.W.) in first and second seasons, respectively, were obtained at irrigation intervals of 4 days. Increasing irrigation intervals to 8 days resulted in steady significant reductions in the total chlorophyll contents (24.35 and 23.85) and total sugars content (6.13 and 6.12 mg g^{-1} F.W.) in the two seasons, respectively.

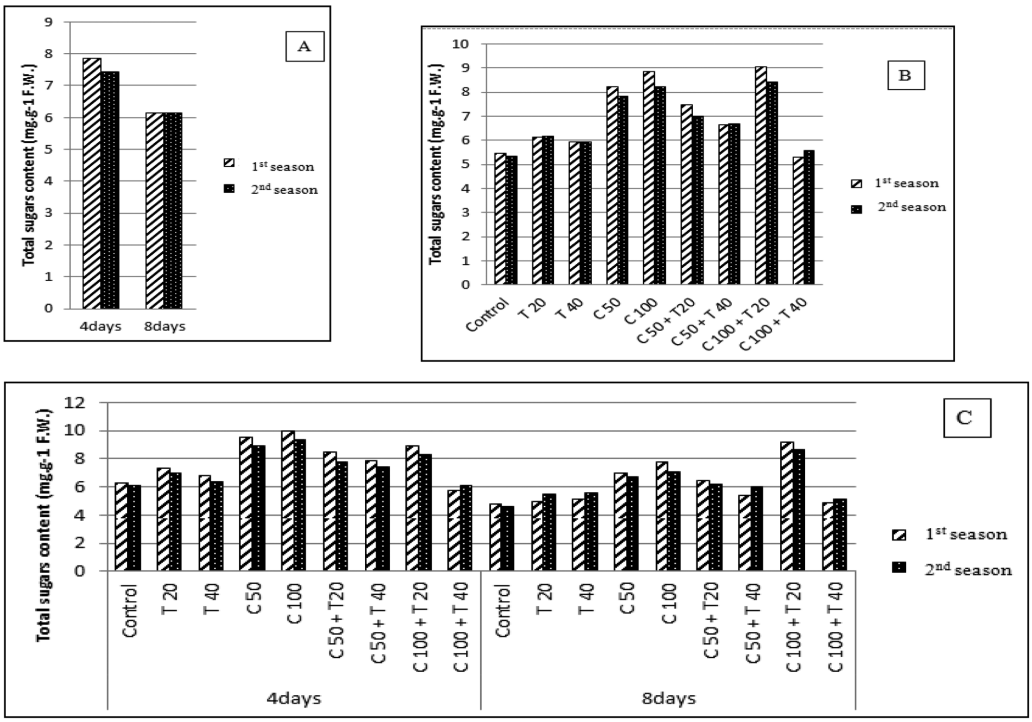
Decrease in photosynthetic activity due to drought may also due to reduce ability of stomatal movement Marci'nska *et al.* [45]. Acute drought stress conditions also cause the damage to Rubisco enzyme and other enzymes associated with photosynthesis and are responsible for loss of photosynthesis pigment content [46]. Our results are in the same line as El-Shanhorey and Sorour, [47], Nahed *et al.* [48], Elsayed

Figure 1



Effect of irrigation treatment (a), botanical extracts [carrot (C) and turmeric (T)] (b) and their interaction (c) on total chlorophyll content in leaves of *A. indica* seedlings.

Figure 2



Effect of irrigation treatment (a), botanical extracts (carrot (C) and turmeric (T)) (b) and their interaction (c) on total sugars content (mgg^{-1} F.W.) in leaves of *A.indica* seedlings.

et al. [38], and El-Sayed *et al.* [39]. Also, the reduction in total sugar% may be due to the fact that during the course of drought stresses tolerance mechanism [49]. Our results are in harmony with the results obtained by Mazher *et al.* [50], Romaisa *et al.* [51], Deligöz and Bayar [52], and Chaturved *et al.* [53].

As for the effect of botanical extracts on the total chlorophyll and total sugars content, data showed that the highest values of total sugars content which gave (9.03 and 8.41 mg g $^{-1}$ F.W.) in both seasons, respectively, and total chlorophyll in the second season (29.87) were obtained by carrot at 100 ml/l +turmeric at 20 ml/l as compared with the untreated plants and the other treatments, while the highest value of total chlorophyll content (28.94) in the first season was obtained by carrot at 100 ml/l. The previous results illustrated that carrot or/ and turmeric extract enhanced chlorophyll content and sugars content. Increasing in the chlorophyll content by carrot treatments may be due to ascorbic acid (one of the components in carrot root extract), is very important for the regulation of photosynthesis, and due to its antioxidants properties [19,54]. Carotenoids, which as major components in carrot root extract, are essential in photosynthesis where they function as energy carriers. The effect of carrot and its extracts may be a result of a variety of interaction factors with chemical properties capable of increasing pigment content. [55]. Carrot root extract contains auxins and cytokinins which have a beneficial effect on carbohydrates accumulation [56,57]. Also, phosphorus, is a component in carrot root extract, plays a role in increasing water-use efficiency, improving leaf expansion, improving photosynthetic surface area, and carbohydrate utilization [58]. In addition, micronutrients such as Fe, Cu, Zn, and Mn (which present in carrot root extract) assist in the formation of chlorophyll, cell division and growth, carbohydrate formation, as well as the maintenance of plant's enzyme system.

Regarding the effect of interaction, the highest values of total chlorophyll content (30.95 and 32.05) and total sugars content (9.96 and 9.35 mg g^{-1} F.W.) were obtained in plants treated with carrot extract at 100 ml/l and irrigated every 4 days in the 1st and 2nd seasons, respectively. Under irrigation every 8 days, the highest values of total chlorophyll content (27.84 and 29.09) and total sugars content (9.17 and 8.59 mg g^{-1} F.W.) were obtained by carrot at 100 ml/l +turmeric at 20 ml/l as compared with the untreated plants and the other treatments under the same irrigation level.

Total flavonoids content (mg g^{-1} F.W.), total phenols (mg g^{-1} F.W.) and total indols (mg 100 g^{-1} F.W).

From the given data in Figs. 3–5 it can be concluded that decreasing irrigation intervals caused an increase in total flavonoids content in both seasons. The highest values of total flavonoids content (7.56 and 8.23 mg g^{-1} F.W.) and total phenols content (3.61 and 3.79 23 mg g^{-1} F.W.) were obtained by irrigation intervals every 4 days in the two seasons, respectively. On the other hand, total indoles content was increased as the irrigation level was sloping downward. The highest values of total indoles content (6.89 and 7.22 mg 100 g^{-1} F.W.), respectively, in the 1st and 2nd seasons were obtained by irrigating every 8 days compared with other treatments. Antioxidants are the first line of defence against free radical damage. There are several compounds which contribute to the antioxidative properties; these include polyphenols, vitamin C, flavonoids, and carotene [59,60].

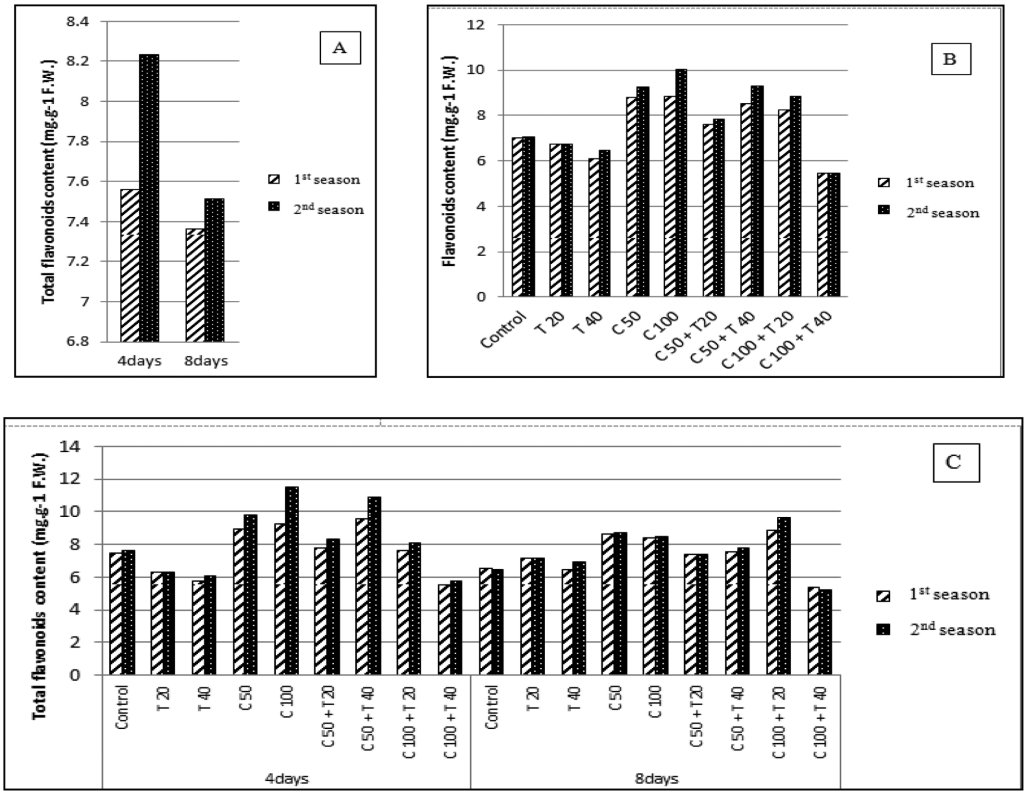
Regarding the effect of botanical extracts on total flavonoids, total phenols and total indoles content, C extract at 100 ml/l gave the highest values of total flavonoids content (8.81 and 9.99 mg g^{-1} F.W.), C extract at 50 ml/l gave the highest values of total phenols (4.48 and 4.65 mg g^{-1} F.W.) and C extract at 50 ml/l+T extract at 20 ml/l gave the highest values of total indoles (8.89 and 9.25 mg100 g^{-1} F.W.), respectively, in first and second season. Flavonoids are known to be synthesized as a response to different environmental stimuli; their ability to act as antioxidant depends on the reduction potentials of their radicals and accessibility of radicals [61]. It has been found that there is a considerable increase in flavonoid levels following abiotic and biotic stress.

Regarding the interaction between watering intervals and the application of plant extracts the highest values of flavonoids in the 1st season and total phenol content in both seasons were provided when treated plants with C extract at 50 ml/l+T at 40 ml/l, which irrigated every 4 days, whereas the highest value of flavonoids was obtained from plant irrigated every 4 days and treated with C extract at 100 ml/l. The highest values of total indoles content were obtained by C extract at 100 ml/l+T extract at 20 ml/l and irrigated every 8 days in both seasons.

Lipid peroxidation (MDA) (μg g^{-1} F.W)

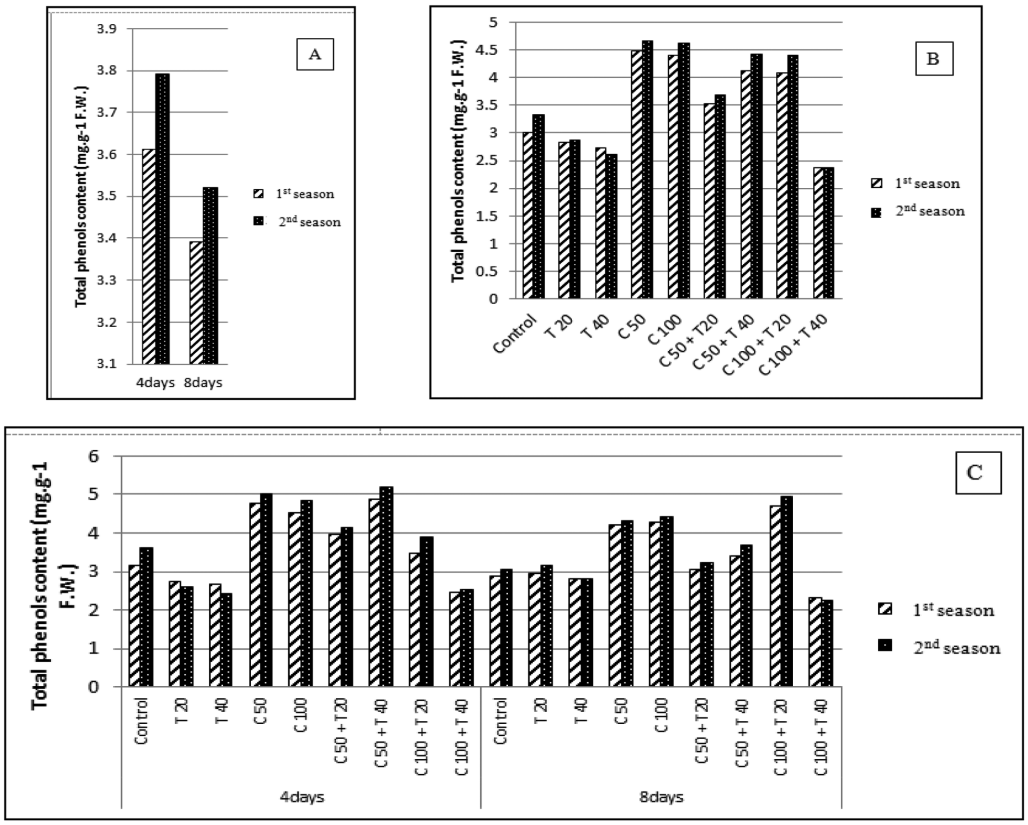
Data recorded in Fig. 6 Illustrated that the *A. indica* plants irrigated every 8 days produced the highest content of MDA giving values of 1.70 and

Figure 3



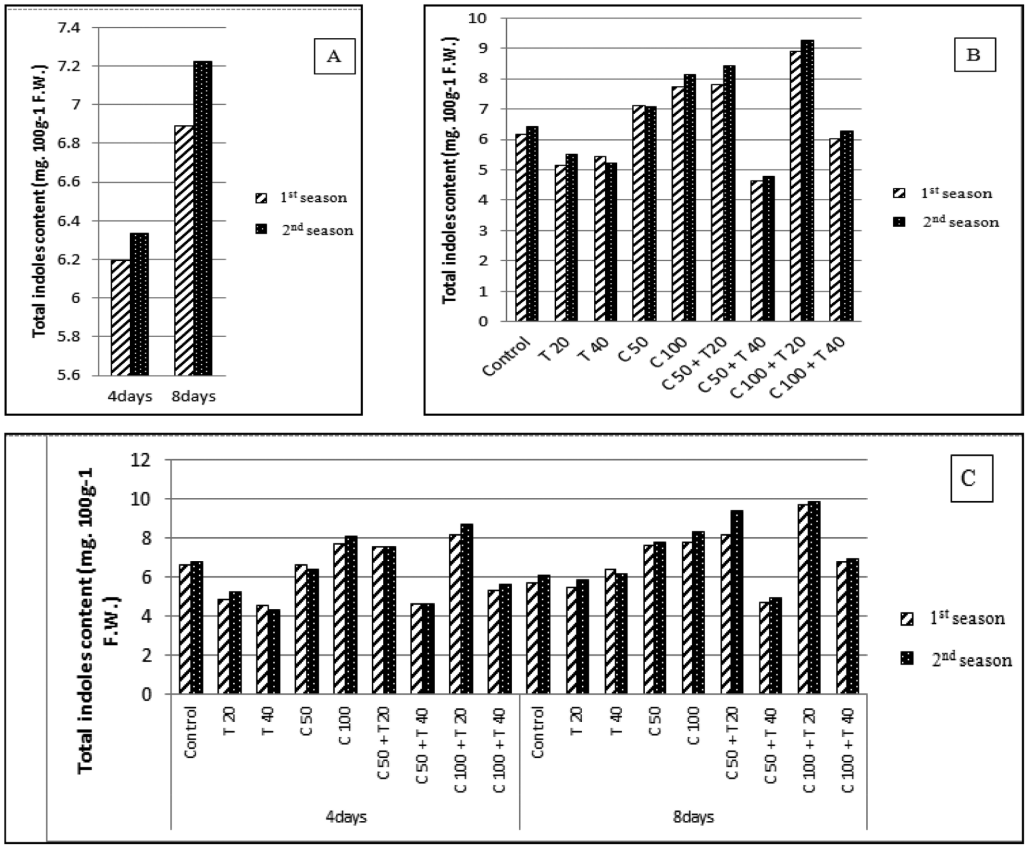
Effect of irrigation treatment (a), botanical extracts (carrot (C) and turmeric (T)) (b) and their interaction (c) on total flavonoids content (mgg^{-1} F.W.) in leaves of *A.indica* seedlings.

Figure 4



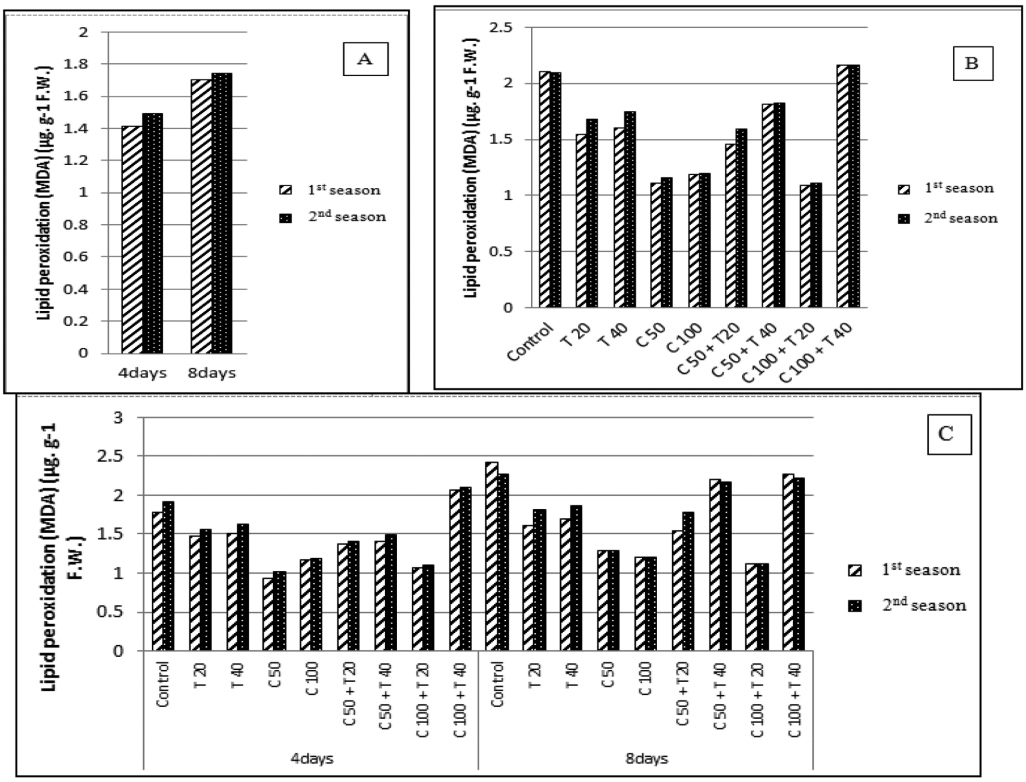
Effect of irrigation treatment (a), botanical extracts (carrot (C) and turmeric (T)) (b) and their interaction (c) on total phenols content (mgg^{-1} F.W.) in leaves of *A.indica* seedlings.

Figure 5



Effect of irrigation treatment (a), botanical extracts (carrot (C) and turmeric (T)) (b) and their interaction (c) on total indoles content (mg 100 g^{-1} F. W.) in leaves of *A. indica* seedlings.

Figure 6



Effect of irrigation treatment (a), botanical extracts (carrot (C) and turmeric (T)) (b) and their interaction (c) on lipid peroxidation (MDA) (µg g^{-1} F.W.) in leaves of *A.indica* seedlings.

1.74 µg g^{-1} F.W., respectively, in both seasons. MDA is a product of peroxidation of unsaturated fatty acids in phospholipids, and lipid peroxidation level is used as indicator of free radicals damage to cell membranes under stress conditions [62,63]. Under drought stress condition, MDA content increased in leaves and roots with increase in the duration of stress. Increase in MDA level under water deficit stress where that water stress could cause highly lipid peroxidation in membranes through ROS production [64]. Leaves displayed higher accumulated MDA than roots. In harmony with these results were those obtained by Guo *et al.* [65] and Sharma *et al.* [66].

Concerning the effect of botanical extracts, plants sprayed with C extract at 100 ml/l+T extract at 40 ml/l gave the highest significant MDA, which showed the same value (2.16 µg g^{-1} F.W.), in both seasons compared with the other treatments and the untreated plants.

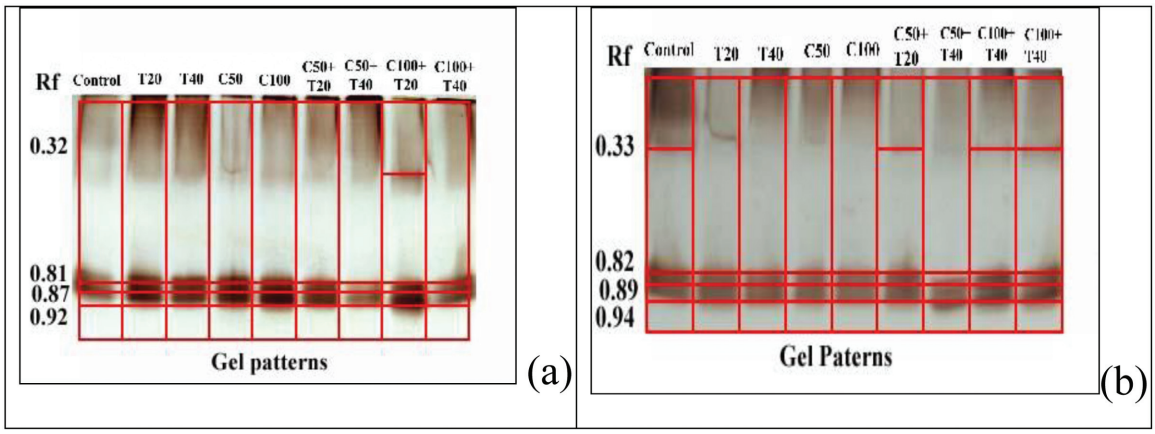
Regarding the interaction, the recorded data clarified that the untreated plants gave the highest values of MDA, followed by plants sprayed with C extract at 100 ml/l+ T extract at 40 ml/l when irrigated every 8 days in both seasons.

Antioxidant isozymes expression

Peroxidase isozyme

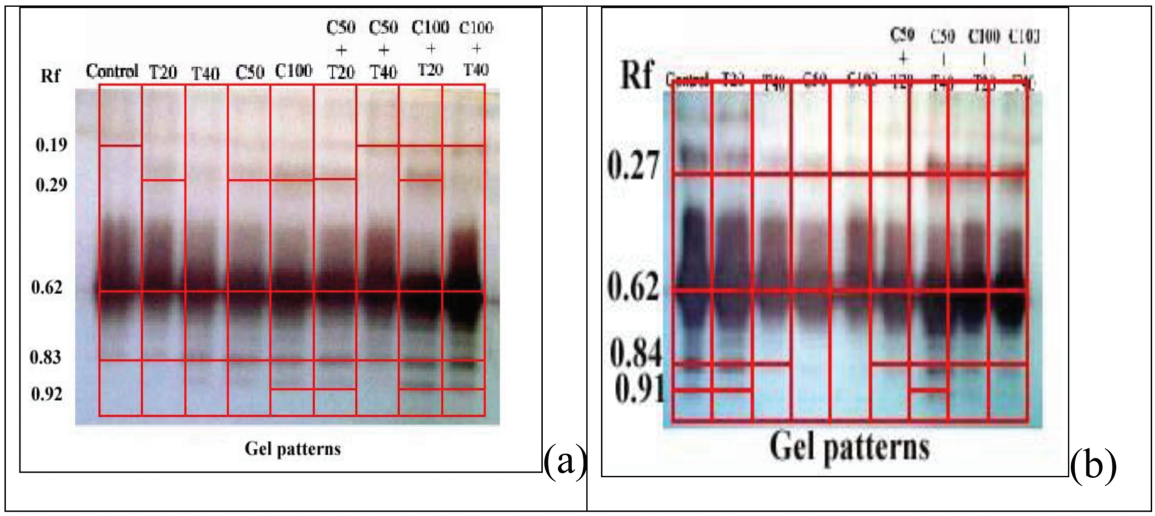
Data in Fig. 7a, b patterns of POD isoforms in leaves with irrigation every 4 days as determined by activity staining in nondenaturating polyacrylamide gels. When comparing all treatments depending on the number of isoforms showed two Pod isoforms were found in treatments (control, T20, T40, C50, C100, C50+ T20, C50+T40, and C100+ T40), while treatment C50+T20 showed 3 POD isoforms, depend on intensity of band treatments C100, C50, and C100+T20 showed the highest value intensity activity, respectively, while treatments C100+T40, Control and T40 recorded the lowest intensity value respectively. Irrigation every 8 days revealed distinct patterns in POD activity in treated and control seedlings. Results showed that treatments (Control, C100+T 20, C100+T40, C50+T 20, and C100+T40) produced four isoforms of POD, while the other treatments produced three isoforms. Treatment C100+T40 recorded the lowest value. Results showed that treatments (Control, C100+T20, C100 +T40, C50+T20, and C100+T40) produced four isoforms of POD, while the other treatments produced three isoforms. Treatment C100+T40 recorded the lowest value. We found that the band

Figure 7



Isozyme banding patterns of peroxidase, where (a): irrigation every 4 days (b): irrigation every 8 days. Control; T extract 20 ml/l; T extract 40 ml/l; C extract 50 ml/l; C extract 100 ml/l, C extract 100 ml/l; C extract 50 ml/l +T extract 20 ml/l: C extract 50 ml/l +T extract 40 ml/l; C extract 100 ml/l +T extract 20 ml/l and C extract 100 ml/l +T extract 40 ml/l.

Figure 8



Isozyme banding patterns of polyphenol where (a): irrigation every 4 days, (b): irrigation every 8 days. Control; T extract 20 ml/l; T extract 40 ml/l; C extract 50 ml/l; C extract 100 ml/l, C extract 100 ml/l; C extract 50 ml/l +T extract 20 ml/l: C extract 50 ml/l +T extract 40 ml/l; C extract 100 ml/l +T extract 20 ml/l and C extract 100 ml/l +T extract 40 ml/l.

intensity in treatment C100+T20 had the highest intensity value, followed by C100 and then C50. The lowest value was obtained from C100+T40 during the treatments (Fig. 8a, b).

Polyphenol oxidase isozyme

The increase of band intensity and appearance of new bands may be an indication of an increase in isozymes POD and PPO activity under drought conditions, based on our results we can reveal that the activity of peroxidase and polyphenol oxidase isozymes increased in all treatments with increasing irrigation periods (4 and 8 days, respectively), but at different rates based on the types of plant extracts and their concentrations, such as a result of the water stress to which the seedlings were exposed in the case of peroxidase enzyme. Our results agree with those of Elsayed *et al.* [38] and El-Sayed *et al.* [39] they said that drought stress causes oxidative stress through the generation of ROS, for example, $O_2\bullet$, hydroxyl radicals ($OH\bullet$), one oxygen ($1O_2$), and H_2O_2, which drive a plant to increase the induction of enzymatic activity to protect cells from oxidative damage. PPO are an important class of specialized metabolites that play physiological roles within the plant such as responses to various environmental stresses. The

phenylpropanoid biosynthesis pathway is usually activated under adverse environmental conditions such as drought, leading to the accumulation of various phenolic compounds according to LiniÌ c *et al.* [67] and Šamec *et al.* [68].

The studied results indicated that the foliar sprays of C extract (50 ml/l and 100 ml/l), followed by foliar application treatments C100 +T 20 then C 50+T 20 gave the best positive enzymatic activity for the seedling as compared with all treatments at irrigation every 4 and 8 days and thus improved the morphological characteristics while foliar application with T extract at 40 ml/l alone or with C extract get negative enzymatic activity these resulted is agreement with Commisso *et al.* [69]; Sharma *et al.* [66] they said that activation of plant antioxidant systems as a result of stimulation of the phenylpropanoid biosynthetic pathway that induces the synthesis of phenolic acids Mattila and Kumpulainen [70] revealed that a carrot extract is an important source of several phenolic compounds (p-hydroxybenzoic acid, protocatechuic acid, vanillic acid, P-coumaric acid, and ferulic acid) that play a role in the effectiveness of powerful antioxidants enzymes that mediate the elimination of oxygen species. play an important role. Plant response activity (ROS) to different abiotic stresses including drought, Kiokias *et al.* [71] and Parvin *et al.* [72] found that Vanillic acid increases tolerance to abiotic stressors by enhancing osmolyte accumulation (e.g., proline), ion regulation (increases K, Ca, and Mg), and antioxidant enzyme activity, on the other hand, Yang *et al.* [73] illustrated turmeric (*Curcuma zedoaria*) extract contains essential oils, polysaccharides, and substances that can act as antioxidants, anti-inflammatory, analgesic and hepatoprotection which are curcumin and sesquiterpenes may to help prevent the deleterious consequences of oxidative stress and prevent oxidative damage caused by Reactive Oxidative Species. Our studies showed that a high concentration of turmeric 40 ml/l had a negative effect on the growth of seedlings, and this may be attributed to the fact that the high concentration of the extract led to increase in the release of free radicals.

Conclusion

As previously mentioned with different concentrations and different irrigation periods; the treatment with carrots at concentration 100 ml/l gave the highest values for most of the traits under a 4 days irrigation period, while the plants were able to maintain their balance in the growth under the conditions of irrigation every 8 days, through the treatment with carrot 100 ml/l+turmeric 20 ml/l compared to nonplants treated under the same irrigation period. Through the results that were explained in this study, we can conclude that the lack of water had negative effects on the vegetative, chemical characteristics and isozymes activity (peroxidase and polyphenol oxidase) of the *Azadirachta indica* seedlings, except for the morphological characteristics of the root, which was positively affected with the lack of water, but the treatment with extracts from turmeric and carrots showed that it had a clear positive effect on the characteristics.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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