Egypt. J. Chem. **Vol. 65,** No. 12 pp. 619- 635 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Improvement the Drought Tolerance of *Eucalyptus citriodora* Seedling by Spraying Basil Leaves Extract and Its Influence on Growth, Volatile Oil Components and Some Enzymatic Activity

Shaimaa I. M. Elsayed[1*]; Azza A. M. Mazhar[2]; Samah M. El-Sayed[2] and Amr Said Mohamed[3]

[1-]Horticultural Crops Technology Dept. National Research Centre, Dokki, Giza, Egypt.

[2-] Ornamental Plants and Woody Trees Dept.. National Research Centre, Dokki, Giza, Egypt.

[3-] Botanical Garden Research Dept., Horticulture Research Institute, Agricultural Research Centre.

Abstract

The experiment trials were conducted in Horticulture Research Institute (HRI), Agricultural Research Centre, Giza, Egypt during two successive seasons of 2020 and 2021 to study the effect of basil leaves extract on growth, chemical composition and different properties for essential oil of *Eucalyptus citriodora* seedlings under different irrigation intervals. Results clarified that the vegetative growth parameters and photosynthetic pigments were increased in plants that were irrigated every 6 days but proline and MDA were decreased at the same treatment. While, leaf area showed high increment in plants irrigated every 4 days, whereas, all root traits, total sugars content in all organs and antioxidant enzymes activity (PPO, SOD, POD and CAT) were raised by irrigation intervals every 8 days. *E. citriodora* plants sprayed with BLE at rate 20% produced the highest value for all vegetative growth parameters and all chemical compositions except proline and MDA which showed the lowest value at the same rate. Irrigation every 6 or 8 days with spraying 20 or 40 % of (BLE) was the best treatments for production of essential oil fresh leaves. Main constituent of essential oil of *Eucalyptus citriodora* fresh leaves is citronellal which reached to (71.54%) by irrigation every 8 days with spraying 40% of (BLE), the oil was monoterpenoid (92.59- 97.93%). Oil and its major monoterpenes exhibited restrained to strong antioxidant activity.

Keywords: Eucalyptus citriodora, *Ocimum basilicum*, basil, extract, lipid peroxidation, enzyme activity, essential oil, GC-MS

1. Introduction

Eucalyptus is a large genus belonging to the family Myrtaceae. It comprises more than 600 species and subspecies of evergreen trees and shrubs, mainly native to Australia, Tasmania, New Guinea and neighboring islands.

Eucalyptus citriodora Hook. commonly known as 'Lemon-Scented Eucalyptus' or 'Lemon-Scented Gum', is an evergreen trees native to Queensland, Australia [1]. This species holds a great value due to its citronellal rich essential oil extracted from its leaves. The species was introduced in various countries such as India due to its commercial cultivation. Its oil is commonly valued for manufacturing of formulations of perfumes, toiletries and also used in disinfectants. Citronellal extracted from oil is used in the manufacture of synthetic menthol as well as citronellol. The leaves reported to possess antiseptic properties and are used in the treatment of various skin diseases. *E. citriodora* oil has been shown to have antibacterial, antifungal, ascaricidal, and insect repellent properties [2, 3, 4& 5]. The oil has also been found to be phytotoxic and has the potential to be used as a herbicide [6, 7, 8, and 9]. The plant's wood is also valuable as a timber and firewood.

It is a tall graceful tree with slender tapering trunk reaching a height up of 60 m with a straight that shows a bole and smooth bark which peels off in long thin strips or sheets. The leaves have a leathery texture and hang vertically or diagonally and are covered in glands that contain a scented volatile oil. The young leaves are broad, opposite, cordate-ovate, glaucous-grey; the adult leaves (after 4 or 5 years) are sword-shaped, 15-30 cm long, bluish-green in color, alternate, vertical.

Several studies have found that basil (*Ocimum basilicum*) is an excellent source of antioxidants. Secondary metabolites of plants, such as flavonoids and phenolic acids, contribute to their

*Corresponding author e-mail: shaimaa_elsayed83@yahoo.com
Receive Date: 15 March 2022, Revise Date: 09 April 2022, Accepted **Date:** 12 April 2022
DOI: 10.21608/EJCHEM.2022.127566.5662

antioxidant properties. The methanolic leaves extract of basil had high antioxidant potential and it is characterized by free radical of scavenging nature [10].

Drought stress is widely regarded as the most severe environmental constraint on the yield and growth of several species of plants in arid and semi-arid areas. Drought may have an adverse effect not only on the morphological level, but also on the physiological, biochemical, and molecular levels. [11]. Drought stress causes the production of excess reactive oxygen species (ROS), that is lipid peroxidation, protein denaturation, DNA mutation, disruption of cellular hemeostasis, and several types of oxidative damage on cellular level , affecting plant growth and yield. [12]. Thus, applying exogenous antioxidants or any strategy that can enhance antioxidant component in plants can be considered as a potent strategy for reducing oxidative stress and to ameliorate drought stress tolerance. This work aims to improve the response of *Eucalyptus citriodora* to cope the drought stress by using basil leaves extract.

2. Experimental

The The experiment trials were carried out in Horticulture Research Institute (HRI), Agricultural Research Center, Giza, Egypt during two successive seasons of 2020 and 2021, and the chemical analysis implemented in Ornamental Plants and Woody Trees Dept. and determination essential oil properties in the laboratory of Horticultural Crops Technology Dep., National Research Centre, Dokki, Giza, Egypt. It intended to find out the individual and combined effect of different irrigation intervals and basil leaf extract on growth, chemical constituent, essential oil content and its properties in leaves of *Eucalyptus citriodora*

Seedlings of *Eucalyptus citriodora* (the average height of seedlings 40-45 cm) were obtained nursery of Timber and Forestry Research department, Horticulture Research Center, Agricultural Research Center. The seedlings were planted on the first 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 [13] The field capacity was 1400 cm^3/pot. The irrigation schedule was for treatment (every 4, 6 and 8 days). After one month from transplanting, the irrigation was started and then harvest in 15^{th} November.

Three rates of basil leaves extract (BLE) (0, 20 and 40%) was used, the plant treated with (BLE) three times of 30 days irrigation regime starting on the first May in both seasons, the second one after another month and so on.

The available commercially fertilizer used through this experimental work Kristalon (NPK 19:19:19) produced Phayzen Company, Holland (Netherlands) the fertilizer rates 5.0 g/pot in four doses 4, 8, 16 and 20 weeks from transplanting.

Preparation of ethanolic basil leaves extract

Basil dried leaves were from National Research Centre in Giza, Egypt, It was extracted according to the techniques described by Sedky [14]. Dried leaves were crudely powdered, then basil leaves powder (100 g) were soaked in 500 ml 70% ethyl alcohol, and kept in tightly sealed vessels at room temperature for three weeks, this mixture was filtered. The extraction of the residue was repeated 3 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^o C until the solvent was completely removed. The extract was stored at 4°C until used. The plant extract was tested for the total phenols content and antioxidant activity and the values shown in Table (2).

Table (1) The physical and chemical properties of the soil mixture

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

Table (2) Analysis the total phenols and antioxidant activity in basil leaves Extract

Total phenolic (Mg/g Extract)	**Antioxidant Activity %**					
	20 µg. ml^{-1}	**50 µg. ml^{-1}**	**100 µg. ml^{-1}**	**150 µg. ml^{-1}**	**200 µg. ml^{-1}**	**IC_{50} (µg/ml)**
122.8 ± 3.39	20.5 ±1.479	27.9 ±3.310	42.7 ±1.283	64.2 ±1.596	74.7 ± 2.566	11.67

Determination of total phenols content (mg. g^{-1}):

Total phenols content (mg. g^{-1}) of basil leaves extract was determined in the representative dry herb samples according Singleton *et al*. [15] as follows:

The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated at room temperature for 45 min. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

Determination of antioxidant activity (%)

The antioxidant activities of (BLE) was assessed in terms of hydrogen-donating or radicals cavenging ability, using the stable radical DPPH (2, 20-Diphenyl-1-picrylhydrazyl) according to Brand-Williams *et al.* [16]. This activity is comparable with that of the standard drug ascorbic acid as strong antioxidant. The DPPH solution was prepared by dissolving 9.85 mg in 100 ml 70% methanol. (20, 50, 100, 150 and 200 µg. ml^{-1}) of (BLE) the mixtures were thoroughly shaken before being placed in the dark at room temperature for 30 minutes. If free radicals have been scavenged the purple color of DPPH will degenerate to yellow, indicating free radical scavenging activity. A spectrophotometer was used to measure the decrease in absorbance at 517 nm. The Absorbance of the radical without sample was used as control (665 µl methanol 70 % and 335 µl DPPH). The amount of sample required to reduce the DPPH absorbance was determined. The inhibition percentage of the DPPH radical by the extracts was calculated according to the following equation:

DPPH inhibition % = [(AB - AS)/AB] x 100

Where, AB=absorbance of blank, and AS= absorbance of a tested sample at the end of the reaction.

Data recorded:

1. **Vegetative growth:**

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

2. **Chemical analysis**

Photosynthetic pigments including chlorophyll a, b and carotenoids (mg. g^{-1} F.W.) were determined according to Saric *et al.* [17], Total sugars content (mg. g^{-1} F.W.) in leaves, stems and roots were determined according to Dubois *et al*. [18], Lipid peroxidation (µg. g^{-1} F.W.) was expressed as malondialdehyde (MDA) content and was determined according to Buege and Aust [19], Proline content (µg. g^{-1}F.W) was determined according to Bates *et al.* [20] and antioxidant enzymes activity of polyphenol oxidase (PPO) [1.10.3.2] was determined according to Matta and Dimond [21], superoxide dismutase (SOD) [1.15.1.1] determined according to Marklund and Marklund [22], peroxidase (POD) [1.11.1.7] determined according to Bergmeyer [23] and catalase (CAT) [1.11.16] determined according to Aebi [24].

Essential oil properties in leaves

Essential oil percentage (%)

Fresh leaves of *Eucalyptus citriodora* seedling were subjected to the essential oil extraction with hydro-distillation (water distillation). Extraction and quantification of the essential oil for each sample in the fresh leaves were separately performed with hydro-distillation for 3 hours using a modified Clevenger apparatus according to the Guenther [25]. The extracted oil was dehydrated over anhydrous sodium sulphate and stored in glass vials at deep freezer in the absence of light until used. That was done in the Central Laboratory in NRC, gas chromatography-mass spectrometry (GC - MS).

Essential oil yield (ml/ plant).

The oil yield per plant was calculated by multiplying the oil percentage by average plant yield of fresh leaves.

Antioxidant activity (%)

It was determined according to Brand-Williams *et al.* [16] the same former steps in basil but in known volume (20 µl) of essential oil of fresh leaves of *Eucalyptus citriodora* was dissolved in (480 µl) 70 % methanol then mixed with DPPH (0.5 ml).

GC-MS analysis

The GC-MS analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Department of Medicinal and Aromatic Plants Research, National Research Center with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TG-WAX MS column (30 m x 0.25 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml/min and a split ratio of 1:10 using the following temperature program: 60 ºC for 1 min; rising at 3.0 ºC /min to 240 ºC and held for 1 min. The injector and detector were held at 240 ºC. Diluted samples (1:100 hexane, v/v) of 1 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40-450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library) and the published data by Adams [26].

Experiment layout and statistical analysis

The experimental layout was set in factorial experiment in complete block design with three irrigation intervals and sprayed with three rates of (BLE) to give 9 treatments with 3 replicates for each season. Obtained results were subjected to statistical analysis by using least significant differences (LSD) at 5% level according to method described by Snedecor and Cochran [27].

3- Results and discussion

Vegetative growth

The results obtained in Table (3, 4, 5& 6) showed that in both seasons the highest value of plant height (cm), stem diameter (cm), no. of leaves/ plant, no. of branches/ plant and leaves and stem fresh and dry weight (g) which gave (109.69, 0.93, 173.89, 8.22, 81.21, 22.60, 67.12 and 31.77) and (118.94, 0.96, 180.11, 9.11, 82.29, 23.65, 72.38, and 34.94) in the first and second seasons respectively. Leaf area decreased slowly when the irrigation intervals were increased and the highest value for this parameter was obtained with irrigation every 4days.

Water stress has decreased stomatal conductance and photosynthesis in quite a few species [28]. When stomatal conductance decreased, the loss of plant water also decreased [29] and photosynthesis efficiency declines. This decrease affected the growth of the plant and its development as the molecules of carbohydrate and energy that are part of the development and growth process of the plant are produced by photosynthesis [30]. The suppression of plant growth under drought stress might be attributed to the metabolic disorders induced by stress, generation of ROS that caused a reduction in division and elongation of cells, meristematic division, cell turgor, cell volume and eventually cell growth, decrease in photosynthetic capacity of plant leaves and / or blocking up the translocation vessels thus hindering any movement of water or nutrients through it [31]. On the contrary, root length, fresh and dry weight of root gave an opposite manner which they gradually increased with increasing irrigation intervals. Irrigation plants every 8 days gave the highest value of root length, fresh and dry weight of root which resulted (83.33, 26.16 and 10.62) in the first season, respectively) and (85.52, 26.48 and 10.51) in the second season, respectively. The lower water supply caused the root system to penetrate deeper and extend wider in the soil with higher root system researching for moisture in lower soil [32]. These results are in line with those obtained by Azza *et al*. [33] and Rania [34].

Data also indicated that in both seasons, spraying *Eucalyptus citriodora* seedling with basil leaves extract at 20% concentration showed high performance of growth parameters including (plant height, stem diameter, no. of leaves/plant, root length, no. of branches/plant, fresh and dry weight of leaves, stem and root).

Such increments were estimated by 21, 28, 15, 23, 67, 44, 22, 21, 34, 33 and 34 % respectively, in the first season and 24, 34, 20, 24, 72, 53, 23, 30, 34, 42, 41 and 50%, respectively, in the second season, compared with untreated plants.

Regarding the interaction between water intervals and basil leaves extract (BLE) rates, data presented in Table (3, 4, 5& 6) revealed that, spraying with (BLE) at 20% concentration under 6 days irrigation intervals gave the highest values of all previous growth parameters except leaf area gave the highest values when seedling spraying with BLE at 20% concentration plus irrigation intervals compared with the other treatments.

Chemical constituents

Photosynthetic pigments (mg. g^{-1} F.W.)

Data in Fig. (1) stated that irrigation every 6 days was the most effective irrigation treatment for promoting the synthesis and accumulation of the three photosynthetic pigments than plants irrigated every 4 days or 8 days intervals in both seasons. The values were (2.00, 1.12 and 1.41) respectively in the first seasons and (2.00, 1.10 and 1.37) respectively, in the second seasons. These results are in accordance with those obtained with those obtained by Azza *et al*. [33], Mazhar *et al*. [35] and El-Quesni *et al*. [36].

The induced reduction of Chlorphyll content by water deficit has been attributed to the loss of chloroplast membranes, excessive swelling, and distortion of the lamellae vesicular and the appearance of lipid droplets [37]

Concerning the effect of the BLE extract on photosynthetic pigments as shown in Fig. (2) the data revealed the positive and active of the BLE extract at 20% on three pigments content in leaves of *Eucalyptus citradora* plants as compared with control plants and other treatment in the first and second seasons.

Regarding the interaction of the two factors under study, application of 20% BLE extract was more effective on pigments content with irrigation every 6 days intervals.

Table (3) Effect of irrigation intervals and BLE on plant height, stem diameter and number of leaves of *Eucalyptus citriodora* seedlings during 2020 and 2021 seasons

BLE (B)	Plant height (cm)				Stem diameter (cm)				No. of leaves/ plant			
	Irrigation intervals (days) (A)											
	4	6	8	Mean	4	6	8	Mean	4	6	8	Mean
	First season											
Control	81.75	111.50	72.00	**88.42**	0.63	0.92	0.69	**0.74**	143.33	163.33	130.67	**145.78**
20%	102.15	127.25	91.22	**106.87**	0.86	1.04	0.94	**0.95**	165.00	187.33	152.67	**168.33**
40%	86.63	90.33	79.41	**85.46**	0.74	0.84	0.78	**0.79**	157.67	171.00	144.00	**157.56**
Mean	**90.18**	**109.69**	**80.88**		**0.74**	**0.93**	**0.80**		**155.33**	**173.89**	**142.45**	
LSD$_{0.05}$	**(A): 4.22** **(B): 4.22** **(A*B): 7.23**				**(A): 0.04** **(B): 0.04** **(A*B): 0.07**				**(A): 5.81** **(B): 5.81** **(A*B): 10.07**			
	Second season											
Control	87.33	109.25	74.33	**90.30**	0.61	0.88	0.70	**0.73**	140.33	167.00	129.67	**145.67**
20%	108.00	131.74	96.12	**111.95**	0.89	1.07	0.97	**0.98**	169.33	193.67	161.33	**174.78**
40%	90.63	115.83	85.26	**97.24**	0.79	0.93	0.81	**0.84**	161.00	179.67	139.67	**160.11**
Mean	**95.32**	**118.94**	**85.24**		**0.76**	**0.96**	**0.83**		**156.89**	**180.11**	**143.56**	
LSD$_{0.05}$	**(A): 3.25** **(B): 3.25** **(A*B): 5.63**				**(A): 0.03** **(B): 0.03** **(A*B): 0.05**				**(A): 4.84** **(B): 4.84** **(A*B): 7.98**			

Table (4) Effect of irrigation intervals and BLE on number of branches, leaf area and root length of *Eucalyptus citriodora* seedlings during 2020 and 2021 seasons

BLE (B)	No. of branches/ plant				Leaf area (cm^2)				Root length (cm)			
	Irrigation intervals (days) (A)											
	4	6	8	Mean	4	6	8	Mean	4	6	8	Mean
	First season											
Control	4.00	6.67	3.67	**4.78**	18.75	18.05	9.38	**15.39**	64.45	67.69	71.24	**68.78**
20%	7.33	9.67	7.00	**8.00**	27.43	21.53	17.45	**22.14**	73.27	82.50	98.25	**84.67**
40%	6.67	8.33	5.00	**6.67**	23.61	17.71	12.15	**18.45**	70.05	77.71	80.49	**77.03**
Mean	**6.00**	**8.22**	**5.22**		**23.26**	**19.10**	**12.99**		**69.26**	**75.97**	**83.33**	
LSD$_{0.05}$	**(A):1.44** **(B): 1.44** **(A*B): 2.49**				**(A): 2.61** **(B): 2.61** **(A*B): 4.53**				**(A): 3.88** **(B): 3.88** **(A*B): 6.65**			
	Second season											
Control	5.67	6.33	4.67	**5.56**	19.22	18.28	10.41	**15.97**	65.61	71.24	75.12	**70.66**
20%	9.00	11.33	8.33	**9.55**	29.50	24.73	19.16	**24.46**	78.11	86.54	97.67	**87.44**
40%	7.67	9.67	6.33	**7.89**	26.42	21.86	13.77	**20.68**	72.49	80.46	83.77	**78.91**
Mean	**7.45**	**9.11**	**6.44**		**25.05**	**21.62**	**14.45**		**72.07**	**79.41**	**85.52**	
LSD$_{0.05}$	**(A):1.46** **(B): 1.46** **(A*B): 2.54**				**(A): 1.89** **(B): 1.89** **(A*B): 3.27**				**(A): 3.25** **(B): 3.25** **(A*B): 5.63**			

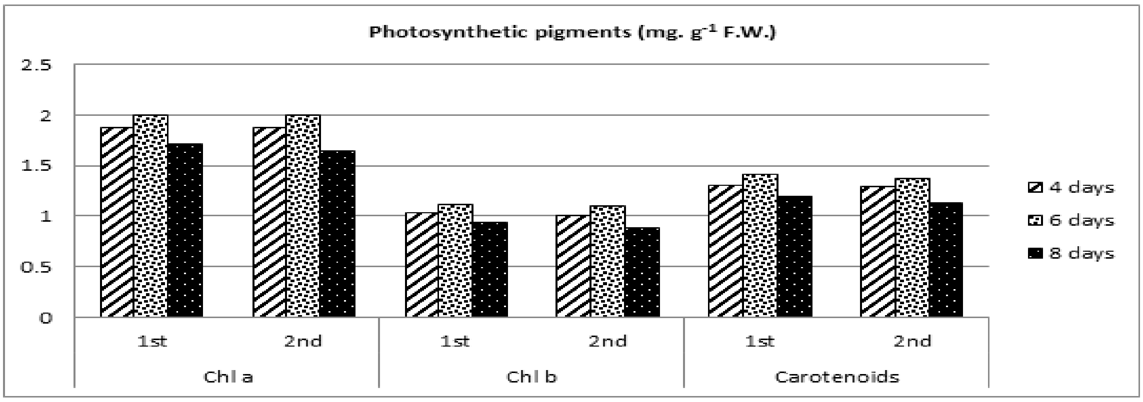

Fig. (1) Effect of irrigation intervals on photosynthetic pigments (chl a, chl b and carotenoids) of *Eucalyptus citriodora* during 2020 and 2021 seasons

Table (5) Effect of irrigation intervals and BLE on fresh weights of leaves, stems and roots of *Eucalyptus citriodora* seedlings during 2020 and 2021 seasons

BLE (B)	Leaves fresh weight (g)				Stems fresh weight (g)				Roots fresh weight (g)			
	Irrigation intervals (days) (A)											
	4	6	8	Mean	4	6	8	Mean	4	6	8	Mean
	First season											
Control	60.73	75.75	53.40	**63.29**	46.27	56.89	50.24	**51.13**	16.42	20.53	21.75	**19.57**
20%	73.77	87.85	69.12	**76.91**	57.94	80.34	67.2	**68.49**	22.62	24.94	29.33	**25.63**
40%	68.05	80.02	59.43	**69.17**	52.22	64.13	51.33	**55.89**	19.73	22.83	27.41	**23.32**
Mean	**67.53**	**81.21**	**60.65**		**52.14**	**67.12**	**56.26**		**19.59**	**22.77**	**26.16**	
LSD$_{0.05}$	**(A): 4.36** **(B): 4.36** **(A*B): 7.56**				**(A): 4.28** **(B): 4.28** **(A*B): 7.42**				**(A): 2.61** **(B): 2.61** **(A*B):4.53**			
	Second season											
Control	64.06	74.33	55.63	**64.67**	48.11	61.73	53.08	**54.31**	16.85	18.62	22.01	**19.16**
20%	78.46	88.62	71.77	**79.62**	64.59	85.03	72.66	**74.09**	22.82	27.07	30.98	**26.96**
40%	70.53	83.93	63.07	**72.51**	55.86	70.37	59.97	**62.07**	21.14	23.66	26.45	**23.75**
Mean	**71.02**	**82.29**	**63.49**		**56.19**	**72.38**	**61.90**		**20.27**	**23.12**	**26.48**	
LSD$_{0.05}$	**(A): 4.33** **(B):4.33** **(A*B): 7.13**				**(A): 3.88** **(B): 3.88** **(A*B): 6.46**				**(A): 2.30** **(B): 2.30** **(A*B):3.98**			

Table (6) Effect of irrigation intervals and BLE on dry weights of leaves, stems and roots of *Eucalyptus citriodora* seedlings during 2020 and 2021 seasons

BLE (B)	Leaves dry weight (g)				Stems dry weight (g)				Roots dry weight (g)			
	Irrigation intervals (days) (A)											
	4	6	8	Mean	4	6	8	Mean	4	6	8	Mean
	First season											
Control	16.73	21.46	13.71	**17.30**	22.05	27.09	23.40	**24.18**	6.58	8.00	8.35	**7.64**
20%	20.98	23.92	18.11	**21.00**	27.31	39.13	30.31	**32.25**	9.05	9.48	12.28	**10.27**
40%	18.23	22.42	15.85	**18.83**	24.01	29.08	24.42	**25.84**	8.01	8.56	11.23	**9.27**
Mean	**18.65**	**22.60**	**15.89**		**24.46**	**31.77**	**26.04**		**7.88**	**8.68**	**10.62**	
LSD$_{0.05}$	**(A): 2.46** **(B): 2.46** **(A*B): 4.26**				**(A): 3.06** **(B): 3.06** **(A*B): 5.29**				**(A):1.64** **(B): 1.64** **(A*B):2.83**			
	Second season											
Control	16.96	20.70	14.42	**17.36**	22.10	29.34	24.49	**25.31**	6.12	6.96	8.50	**7.19**
20%	22.06	26.09	19.76	**22.64**	30.91	41.61	35.44	**35.99**	8.89	10.85	12.55	**10.76**
40%	19.16	24.16	16.45	**19.92**	25.96	33.86	28.29	**29.37**	8.06	9.25	10.49	**9.27**
Mean	**19.39**	**23.65**	**16.88**		**26.32**	**34.94**	**29.41**		**7.69**	**9.02**	**10.51**	
LSD$_{0.05}$	**(A): 2.28** **(B):2.28** **(A*B): 3.96**				**(A):2.41** **(B): 2.41** **(A*B): 5.90**				**(A): 1.94** **(B): 1.94** **(A*B):3.36**			

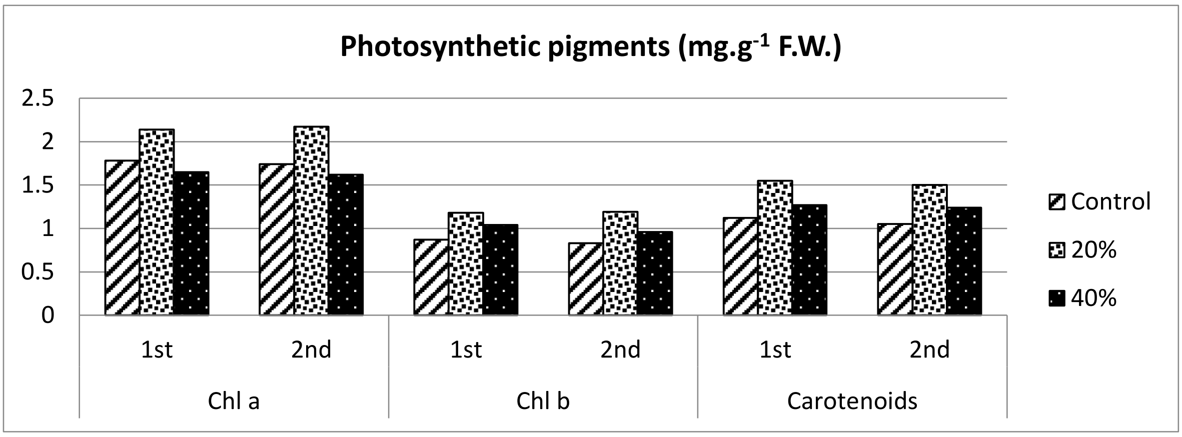

Fig. (2) Effect of BLE on photosynthetic pigments (chl a, chl b and carotenoids) of *Eucalyptus citriodora* during 2020 and 2021 seasons

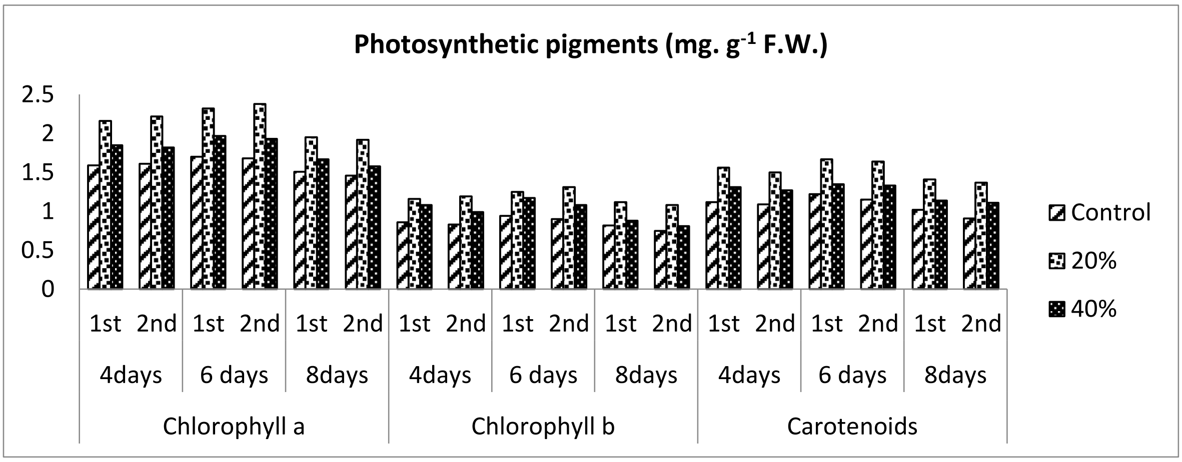

Fig. (3) Effect of Interaction between irrigation intervals BLE on photosynthetic pigments (chl a, chl b and carotenoids) of *Eucalyptus citriodora* during 2020 and 2021 seasons

Total sugar content (mg. g^{-1} F.W.)

It was clear from Table (4) that total sugar content in all plants organ (leaves, stem and root) was gradually augmented as the irrigation level was sloping downward. This may be due to the fact that during the course of drought stress active solute accumulation carbohydrates is claimed to be an effective stress tolerance mechanism [38]. Our results are in harmony with the results obtained by Azza *et al*. [39], Azza *et al*.[33], Romaisa *et al*.[40] and Ayse and Esra[41].

As for the effect of BLE extract on total sugars percentage, data showed that BLE extract application either 20 or 40 % increased total sugars percentage in the different plant organs of *Eucalyptus citriroda* seedlings as compared with untreated seedlings.

In this context, the interaction between irrigation intervals and BLE extract, revealed that combination of both factors on total sugars percent was more effective than application of each of them alone. The highest values were obtained by the application of 20% BLE extract and irrigated 8 days in the two seasons.

Proline in leaves (µg.g^{-1} F.W.)

From the given data in Table (7) it can be concluded that decreasing soil moisture level caused an increase of proline content in leaves in both seasons.

Regarding effect of BLE extract, results indicated that these treatments caused a reduction in proline content compared to untreated plants in both seasons. The highest depression was recorded with the application of BLE extract at 20% compared with the other treatments in the first and second seasons.

In this respect, interaction between irrigation intervals and BLE extract applications, the data revealed that the combination of the both factors on proline was more effective than the effect of each factor when tested alone. The lowest values of proline content were provided when spraying BLE at 20% and irrigation every 8 days in the first and second seasons.

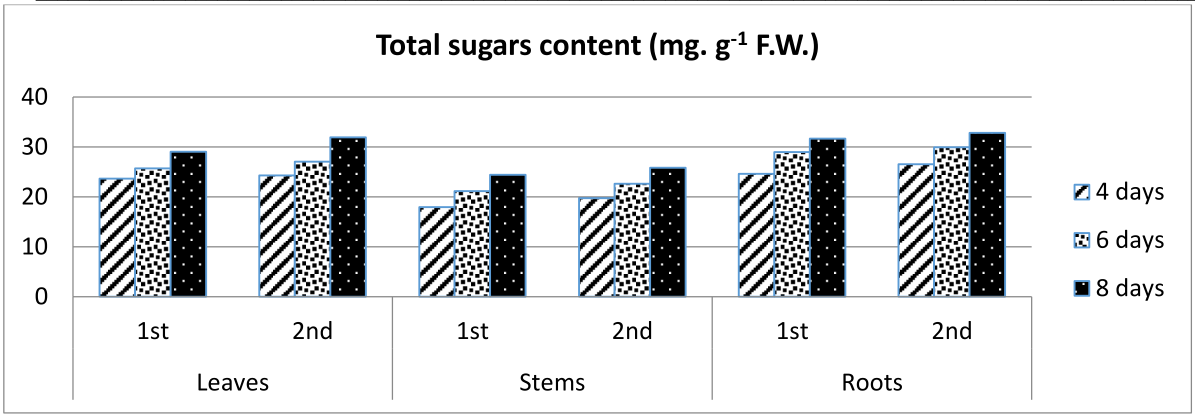

Fig. (4) Effect of irrigation intervals on total sugar content in leaves, stems and roots of *Eucalyptus citriodora* during 2020 and 2021 seasons

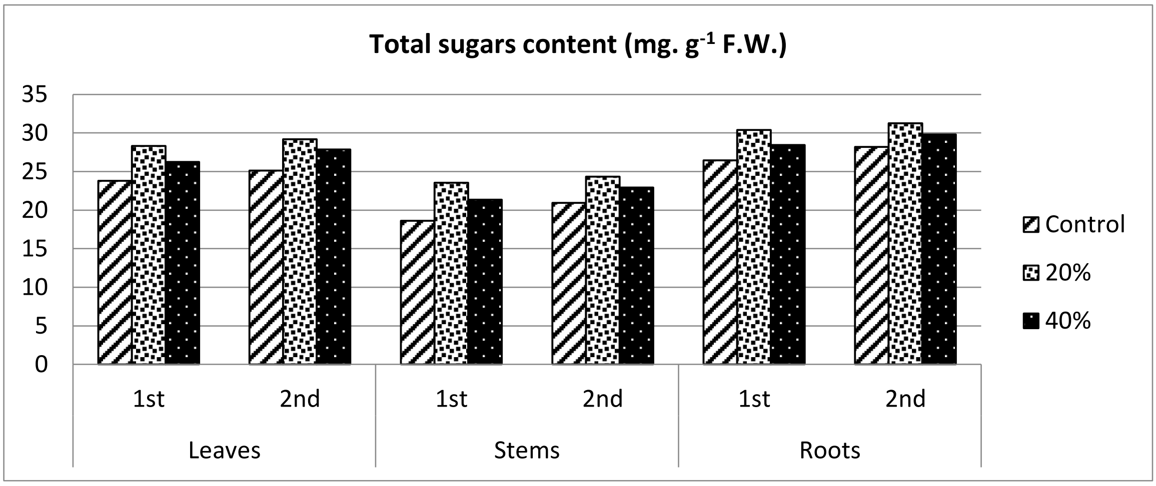

Fig. (5) Effect of BLE on total sugar content in leaves, stems and roots of *Eucalyptus citriodora* during 2020 and 2021 seasons

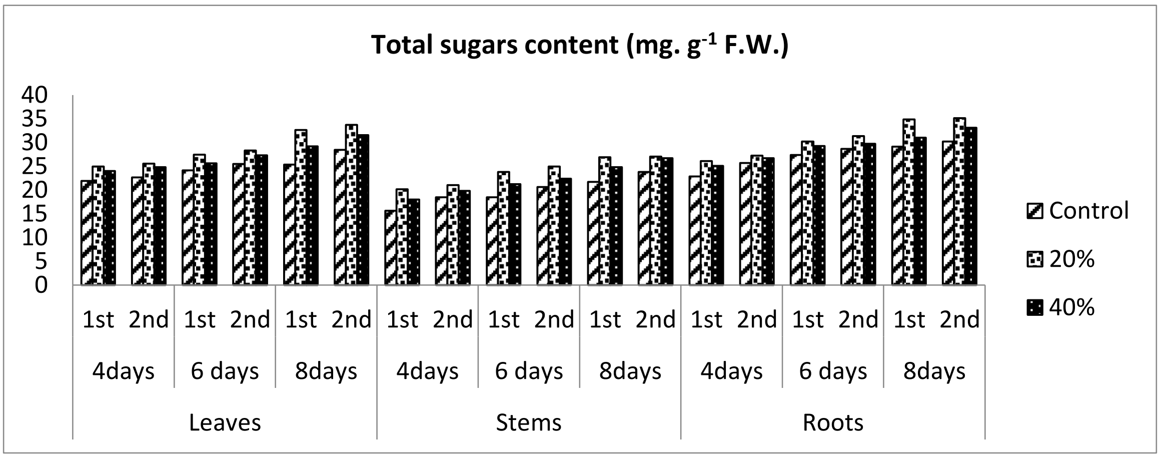

Fig. (6) Effect of Interaction between irrigation intervals BLE on total sugar content in leaves, stems and roots of *Eucalyptus citriodora* during 2020 and 2021 seasons

Proline has an important biological role in response to stress [42]. Many plant species accumulate proline in response to drought stress [43&44]. Proline accumulation can influence stress tolerance in various ways [45]. Generally, osmolit accumulation in plant cell results in a decrease in cell osmotic potential and therefore in maintenance water absorption and cell turgor pressure, helping physiological process such as stomatal opening, photosynthesis, and growth under dry conditions [46]. In agreement with this results concerning irrigation were the results of Soad [47], Azza *et al*.[39] and Salwa and Osama [48].

Table (7) Effect of irrigation intervals and BLE on proline content and lipid peroxidation (MDA) in leaves of *Eucalyptus citriodora* seedlings during 2020 and 2021 seasons

BLE (B)	Proline (µg.g^{-1} F.W.)				Lipid peroxidation (MDA) (µg.g^{-1} F.W.)			
	Irrigation intervals (days) (A)							
	4	6	8	Mean	4	6	8	Mean
	First season							
Control	561.21	527.66	598.45	**562.44**	1.92	0.65	3.29	**1.95**
20%	443.06	398.34	492.18	**444.53**	0.86	0.35	2.17	**1.13**
40%	530.47	477.85	573.14	**527.15**	1.28	0.46	2.86	**1.53**
Mean	**511.58**	**467.95**	**554.59**		**1.35**	**0.49**	**2.77**	
LSD$_{0.05}$	**(A): 1.46**				**(A): 0.023**			
	(B): 1.46				**(B): 0.023**			
	(A*B): 2.53				**(A*B): 0.051**			
	Second season							
Control	542.65	510.78	586.22	**546.55**	1.95	0.78	3.36	**1.95**
20%	429.91	381.11	488.15	**433.06**	0.91	0.41	2.24	**0.91**
40%	521.22	465.07	558.80	**515.03**	1.33	0.49	2.95	**1.33**
Mean	**497.93**	**452.32**	**544.39**		**1.39**	**0.56**	**2.85**	
LSD$_{0.05}$	**(A): 1.22**				**(A): 0.011**			
	(B): 1.22				**(B): 0.011**			
	(A*B): 2.12				**(A*B): 0.20**			

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

Malondialdehyde (MDA) was one of the key of the lipid peroxidation by thiobarbituric acid reaction. The MDA content is an indicator of membrane lipid peroxidation which could reflect the degree of damage at adverse condition [49]. As shown in Table (7), the MDA content of *Eucalyptus citriodora* seedlings leaves increased on the 8 days of drought stress in both seasons. It is considered a major type of oxidative damage. It decreases cell membrane fluidity, ion channels, membrane proteins and enzyme activities and induce the leakiness of the cell membrane [50]. On the other hand, spraying with BLE reduced MDA level in leaves of *Eucalyptus citriodora* seedlings plants when compared with the control in the two seasons. Spraying seedling with BLE at 20% concentration gave the lowest content of MDA, which resulted (1.13 and 0.91µg.g^{-1} F.W.) in the first and second seasons, respectively, in comparison with control and other treatments.

As regard the effect of interaction, irrigating water every 8 days plus without spraying BLE gave the highest values of MDA which recorded 3.29 and 3.36 µg.g^{-1} F.W. in the two seasons, respectively, and the lowest values were obtained by seedlings irrigated every 6 days intervals and spraying with BLE at 20% which gave 0.35 and 0.41 µg.g^{-1} F.W. in both seasons, respectively, compared with the other treatments.

Antioxidant enzymes activity (µg.g^{-1} F.W.)

Data recorded in Fig (7) indicated that Catalase (CAT), Polyphenol Oxidase (PPO), Peroxidase (POD) and Superoxide dismutase (SOD) in leaves activities of *Eucalyptus citriodora* seedlings were positively affected under water stress conditions. Enzymes activities were gradually increased as the level of water irrigation was sloping down-word. The highest values for all these enzymes were obtained due to the use of irrigation every 8 days. The values of CAT, PPO, SOD and POD were (0.061, 0.73, 0.55 and 0.37 µg.g^{-1} F.W.) in the first season, respectively and were (0.065, 0.71, 0.58 and 0.40 µg.g^{-1} F.W.) in the second season, respectively. Tolerance to drought-stress in higher plants correlates to the levels of antioxidant systems and substrates [51]. To combat the effect of drought-induced oxidative stress, plant develops a complex mechanism of antioxidant system. Relatively higher activities of reactive oxygen species (ROS)-scavenging enzymes have been reported in tolerant genotypes when compared to susceptible ones, suggesting that the antioxidant system plays an important role in plant tolerance against environmental stress. This indicated plants will produce more CAT, PPO, SOD and POD under drought conditions to remove the extra ROS in cells. The CAT is one of the highest turnover rates for all enzymes with the potential to directly dismutate H_2O_2 in H_2O and O_2 and is indispensible for ROS detoxification in peroxisomes during stress condition [52]. The SOD detoxifies superoxide anion free radicals (O^-_2) by forming H2O2 and then H_2O_2 can be eliminated by CAT and POD [53]. Moreover, POD also involved in various plant processes, including lignification [54], oxidation of phenolics [55], regulation of cell elongation [56] and detoxification of toxic compounds such as H_2O_2 [57]. Also, this indicates that polyphenolic oxidase PPO plays an important role in the adaptation mechanism to water deficit. Evidence is accumulating to support the idea that phenolics also play a role in defending the plant against various biotic and abiotic stresses [58 & 59]. These results are in accordance with those obtained by Sonja *et al* [60], Tayebeh and Hassan [61] .

According to the different BLE extract levels values of the antioxidant enzymes (CAT, SOD, POD and PPO) increased by all BLE extract concentrations. The highest values of all previous antioxidant enzymes in the two seasons were obtained by BLE at 20%. The values were 0.06, 0.72, 0.53 and 0.36) in the first season, respectively and (0.064, 0.71, 0.56 and 0.39) in the second season, respectively.

In this context, the interaction between different factors (irrigation X BLE) was almost increased for all antioxidant enzymes that have been studied in both seasons. The highest values due to the irrigation regime and BLE were obtained due to irrigation every 8 days and 20% BLE for CAT, SOD, POD and PPO in the two seasons.

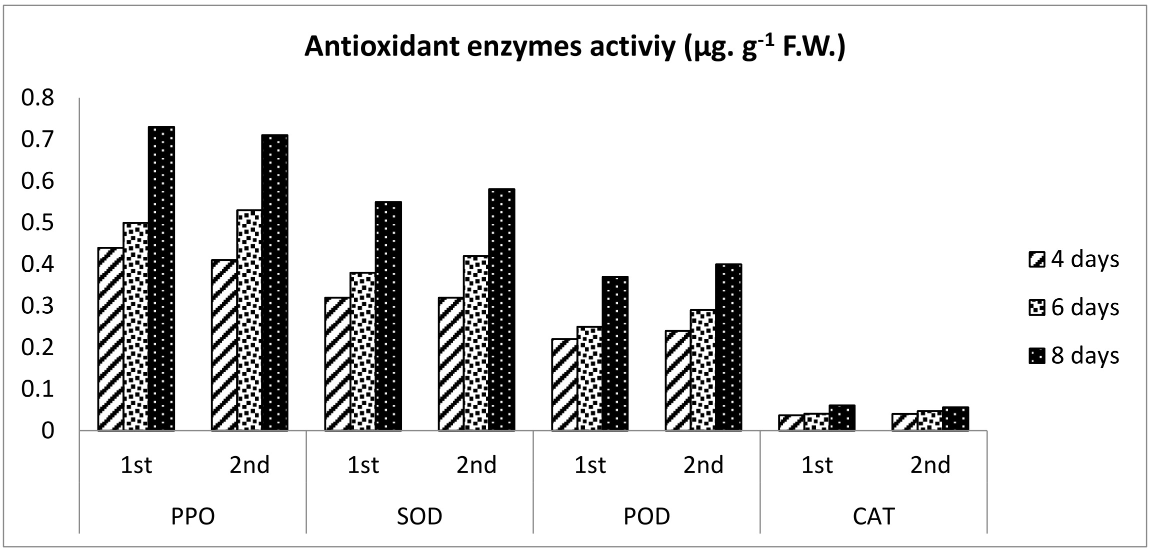

Fig. (7) Effect of irrigation intervals on antioxidant enzymes activity (PPO, SOD, POD and CAT) in leaves of *Eucalyptus citriodora* during 2020 and 2021 seasons

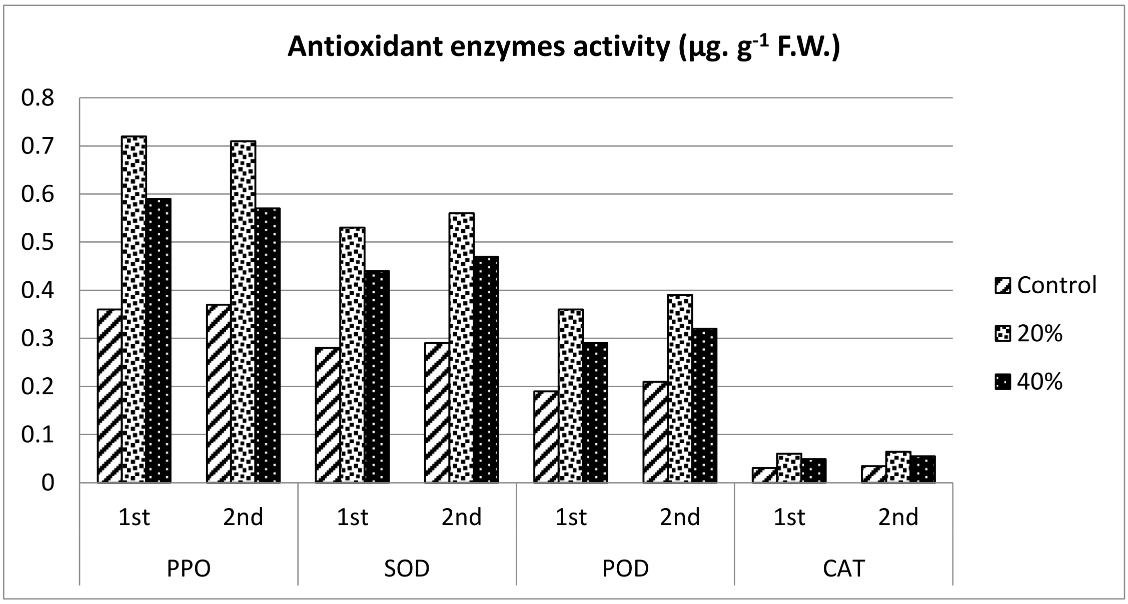

Fig. (8) Effect of BLE on antioxidant enzymes activity (PPO, SOD, POD and CAT) in leaves of *Eucalyptus citriodora* during 2020and 2021 seasons

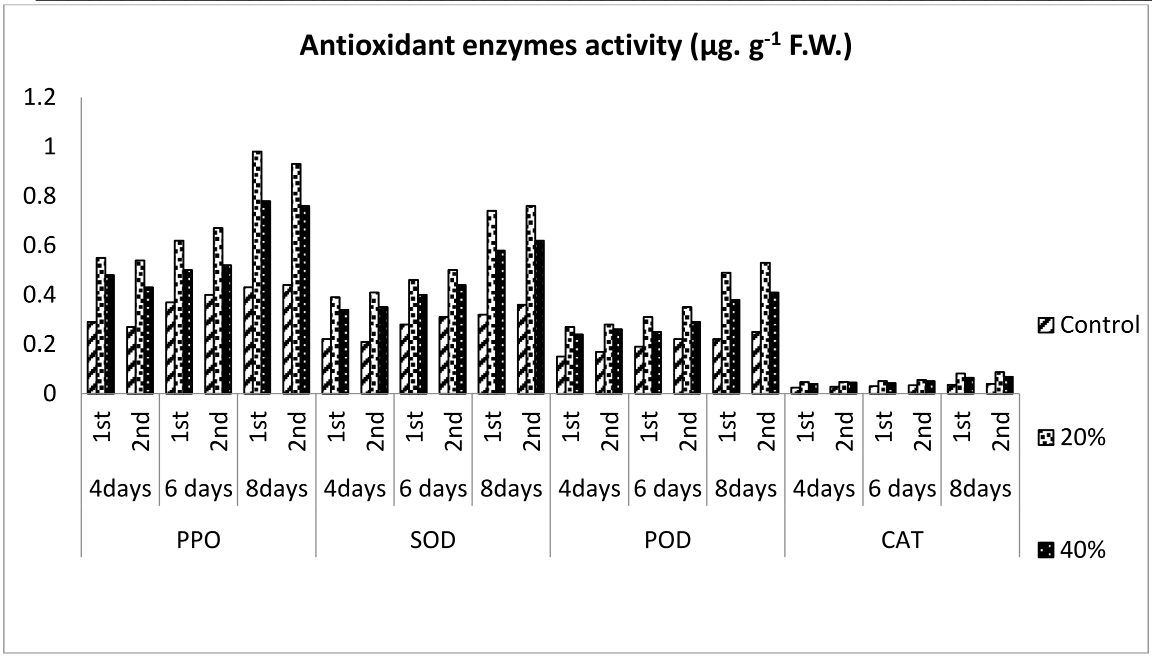

Fig. (9) Effect of Interaction between irrigation intervals BLE on antioxidant enzymes activity (PPO, SOD, POD and CAT) in leaves of *Eucalyptus citriodora* during 2020 and 2021 seasons

Essential oil percentage (%)

The data presented in Table (8) illustrated that the *E. citriodora* plants were irrigated every 8 days produced the highest percentage of essential oil giving values 1.21 and 1.34%, respectively, in the first and second seasons. Our results were in agreement with Gerami *et al*. [62] on *Origanum vulgaris* L. and Mazrou *et al*. [63] on lemongrass plant.

The plants received BLE at rate 20%showed the highest percentage of essential oil with values 1.13 and 1.20% in the first and second seasons, respectively.

Regarding the interaction treatment, it was noticed from data tabulated in Table (8) that the plants irrigated every 8 days and sprayed with BLE at rate 20% gave the highest percentage of essential oil (1.28%) in the first season. While; the *E. citriodora* plants were irrigated every 8 days and sprayed with BLE at rate 20 or 40% gave the highest percentage with the same value (1.42%) in the second season.

Essential oil yield (ml/ plant)

The data showed in Table (8) revealed that irrigation intervals every 6 days for *E. citriodora* plants gave the highest yield of essential oil with values 0.89 and 0.95 ml/ plant as compared with control plants in the first and second seasons, respectively. The obtained results were in the same line with Mazrou *et al*. [63] on lemongrass plant and Al-Azzony and Khater [64] on funnel plant.

It was clear from data presented in Table (8) that the essential oil yield in leaves of *E. citriodora* plants was affected by application of BLE. The plants received BLE at rate 20% gave the highest yield with values 0.87 and 0.96 ml/ plant in both seasons, respectively.

The interaction between irrigation intervals and BLE treatments showed different effects on essential oil yield in leaves of *E. citriodora* plants. The plants were irrigated every 6 days and sprayed with BLE at rate 20% produced highest yield of essential oil giving values 1.07 and 1.13 ml/ plant.

Antioxidant activity (%) of essential oil

The showed data in Table (8) stated that the irrigation every 8 days increased antioxidant activity in leaves of *E. citriodora* seedlings giving values 79.10 and 78.57% in the first and second seasons, respectively. These results agree with Singh *et al*. [65] found that the antioxidant activity of essential oil of *E. citriodora* has been reported against a range of free radicals, is due to the synergistic effect of citronellal and citronellol. Citronellal exhibited strong scavenging activity against DPPH.

It was clear from data presented in Table (8) that the antioxidant activity in essential oil increased in seedlings treated with BLE at rate 40%.

The data presented in Table (8) cleared that *Eucalyptus citriodora* seedlings irrigated every 8 days with spray 40% of (BLE) gave highest mean values of antioxidant activity (%) for essential oil of fresh leaves at first and second seasons. The lowest mean value of antioxidant activity (%) for essential oil of fresh leaves at first and second seasons were obtained from plants irrigated every 4 days without spraying BLE.

Essential oil Composition

Results in Table (9) represented the main constituents of the essential oil of the *Eucalyptus citriodora* seedlings leaves as affected by basil leaves extract in different concentration.

Nineteen components were identified in the essential oil of lemon eucalyptus leaves by GC-MS at seedling stage. The total identified compounds ranged from 96.06 to 99.99% from the separated compounds.

The major compound was found to be Citronellal in all treatments and ranged from (57.02%) by irrigation every 4 days without spraying of (BLE) and increased by increasing irrigation treatments with spraying basil leaf extract to (71.54%) by irrigation every 8 days with spraying 40% of (BLE) treatment in the essential oil of lemon eucalyptus leaves at seedling stage.

The second major compound was identified as β-Citronellol in the essential oil of lemon eucalyptus leaves. The maximum β-Citronellol content (17.44) was found in the leaves of lemon eucalyptus which irrigated at 4 days with spraying 40% of (BLE) treatment, the content of β-Citronellol decreased with the drought rates, even with spraying the extract of basil leaves. It reached 10.14% by irrigated at 8 days with spraying 40% of (BLE) in the essential oil leaves of lemon eucalyptus at seedling stage.

Table (8) Effect of irrigation intervals and BLE on essential oil percentage, yield and antioxidant activity in leaves of *Eucalyptus citriodora* seedlings during 2020 and 2021 seasons

BLE (B)	Essential oil percentage (%)				Essential oil yield (ml/ plant)				Antioxidant activity (%)			
	Irrigation intervals (days) (A)											
	4	**6**	**8**	**Mean**	**4**	**6**	**8**	**Mean**	**4**	**6**	**8**	**Mean**
					First season							
Control	0.82	0.92	1.10	**0.95**	0.50	0.70	0.59	**0.59**	70.7	73.4	76.3	**76.13**
20%	0.90	1.22	1.26	**1.13**	0.66	1.07	0.87	**0.87**	78.2	78.6	79.9	**76.23**
40%	0.87	1.14	1.28	**1.10**	0.59	0.91	0.76	**0.76**	75.7	80.1	81.1	**78.97**
Mean	**0.86**	**1.09**	**1.21**		**0.59**	**0.89**	**0.74**		**74.87**	**77.37**	**79.10**	
LSD0.05	**(A): 0.055**				**(A): 0.048**				**(A): 5.29**			
	(B): 0.055				**(B): 0.048**				**(B): 5.29**			
	(A*B): 0.096				**(A*B): 0.085**				**(A*B): 9.17**			
					Second season							
Control	0.84	0.95	1.18	**0.99**	0.54	0.71	0.66	**0.63**	70.0	73.0	76.1	**73.03**
20%	0.91	1.28	1.42	**1.20**	0.71	1.13	1.02	**0.96**	74.7	79.0	78.9	**77.53**
40%	0.89	1.20	1.42	**1.17**	0.63	1.01	0.90	**0.84**	76.1	79.7	80.7	**78.83**
Mean	**0.88**	**1.14**	**1.34**		**0.63**	**0.95**	**0.86**		**73.60**	**77.23**	**78.57**	
LSD0.05	**(A): 0.062**				**(A): 0.061**				**(A): 2.83**			
	(B):0.062				**(B): 0.061**				**(B): 2.83**			
	(A*B): 0.11				**(A*B): 0.10**				**(A*B): 4.91**			

Table (9) The main constituents of the essential oil of fresh leaves of *Eucalyptus citriodora* plant affected by drought and spraying of basil leaf extract (BLE) at seedling stage

R.T.	Components	Area %								
		4 days			6 days			8 days		
		Control	BLE 20%	BLE 40%	Control	20% BLE	BLE 40%	Control	BLE 20%	BLE 40%
4.16	**α-Pinene**	0.18	0.31	0.29	0.16	0.20	0.22	0.31	0.25	0.34
5.25	**β-Pinene**	0.56	1.10	1.03	0.85	1.03	1.41	1.27	1.01	1.69
5.51	**β-Myrcene**	0.13	0.09	0.10	Trace	Trace	Trace	0.14	Trace	0.11
6.72	**Limonene**	0.21	0.27	0.30	0.37	0.25	Trace	0.23	Trace	0.19
6.83	**Eucalyptol (1,8-Cineole)**	Trace	0.34	0.31	Trace	0.15	Trace	0.14	0.63	-
7.01	**O-Menthan-8-ol**	0.14	Trace	Trace	0.25	0.18	0.1	0.21	0.29	0.16
7.71	**5-Heptenal, 2,6-dimethyl-**	0.40	0.14	0.16	0.39	0.28	0.12	0.23	0.3	0.22
9.27	**Linalool**	Trace	Trace	Trace	Trace	0.18	Trace	0.21	0.14	Trace
10.46	**5-Hepten-1-ol, 2,6-dimethyl-**	Trace	Trace	0.10	0.35	0.23	0.12	0.21	0.25	0.18
11.17	**1,8 -Cineole**	8.97	9.03	9.39	10.67	11.62	7.40	7.38	7.19	7.01
11.45	**Citronellal**	57.02	58.06	57.69	60.19	64.68	71.40	60.89	65.63	71.54
11.98	**Isopulegol**	3.81	2.38	1.87	5.40	1.59	1.81	3.87	1.17	1.11
14.41	**β-Citronellol**	14.49	17.2	17.44	14.49	12.97	11.14	14.45	12.52	10.14
19.45	**Citronellol acetate**	5.51	4.21	4.43	3.16	3.99	2.22	5.16	5.93	3.11
20.74	**Geranyl acetate**	1.35	0.40	0.39	0.18	0.22	-	3.23	0.16	-
21.99	**Caryophyllene**	2.78	5.51	5.30	0.92	1.99	3.23	1.73	2.88	2.58
23.51	**Humulene**	0.15	0.41	0.40	-	0.11	0.16	-	0.17	0.15
24.57	**GERMACRENE-D**	0.14	0.24	0.24	-	-	0.15	-	-	0.28
28.64	**Caryophylene oxide**	0.40	0.25	0.27	0.29	0.23	0.36	0.33	0.67	0.37
	Monoterpenes	**92.59**	**93.53**	**93.50**	**96.46**	**97.57**	**95.94**	**97.93**	**95.47**	**95.90**
	Sesquiterpenes	**3.47**	**6.41**	**6.21**	**1.21**	**2.33**	**3.90**	**2.06**	**3.72**	**3.38**
	Oxygenated compounds	**92.09**	**92.01**	**92.05**	**95.37**	**96.32**	**94.67**	**96.31**	**94.88**	**93.94**
	Non-oxygenated compounds	**3.97**	**7.93**	**7.66**	**2.30**	**3.58**	**5.17**	**3.68**	**4.31**	**5.34**
	Total	**96.06**	**99.94**	**99.71**	**97.67**	**99.90**	**99.84**	**99.99**	**99.19**	**99.28**

Monoterpene 1,8-Cineole was observed as the third major compound identified in the essential oil of lemon eucalyptus seedling leaves. The 1,8-Cineole content in essential oil tended to increase by increasing the drought treatments up to 6 days with spraying of (BLE) and it reached the highest content (11.62%) with irrigation of seedling every 6 days with spraying 20% of (BLE) treatment, then it decreases with increase in the irrigation intervals to reach its lowest content (7.01%) with irrigation at 8 days with spraying 40% of (BLE) treatment.

Citronellol acetate is an oxygenated monoterpene component increased to (5.93%) in the essential oil leaves of seedling lemon eucalyptus sprayed with 20% of (BLE) and irrigated at 8 days.

Data in Table (9) presented that the oil had lower relative concentrations of sesquiterpene compound (1.21 to 6.41%) and the non- oxygenated compounds ranged from (2.30 to 7.93 %). Caryophyllene sesquiterpene component gave the maximum content (5.51%) with irrigation every 4 days with spray basil leave extract at 20% treatment but the minimum content (0.92%) was found in essential oil leaves of seedling irrigated at 6 days without spray of (BLE).

The study concluded that the essential oil of *E. citriodora* is a very good source of monoterpenes as more than 94% of the oil is monoterpenoid in nature. The oil and its selected components exhibited moderate to strong antioxidant activity thereby implying its potential in providing protection against oxidative diseases and its use as a natural antioxidant in the food and confectionery industries.

These results agreed with Leicach, *et al*. [66] they found that drought stress has different effects on the quantity and quality of essential oil of Eucalyptus species including *Eucalyptus camaldulensis* Dehnh, mild drought stress improved the production of (the main constituent) 1, 8-cineol, however it was observed that it decreased or stopped the production of many other compounds. And with Singh *et al*., [65] them study concludes that, the essential oil of *E. citriodora* is a very good source of monoterpenes as more than 94% of the oil is monoterpenoid in nature. The oil and its selected components exhibited moderate to strong antioxidant activity there by implying its potential in providing protection against oxidative diseases and its use as a natural antioxidant in the food and confectionery industries.

4. Conclusions

It is concluded from this study that the *E. citriodora* seedlings irrigated every 6 days and sprayed with BLE at rate 20% showed the best performance of vegetative growth and decreased the activity of antioxidant chemical composition as results to growth of these plants under the optimum irrigation condition. It is clear that increasing irrigation treatments increased the content of citronellal. This could be attributed to the stress effect on the secondary metabolites which increase most of secondary metabolites i.e. essential oil and their constituents. Application of basil leaves extract did not affect the content of citronellal in treatments irrigated at 4 days intervals; on the other hand application of 40% basil leaves extract increased citronellal content in both 6 days and 8 days irrigation intervals.

5. Conflicts of interest

"There are no conflicts to declare".

6. Formatting of funding sources

List funding sources in a standard way to facilitate compliance to funder's requirements.

7. Acknowledgments

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

7- References

[1] Chen J., Craven L.A., *Myrtaceae*: Flora of China. *Science Press (Beijing) and Missouri Botanical Garden Press*, **13**: 321(2007).

[2] Ramezani H., Singh H.P., Batish D.R., Kohli R.K., Dargan J.S., Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronellal. *New Zealand Plant Protection*, **55**, 327-330 (2002).

[3] Singh H., Antifungal activity of the volatile oil of *Eucalyptus citriodora*. *Phytother*, **73**(3), 261-262 (2002).

[4] Verbel J.O., Nerioa L.S., Stashenkob E.E., Bioactivity against *Tribolium castaneum* Herbst (Coleoptera: tenebrionidae) of *Cymbopogon citratus* and *Eucalyptus citriodora* essential oils grown in Colombia. *Pest Manag. Sci.,* **66**, 664–668(2009).

[5] Luqman S., Dwivedi G.R., Darokar M.P., Kalra A., Khanuja S.P.S., Antimicrobial activity of *Eucalyptus citriodora* essential oil. *Int. J. Essential Oil Therap*., **2**(2), 69-75 (2008).

[6] Batish DR, Setia N, Singh HP, Kohli RK., Chemocal composition and inhibitory activity of essential oil from decaying leaves of *Eucalyptus citriodora.* Z Naturforsch C., **61c**: 52-56 (2006).

[7] Batish DR, Singh HP, Setia N, Kohli RK, Kaur S, Yadav S.S., Alternative control of little seed canary grass using euclypt oil. Agron. Sustain. Dev., **27**: 171-177 (2007).

[8] Singh HP, Batish DR, Setia N, Kohli R.K., Herbicidal activity of volatile oils from *Eucalyptus citriodora* against parthenium hysterophorus. Annals of Applied Biol., **146**(1): 89-94 (2005).

[9] Singh HP, Batish DR, Kaur S, Kohli RK, Arora K., Phytotoxicity of the volatile monoterpene citronellal against some weeds. Biology Biochemistry Biophysics virology. Part C, J. Nat. Res., **61** (5-6): 334-340(2006).

[10] Kaurinovic B., Popovic M., Vlaisavljevic S. and Trivic S., Antioxidant Capacity of Ocimum basilicum L. and Origanum vulgare L. Extracts. Molecules, **16**,7401-7414 (2011).

[11] Fathi and Tari, Effect of drought stress and its mechanism in plants. Inter. J. Life Sci., **10** (1): 1- 6 (2016).

[12] Pandey V, Shukla A., Acclimation and tolerance strategies of rice (*Oryza sativa L.*) under drought stress. Rice Sci; **22**(4):147-61 (2015).

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

[14] Sedky D., Mohamed A. M., Fouad R., Khafagi M.H.M., Elsayed A. O., Elbayoumy M.K., Mohammad M. E., Abou-Zeina H.A.A., Assessment of Phytochemical, Antioxidant and Antibacterial Activity of Balanites Aegyptiaca and Curcuma Longa against Some Bacterial Pathogens Isolated from Dairy Cow Infected with Mastitis. *Advances in Animal and Veterinary Sciences.*, **10** (1), 160-169 (2022).

[15] Singleton, V.L.; R. Orthofer; R.M. Lamuela-R., Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol., **299**, 152-178 (1999).

[16] Brand-Williams W, Cuvelier M.E, Berset C., Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., **28**(1): 25-30. (1995). https://doi.org/10.1016/S0023- 6438(95) 80008-5.

[17] 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).

[18] 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).

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

[20] 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).

[21] Matta, A.; Dimond, A.E., Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems.*Phytopathology*, **53**, 574 (1963).

[22] Marklund, S.; Marklund, G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur. J. Biochem.*, **47**, 469- 474. (1974).

[23] Bergmeyer, H.U. Determination with glucose oxidase and peroxidase. *Methods Enzym. Anal.*, ***15,*** 1205–1215. (1974).

[24] Aebi, H. Catalase in vitro. In *Methods in Enzymology*; Elsevier: Amsterdam, The Netherlands, **105**, 121–126, (1984). ISBN 0076-6879.

[25] Guenther, G. The essential oils VIII. Robert E.D. Nastrand Comp. Inc. Toronto, New York, London. (1961).

[26] Adams, R. Identification of essential oil components by gas chromotography-mass spectrometry 4th ed., USA: Allured Publishing Cooperation, Carol Stream, IL**.** (2005).

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

[28] Peguero-Pina JJ, Sancho-Knapik D, Morales F, Flexas J, Gil-Pelegrín E., Differential photosynthetic performance and photoprotection mechanisms of three Mediterranean evergreen oaks under severe drought stress. Funct Plant Biol **36**,453-462 (2009).

[29] Raftoyannis Y, Radoglou K.,. Physiological responses of beech and sessile oak in a natural mixed stand during a dry summer. Ann Bot **89,** 723-730 (2002).

[30] Ozturk N.Z.,. Bitkilerin kuraklık stresine tepkilerinde bilinenler ve yeni yaklaşımlar. Türk Tarım-Gıda Bilim ve Teknoloji Dergisi **3** 307-315. (2015). (in Turkish)

[31] Banon, S.J., Ochoa, J. Franco, J.A., Alarcon, J.J. and Sanchez-Blanco, M.J., Hardening of oleander seedlings by deficit irrigation and low air humidity. Environmental and Experimental Botany, **56**, 36–43 (2006).

[32] Burman, U.; S. Kathje, B.A. Garg and Lahiri A.N.,, Water management of transplant seedling of Azadirachta indica in aird areas. Forest-Ecology and Management, **40**, 51-63 (1991).

[33] Azza A.M.Mazher, Mona H. Mahgoub, Kh. M. Abd El-Rheem and Sahar M. Zaghloul . Influence of Nile Compost Application on Growth, Flowering and Chemical Composition of Amaranthus tricolor under Different Irrigation Intervals. Middle-East Journal of Scientific Research 12 (6): 751-759 (2012) ISSN 1990-9233 © IDOSI Publications, (2012) DOI: 10.5829/idosi.mejsr.2012.12.6.1751

[34] Rania S. Hanafy . Using *Moringa olifera* Leaf Extract as a Bio-fertilizer for Drought Stress Mitigation of Glycine max L. Plants. Egypt. J.Bot. **57** (2) 281-292 (2017).

[35] Mazher A.A.M., A.A. Yassen and Sahar, M. Zaghloul, Influence of foliar application of potassium on growth and chemical composition of Bauhinia variegata seedlings under different irrigation intervals. World. J. Agric. Sci., **3**(1) 23-31 (2007).

[36] El-Quesni, Fatma, E.M., Azza A.M. Mazher, Nahed, G. Abd El-Aziz and S.A. Metwally, Effected of compost on growth and chemical composition of Matthiola incana (L.) R. Br. Under different water intervals. Journal of Applied Sciences Research, **8** (3) 1510-1516 (2012).

[37] Kaiser, W.M., G. Kaiser, S. Schoner and Neiments S.,. Photosynthesis under osmotic stress. Differential recovery of photosynthetic activities of stroma enzymes, intact chloroplasts and leaf slices after exposure to high solute concentrations. Planta. **153**, 430-435 (1981).

[38] McKersie, B.P. and Lashem Y.Y.,, Stress and Stress Coping in Cultivated Plants. Kluwer Academic, Publishers, London (1994).

[39] Azza, A.M. Mazher, Sahar, M. Zaghloul and Yassen A.A.,. Impact of Boron fertilizer on growth and chemical constituents of Taxodium distichum grown under water regime. World Journal of Agricultural Sciences **2** (4): 412-420 (2006).

[40] Romaisa Altaf, Khalid Hussain, Ujala Maryam, Khalid Nawaz, Naima Munir and Ejaz

Hussain Siddiq. Effect of Different Levels of Drought on Growth, Morphology and Photosynthetic Pigments of Lady Finger (*Abelmoschus esculentus*). World Journal of Agricultural Sciences **11**(4), 198-201, (2015). ISSN 1817-3047 © IDOSI Publications, 2015 DOI: 10.5829/idosi.wjas.2015.11.4.1856.

[41] Ayse Deligoz, Esra BAYAR . Drought stress responses of seedlings of two oak species (Quercus cerris and Quercus robur). Turkish Journal of Agriculture and Forestry, **42**114-123(2018). doi:10.3906/tar-1709-29.

[42] Liang X, Zhang L, Natarajan SK, Becker D.F., Proline mechanisms of stress survival. Antioxid Redox Signal **19**, 998- 1011 (2013).

[43] Bhaskara G.B, Yang T, Verslues P.E., Dynamic proline metabolism: importance and regulation in water limited environments. Front Plant Sci **6**, 484 (2015).

[44] Deligoz, A., Gur, M., Morphological, physiological and biochemical responses to drought stress of Stone pine (*Pinus pinea* L.) seedlings. Acta Physiol Plant **37**, 243 (2015).

[45] Szabados, L.; Savoure, A., Proline A multifunctional amino acid. Trends Plant Sci,**15**, 89–97(2010).

[46] Blum A., Crop responses to drought and the interpretation of adaptation. Plant Growth Regul., **20**, 135-148 (1996).

[47] Soad, Ibrahim M.M., Responses of vegetative growth and chemical composition of jojopa seedlings to some agriculture treatments. Ph.D. Thesis, Fac. Of Agric. Minia Univ. Egypt (2005).

[48] Salwa A.R. H., Osama A.M. A., Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. Annals of Agricultural Science .**59**(1),133-145 (2014).

[49] Jain, M., Mathur, G., Koul, S. and Sarin, N.B., Ameliorative effects of proline on salt stressinduced lipid peroxidation in cell lines of groundnut (Arachis hypogaea L.). Plant Cell Reports, **20**, 463 - 468 (2001).

[50] Shehab, G.G. Ahmed, O.K. and El-Beltagi, H.S., Effects of various chemical agents for alleviation of drought stress in rice plants (*Oryza sativa* L.). Notulae Botanicae Horti Agrobotanici Cluj Napoca, **38** (1), 139-148 (2010).

[51] Athar, H., Khan A. and Ashraf M., Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. Environ. Exp. Bot. **63**, 224 -231 (2008).

[52] Sairam, R.K. and Srivastava G.C., Water stress tolerance of wheat (*Triticum aestivum* L.) variations in hydrogen peoxide accumulation and antioxidant activity in tolerant and susceptible genotypes. J. Agron. Crop Sci. **186**, 63-70 (2001).

[53] Hasheminasab, H., Assad M.T., Aliakbari A. and Sahhafi R., Influence of drought stress on oxidative damage and antioxidant defense systems in tolerant and susceptible wheat genotypes. J. Agric. Sci. **4**(8, 20-30 (2012).

[54] Hendriks, T., H.J. Wijsman and Van Loon L.C., Petunia peroxidase a: Isolation, purification and characteristics. European J. Biochem. **199**, 139-146 (1991).

[55] Liszkay A., Kenk B., Schopher P. Evidence for the involvement of cell wall peroxidase in the generation of hydroxyl radicals mediating extension growth. Planta, 217, 658 - 667 (2003).

[56] Mohammad khani, N. and Heidari R., Drought induced accumulation of soluble sugars and proline in two maize varieties. World Appl. Sci. J. **3**(3), 448-453 (2008).

[57] Chaparzadeh, N., D'Amico, M.L., Khavari-Nejad R.A, Izzo R. and Navari-IzzoF. ,Antioxidative responses of Calendula officinalis under salinity conditions. Plant Physiol. Biochem., **42**, 695–701 (2004).

[58] Munne-Bosch S., Alegre L., Die and let live: leaf senescence contributes to plant survival under drought stress. Funct Plant Biol 3,1203 - 216 (2004).

[59] Pourcel L, Routaboul J.M., Cheynier V., Lepiniec L., Debeaujon I., Flavonoid oxidation in plants: from biochemical properties to physiological functions. Trends Plant Sci., **8**, 29–36 (2006).

[60] Sonja Veljovic-Jovanovica, Biljana Kukavicaa and Flavia Navari-Izzob, Characterization of polyphenol oxidase changes induced by desiccation of *Ramonda serbica* leaves. Physiologia Plantarum **132**,407–416 (2008). doi: 10.1111/j.1399-3054.

[61] Tayebeh, A., Hassan, P. Antioxidant enzymes changes in response to drought stress in ten cultivars of oilseed rape (*Brassica napus* L.) Czech journal of genetics and plant breeding **46**(1) 27-34 (2010).

[62] Gerami1 F., Moghaddam P. R., Ghorbani, R. and Hassani A., Effects of irrigation intervals and organic manure on morphological traits, essential oil content and yield of oregano (*Origanum vulgare* L.). Annals of the Brazilian Academy of Sciences. **88**(4), 2375-2385 (2016).

[63] Mazrou, K. E. , Ibrahim I. A., Alabsawy, S.A. , Gharib, K. G., Effect of water deficit on the growth, yield and essential oil content of lemon grass in vivo., Menoufia J. Plant Prod., **3**, 335 – 350 (2018).

[64] Esam, A., Al-Azzony, A.and Rania M.R. Khater., Effect of Irrigation Intervals and Sodium Selenite on Growth, Seed Yield and Essential Oil of Fennel. Middle East J. Agric. Res., **10** (1), 391-399 (2021).

[65] Singh H. P., Shalinder Kaur, Kirti Negi , Savita Kumari , Varinder Saini , Daizy R. Batish , Ravinder Kumar Kohli., Assessment of in vitro antioxidant activity of essential oil of Eucalyptus citriodora (lemon-scented Eucalypt; Myrtaceae) and its major

constituents**.,** LWT - Food Science and Technology **48**, 237- 241 (2012). doi:10.1016/j.lwt.2012.03.019

[66] Leicach, Silvia, Ana M. Garau, Ana Guarnaschelli, Margarita Yaber Grass, Norberto D. Sztarker, Analia Dato, Changes in Eucalyptus camaldulensis essential oil composition as response to drought preconditioning, Journal of Plant Interactions., **5**(3), 205-210 (2010). DOI: 10.1080/17429145.2010.483744