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MITIGATING SALT STRESS EFFECTS BY EXOGENOUS APPLICATION OF PROLINE AND YEAST EXTRACT ON MORPHO-PHYSIOLOGICAL, BIOCHEMICAL AND ANATOMICAL CHARACTERS OF CALENDULA PLANTS

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ABSTRACT: To avoid the hazard effect of salinity on calendula (*Calendula officinalis* L.), two field experiments were carried out at Sahl El-Husseinieh Research Station, Al-Sharqia Governorate, Egypt during two successive winter seasons of 2017/2018 and 2018/2019 in silty clay soil to evaluate the effects of foliar spraying with yeast at 0, 4, 8 and 12 g l^{-1}, proline at 0, 50, 75 and 100 mg l^{-1} and the interaction between them. The obtained results showed that foliar application of proline, yeast and their interactions led to improve and increase plant growth, floral, physiological and biochemical characters of calendula plants significantly more than those obtained by control, such as plant height, number of branches $plant^{-1}$, plant fresh and dry weights, number of flowers $plant^{-1}$, flowers fresh and dry weight, relative water content, carotenoid, total chlorophyll, proline content in plant leaves, as well as the activity of PPO, POX and CAT. Also, the above treatments led to improve calendula plant growth by reducing the activity of both superoxide and hydrogen peroxide and decreasing EL. Application of proline, yeast and their interactions increased the elements content of calendula plant for N, P, K, Ca and Mg %. In the contrary, they decreased Na % in the plant. Results showed that vegetative growth, floral parameters as well as anatomical characters of leaves were significantly decreased under salt stress conditions. The physiological and biochemical characters such as photosynthetic pigments content, relative water content (RWC %) and mineral elements (N, P, K, Ca and Mg %) in the leaves also significantly decreased. Whereas, Na %, proline concentration, the activity of (O-2 and H2O2) and electrolyte leakage (EL %) were increased. Moreover, available N, P and K (mg kg^{-1}) in the soil is negatively affected by salt stress. The combined treatments of proline at 100 mg l^{-1} + yeast at 12 g l^{-1} followed by proline at 100 mg l^{-1} + yeast at 8 g l^{-1} gave the higher values of plant growth, biochemical and elemental content in plant besides improved the anatomical characters, consequently, increased the tolerance of calendula plants to salt stress under field conditions.

Key words: *Calendula officinalis* L., Salt stress, Proline, Anatomical structure, Enzymes activity.

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INTRODUCTION

Calendula (*Calendula officinalis* L.), belongs to Asteraceae (Composite) family, commonly known as pot marigold. It is an annual flowering winter plant in Egypt, it has an attractive colour of flower which are used as a cut flower and also grown for medicinal use (Rigane *et al.,* 2013). Marigold is used as a source of natural pigment in the foods industry and in the cure of pain and skin disorders as well as a bactericide, antiseptic and anti-inflammatory (El-Gamal, 2015).

Salinity stress seriously affects more than a third of the world's cultivated land (Bayat *et al.,* 2012), causes many harmful effects on plants, a sharp decrease in the yield of many plants and biomass, leads to growth reduction as well as metabolic changes similar to those caused by water stress (Abdelaal, 2015a; Abdelaal *et al.,* 2017; El-Banna and Abdelaal, 2018 and Hafez *et al.,* 2020). Furthermore, salt stress causes ion imbalance, osmotic stress, especially with Ca, K and the direct poisonous effects of ions on the metabolic operation (Hashish *et al*., 2015). The high saline levels can cause hyper osmotic and hyper ionic effects on plants, leading to increment reactive oxygen species (ROS) levels and membrane damage (El-Banna and Abdelaal, 2018 and Abdelaal *et al.*, 2020a). The harmful effects of salinity on plants are attributed to oxidative and osmotic stresses (Helaly *et al*., 2017; Mansour and Ali, 2017 and Abdelaal *et al*., 2020b). Salty soil has injurious effects on soil chemical and physical characters and causes nutrient shortages. Naher *et al.* (2011) indicated that salt stress led to increase soil pH, EC and commutable Na nutrient, shortages of total nitrogen, potassium and phosphorous were very predominant. Potassium, phosphorus, nitrogen and calcium contents in tissues significantly reduced when soil salinity increased.

Currently, a great attention has been focused on the application of natural and safe components to counteract the deleterious effects of salt stress on plant growth and improve plant growth as well as yield production. Proline is an amino acid, it has a main role in primary metabolism as a component of proteins. It is one of the most widely distributed compatible solutes accumulate during various stress conditions such as salinity, drought or low temperatures (Lehmann *et al.*, 2010). The suggested roles of proline under stress are an antioxidative defense molecule and as a signaling molecule to control mitochondrial functions, influence cell death and release special gene expression which can be necessary for plant recovery under stress (Hayat *et al.*, 2012). In addition to its role in keeping membrane stability, osmoregulation, seed germination and plant growth (Hare *et al.*, 2003). Under salinity stress, Sadak and Mostafa (2015) stated that proline treatment led to decrease oxidative stress and improve the plant growth as well as yield production in sunflower plants. Also, enzymes activity such as catalase (CAT) and peroxidase (POX) were significantly increased in salt-stressed *Pancratium maritimum* plants treated with proline compared with untreated plants (Khedr *et al.*, 2003). Exogenous application of proline alleviates the adverse effect on fresh and dry weights as well as chlorophyll content of salt-stressed Cucumis seedlings (Yan *et al.*, 2011), increases N, P, K^+ and Ca^{+2} %, the K^+/Na^+ ratio, decreases Na^+, Cl^- and reduces the harmful effect of salinity on the anatomical structure in *Vicia faba* (Dawood *et al.*, 2014) and reduces the harmful effect of NaCl stress on tobacco plants (Hoque *et al.,* 2008).

Yeast is a natural, safe biofertilizer has a pivotal role in improving plant growth. It is considered as a natural source of cytokinins and B-vitamin (Amer, 2004 and Matter and Abou-Sreea, 2016), organic compounds (protein, carbohydrates, nucleic acid, lipids and most nutrient elements) as well as growth substances such as thiamine, riboflavin, pyridoxine, folic acid and vitamin B12 (Nagodowithana, 1991). Application of yeast extract to calendula decreased conspicuously the risk effects of salinity and improved vegetative and growth characters as well as floral characters under saline and

non-saline conditions of calendula (Nofal *et al.*, 2015) and leucaena plants (Nassar *et al.*, 2016). The application of yeast extract led to enhance plant growth, increase potassium content, but, reduce sodium and proline under salt stress conditions in fennel (Mostafa, 2015).

In the recent years, there is a global direction to use safe and natural products in agricultural production to protect human health and environmental balance. Therefore, the aim of this study aimed to use osmoprotectant (proline) and yeast extract (natural material) for improving growth and flowering of *Calendula officinalis* as well as physiological, biochemical and anatomical characters under salt stress conditions.

MATERIALS AND METHODS

Two field experiments were carried out at Sahl El-Husseinieh Research Station, Agriculture Research Center, Sharqia Governorate during two successive seasons of 2017/2018 and 2018/2019, and the laboratory investigations were carried out at (EPCRS) Excellence Center and Plant Pathology and Biotechnology Lab., Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University, Egypt, the aim of this study was to reduce the harmful effect of soil salinity on the growth and physiological parameters as well as the flowering of *Calendula officinalis* by foliar spraying with proline and yeast extract.

Plant materials and experimental design:

The experiment was conducted in a split plot in randomized complete block design, the main plot which was yeast extract at four levels (0, 4, 8, 12 g l^{-1}) and the subplot which was proline (L-Proline 99%) at four levels (0, 50, 75, 100 mg l^{-1}). All treatments were sprayed on the foliage of calendula plants till falling of the first drop for three times (every two weeks). The experimental treatments were 16 treatments with three replicates. The experimental area (plot) was 10.8 m^2 (3 m × 3.6 m) containing 3 rows. Every row contained nine plants.

Seeds of calendula were obtained from Elzoharia garden, Hort. Res. Inst., ARC, Ministry of Agriculture, Egypt. Seeds were sown on 28 September during two seasons in the hills at 40 cm distance between the hills. Thinning for one plant $hill^{-1}$ was done at 45 days after sowing, the irrigation was executed out whenever plants needed and weeds were removed by hand. The experiment was fertilized by the recommended rates from the Egyptian Ministry of Agriculture. The rate of N was 300 kg fed^{-1} (771 g $plot^{-1}$ ammonium sulfate) at three doses, the period between them is one month, the first one after one month of cultivation. The rate of P was 200 kg fed^{-1} (514 g $plot^{-1}$ calcium superphosphate) added before cultivation. K was 100 kg fed^{-1} (257 g $plot^{-1}$ potassium sulfate) at two doses added with the first and second doses of nitrogen.

Soil chemical and mechanical analysis:

A composite soil sample was taken from the surface soil (0-30 cm) of the experimental sites before any practices and was air-dried, sieved by 2 mm sieve and analyzed. Physico-chemical properties were conducted as the following. Distribution of particle size by utilizing the pipette method according to Dewis and Fertias (1970), electrical conductivity and the soil pH of saturated soil paste extract according to Jackson (1967) and Richards (1954). Data of soil analysis were recorded in Table (1).

Morphological growth characters:

At maturity (after 105 days from planting) nine plants were randomly taken from each plot for determining the following characters. Plant height (cm), number of branches $plant^{-1}$, fresh and dry weights of plant (g $plant^{-1}$).

Floral characters:

Number of flowers, flower diameter (cm), flowers fresh and dry weights (g $plant^{-1}$) as well as vase life of flower (days).

Table 1. Chemical and mechanical analysis of experimental soil.

Seasons	Mechanical analysis (%)				Chemical analysis			
	Sand (%)	Silt (%)	Clay (%)	Texture grade	Organic matter	$CaCO_3$ (%)	pH (1.2.5)	E.C. dS m^{-1} (1. 5)
2017/2018	19.5	28.5	52	Clay	1.14	3.2	8.15	3.64
2018/2019	19	28	53	Clay	1.09	2.1	8.25	4.33

	Available (mg kg^{-1})			Cations (meq l^{-1})				Anions (meq l^{-1})			
	N	P	K	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{--}	HCO_3^{-}	Cl^{-}	SO_4^{--}
2017/2018	56	8.5	271.03	6.70	4.98	24.19	0.30	-	1.50	19.67	15.00
2018/2019	52	8.0	215.48	9.05	6.25	27.30	0.41	-	2.43	22.23	18.35

Physiological and biochemical studies:

Determination of chlorophylls and carotenoids. Total chlorophylls in the leaves and β-carotene contents in flowers were determined according to Sumanta *et al*. (2014).

Determination of proline, reactive oxygen species (superoxide and hydrogen peroxide) and enzymes activity. Proline was determined according to Bates *et al*. (1973). Superoxide and hydrogen peroxide were determined according to Adam *et al*. (1989) and Hückelhoven *et al*. (1999). Activities of catalase (CAT) enzyme were assayed according to Aebi (1983). POX was assayed according to (Hammerschmidt *et al*.,1982). PPO activity was assayed according to Malik and Singh (1980).

Relative water content and electrolyte leakage:

Relative water content (RWC %) was measured according to Sanchez *et al.* (2004) using the following equation RWC = (fresh weight – dry weight) / (turgid weight – dry weight) × 100. Electrolyte leakage (EL %) was estimated according to Dionisio-Sese and Tobita (1998) by calculating initial electrical conductivity/final electrical conductivity x 100. EL% = EC1/EC2×100.

Mineral contents:

Determination of nitrogen, phosphorus, potassium, calcium, magnesium and sodium contents in plants. The samples were dried at 70 ℃ then crushed and digested using H_2SO_4 and $HClO_4$ acids according to Cottenie *et al*. (1982). Nitrogen (N %) was determined as described by Official Analytical (A.O.A.C., 1990). Phosphorus (P %) was determined as described by Olsen and Sommers (1982). Potassium (K %) and sodium (Na^{+} %) were determined as described by Jackson (1967). Calcium (Ca %) and magnesium (Mg %) were determined using inductively coupled spectrometry plasma (ICP) Model Ultimate-Jobin Yvon.

Anatomical characters:

The samples (5 mm length) were taken at the age of 70 days from the leaves during the second season (2018/2019). Was determined according to (Nassar and El-Sahhar, 1998). Slides were investigated and photomicrographed.

Statistical analysis:

Statistical analysis was done by using the analysis of variance technique by means of the Co-Stat Computer Software version 6.303. The mean was compared by Duncan's Multiple Range Test (Duncan, 1955) (DMRT, P≤0. 05) (Gomez and Gomez, 1984).

RESULTS

Morphological growth characters:

Data presented in Table (2) showed that proline, yeast extract and their combination caused a significant increase in plant height of *Calendula officinalis*, number of branches, fresh and dry weights of plants compared with control plants. Foliar application of 100 mg l^{-1} proline resulted in significant increases in the different growth parameters, compared with other proline concentrations and control (81.06 and 79.97 cm for plant height, 17.14 and 16.22 branches $plant^{-1}$ for the number of branches,

Table 2. Effect of proline, yeast and their interaction on plant height (cm), number of branches/plant, plant fresh and dry weights (g) of calendula plants in the two seasons.

Yeast (g l^{-1})	Proline (mg l^{-1})					Proline (mg l^{-1})				
	Control	50	75	100	Mean	Control	50	75	100	Mean
	1st season					2nd season				
	Plant height (cm)									
Control	37.67h	62.83fg	68.67c-e	72.33c	60.38c	37.33g	58.89ef	66.89cd	70.89c	58.50c
4	60.50g	63.67e-g	71.17cd	80.67ab	69.00b	56.78f	61.33ef	69.39c	79.67ab	66.79b
8	60.67fg	78.33b	83.17ab	85.22a	76.85a	58.17ef	76.87b	81.83ab	83.83a	75.18a
12	66.17d-f	80.83ab	84.28a	86.00a	79.32a	63.17de	80.17ab	83.67a	85.50a	78.13a
Mean	56.25d	71.42c	76.82b	81.06a		53.86d	69.31c	75.44b	79.97a	
	No. of branches									
Control	7.00i	10.33gh	11.83e-g	12.67ef	10.46d	6.33l	9.67i-k	10.67g-i	12.00e-g	9.67d
4	9.33h	11.33fg	12.17ef	14.67cd	11.88c	8.67k	10.00i-k	11.67f-h	13.33e	10.92c
8	9.67h	13.17de	18.17b	20.33a	15.33b	9.00jk	12.67ef	17.33c	18.89b	14.47b
12	11.67e-g	14.83c	19.50ab	20.89a	16.72a	10.33h-j	15.33d	18.56bc	20.67a	16.22a
Mean	9.42d	12.42c	15.42b	17.14a		8.58d	11.92c	14.56b	16.22a	
	Plant fresh weight (g)									
Control	225.67h	539.40fg	575.40d-f	594.23c-f	483.68d	229.89g	525.26e	511.10ef	696.01ab	490.56b
4	486.00g	544.70f	578.03d-f	608.67c-e	554.35c	450.31f	536.10de	552.77de	603.37cd	535.64b
8	538.17fg	552.97ef	642.40c	719.85b	613.35b	527.01e	600.43cd	697.67ab	729.20a	638.78a
12	564.42d-f	619.20cd	708.90b	785.05a	669.38a	538.81de	659.62bc	701.42ab	755.93a	663.95a
Mean	453.56d	564.06c	626.18b	676.95a		436.51d	580.35c	615.74b	696.13a	
	Plant dry weight (g)									
Control	42.43i	93.90g	102.40ef	106.27e	86.25d	43.22g	97.75de	90.58e	123.64b	88.80c
4	81.47h	96.50fg	104.73e	109.00e	97.93c	77.14f	98.00de	101.17d	110.22c	96.63b
8	93.60g	107.55e	128.35c	137.77b	116.82b	91.78e	115.58c	138.56a	139.78a	121.42a
12	101.93ef	118.40d	138.28b	147.80a	126.60a	97.04de	125.31b	136.07a	139.86a	124.57a
Mean	79.86d	104.09c	118.44b	125.21a		77.30d	109.16c	116.59b	128.37a	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's multiple range test.

676.95 and 696.13 g plant^{-1} for plant fresh weight, 125.21 and 128.37 g plant^{-1} for plant dry weight during the two seasons, respectively). The obtained results in Table (2) revealed that application of yeast at 12 g l^{-1} recorded the highest mean values in plant height, number of branches, plant fresh and dry weights in both seasons respectively.

Concerning the effect of the interactions between proline and yeast extract on morphological growth characters, data presented in the same Table showed that there were significant differences between treatments. The plants sprayed with proline at 100 mg l^{-1} in combination with yeast extract at 12 g l^{-1} resulted in a maximum plant height (86.00 and 85.50 cm), number of branches plant^{-1} (20.89 and 20.67), plant fresh weight (785.05 and 755.93 g plant^{-1}) and plant dry weight (147.80 and 139.86 g plant^{-1}) in the two seasons, respectively.

Floral characters:

Application of the different proline treatments significantly promoted the production of flowers, flowers fresh and dry weights, flower diameter and vase life of flowers in both seasons as shown in Table (3). Proline treatment at 100 mg l^{-1} had the most promising effect in improving flowering quality. Also, this treatment gave a greatest number of flowers (163.64 and

Table 3. Effect of proline, yeast and their interaction on number of flowers, flower fresh and dry weight (g/flower), flower diameter (cm) and vase life (day) of calendula plants in the two seasons.

Yeast (g l^{-1})	Proline (mg l^{-1})					Proline (mg l^{-1})				
	Control	50	75	100	Mean	Control	50	75	100	Mean
	1st season					2nd season				
	No. of flowers									
Control	54. 67h	115.00f	119.67f	140.00de	107.33d	52.67i	112.67fg	118.00e-g	134.00de	104.33d
4	91.00g	117.33f	132.00e	148.23d	122.14c	89.00h	116.67e-g	131.33d-f	145.67cd	120.67c
8	112. 67f	142.33d	149.67d	175.00b	144.92b	107.67g	139.33d	150.67cd	170.33b	142.00b
12	119.33f	161.33c	165.33c	191.33a	159.33a	117.33e-g	158.67bc	163.33bc	188.33a	156.92a
Mean	94.42d	134.00c	141.67b	163.64a		91.67d	131.83c	140.83b	159.58a	
	Flowers fresh weight (g)									
Control	117.17e	394.00cd	407.30c	434.10bc	338.14b	112.67g	379.81de	398.94c-e	413.38b-d	326.20c
4	345.47d	399.87c	409.80c	441.97a-c	399.28a	325.07f	384.63c-e	400.50c-e	439.73b	387.48b
8	387.67cd	400.90c	414.47bc	464.90ab	416.98a	372.57e	393.40c-e	409.77b-d	470.73a	411.62a
12	389.07cd	401.23c	422.47bc	491.60a	426.10a	380.83de	396.03c-e	417.87bc	478.73a	418.37a
Mean	309.84c	399.00b	413.51b	458.14a		297.78d	388.47c	406.78b	450.65a	
	Flowers dry weight (g)									
Control	33.49f	98.34de	106.79cd	117.77b-d	89.10c	32.97j	94.31h	104.12d-h	111.65c-e	85.76d
4	85.82e	101.73de	106.92cd	121.76a-c	104.06b	82.62i	97.31gh	104.17d-h	120.75bc	101.21c
8	103.82c-e	106.44cd	111.07b-d	127.01ab	112.09ab	100.74f-h	104.64d-g	108.92df	127.57ab	110.47b
12	104.74cd	109.10b-d	115.25b-d	136.42a	116.37a	101.68e-h	106.94d-g	113.12cd	132.13a	113.47a
Mean	81.97c	103.90b	110.01b	125.74a		79.50d	100.8c	107.58b	123.03a	
	Flower diameter (cm)									
Control	4.84i	6.77gh	6.87e-h	7.03c-h	6.38c	5.17j	6.63g-i	6.80e-h	7.07c-f	6.42d
4	6.53h	6.80f-h	6.97d-h	7.30b-f	6.90b	6.40i	6.73e-i	6.93d-g	7.27b-d	6.83c
8	6.63h	7.17b-g	7.43b-d	7.63ab	7.22a	6.50hi	7.10c-e	7.33bc	7.53ab	7.11b
12	6.80f-h	7.37b-e	7.50a-c	7.97a	7.41a	6.70f-i	7.30b-d	7.43bc	7.83a	7.31a
Mean	6.20c	7.03b	7.19b	7.48a		6.19d	6.94c	7.13b	7.43a	
	Vase life (days)									
Control	6.33g	6.83g	8.33f	9.00ef	7.63c	6.00e	6.67e	8.11d	8.67cd	7.36c
4	6.67g	7.00g	8.50f	10.00cd	8.04c	6.33e	6.89e	8.33d	9.67bc	7.81c
8	6.67g	9.33de	10.67bc	11.33ab	9.50b	6.56e	9.11cd	10.33ab	11.00a	9.25b
12	7.00g	10.83b	10.67bc	11.67a	10.04a	6.89e	10.67ab	10.56ab	11.33a	9.86a
Mean	6.67d	8.50c	9.54b	10.50a		6.45d	8.33c	9.33b	10.17a	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's multiple range test.

159.58 flower plant^{-1}), maximum fresh weight of flowers (458.14 and 450.65 g plant^{-1}), flowers dry weight (125.74 and 123.03 g plant^{-1}), flower diameter (7.48 and 7.43 cm) and flower vase life (10.50 and 10.17 days) in the two seasons, respectively. The presented data in Table (3) revealed that all the mean values of floral parameters significantly increased with the different concentrations of yeast extract compared with the untreated plants. Yeast extract at 12 g l^{-1} represented the highest values among all levels which gave the highest flower number (159.33 and 156.92 flower plant^{-1}), fresh weight of flower (426.10 and 418.37 g plant^{-1}), dry weight of flowers (116.37 and 113.47 g plant^{-1}), flower diameter (7.41 and 7.31 cm) and flower vase life (10.04 and 9.86 days) respectively, in the two seasons. Regarding the effect of interactions between

proline and yeast on flowering characters, there was a significant difference between treatments in both seasons (Table, 3). The best treatment was proline at 100 mg l^{-1} + yeast extract at 12 g l^{-1} which gave the highest values in all floral parameters in the two seasons.

Physiological and biochemical studies:

1. Photosynthetic pigments content:

Application of proline at all concentrations caused significant increases in carotenoids and total chlorophyll in both seasons as presented in Table (4). The highest values of carotenoids (26.70 and 27.25 mg 100 g^{-1} fw) and total chlorophylls (0.917 and 0.895 mg g^{-1} fw) were recorded for plants treated with proline at 100 mg l^{-1} in the two seasons, respectively. As shown in the same table, yeast extract treatments significantly affected carotene and chlorophyll content in the two seasons. Yeast extract treatment at 12 g l^{-1} gave the highest values of carotene (26.55 and 27.30 mg 100 g^{-1} fw) and chlorophyll content (0.909 and 0.884 mg g^{-1} fw) in the two seasons respectively. It is quite clear from the data presented in the same Table that the interactions between the two factors significantly affected carotene and chlorophyll content. The plants treated with 100 mg l^{-1} proline +12 g l^{-1} yeast resulted in a maximum carotene (27.70 and 28.70 mg 100 g^{-1} fw) and total chlorophyll (0.997 and 0.985 mg g^{-1} fw) in the two seasons, respectively.

2. Proline content:

Results in Table (4) showed that proline content was decreased in the plants treated with yeast in saline soil. Contrariwise, plants gave the highest response to proline accumulation in the leaves when treated with proline at all concentrations and control plants. Data showed also that plants sprayed with proline at 100 mg l^{-1} gave the highest

Table 4. Effect of proline, yeast and their interaction on carotene (mg 100 g^{-1} fw, total chlorophyll (mg g^{-1} fw) and proline (µg g^{-1} fw) in calendula plants in the two seasons.

Yeast (g l^{-1})	Proline (mg l^{-1})					Proline (mg l^{-1})				
	Control	50	75	100	Mean	Control	50	75	100	Mean
	1st season					2nd season				
	Carotene (mg g^{-1} fw)									
Control	24.20k	25.10i	25.60g	26.00f	25.23d	25.30h	25.90g	26.30ef	26.40de	25.98d
4	24.90j	25.40h	25.60g	26.20e	25.53c	25.40h	26.10fg	26.40de	26.60d	26.13c
8	25.00ij	26.20e	26.50d	26.90b	26.15b	25.90g	26.60d	27.20b	27.30b	26.75b
12	25.50gh	26.30e	26.70c	27.70a	26.55a	26.30ef	26.90c	27.30b	28.70a	27.30a
Mean	24.90d	25.75c	26.10b	26.70a		25.73d	26.38c	26.80b	27.25a	
	Total chlorophyll (mg g^{-1} fw)									
Control	0.537o	0.707l	0.800i	0.827h	0.718d	0.480m	0.639j	0.773h	0.785g	0.669d
4	0.606n	0.737k	0.825h	0.868f	0.759c	0.570l	0.641j	0.778h	0.836e	0.706c
8	0.681m	0.861g	0.927d	0.974b	0.861b	0.581k	0.800f	0.962c	0.974b	0.829b
12	0.767j	0.915e	0.957c	0.997a	0.909a	0.657i	0.931d	0.963c	0.985a	0.884a
Mean	0.648d	0.805c	0.877b	0.917a		0.572d	0.753c	0.869b	0.895a	
	Proline (µg g^{-1} fw)									
Control	7.52d	8.62c	9.12b	9.62a	8.72a	7.50d	7.96c	8.77b	9.51a	8.43a
4	6.27h	6.42g	7.22f	7.41e	6.83b	6.29g	6.63f	7.00e	7.31d	6.81b
8	5.96j	5.99j	6.02ij	6.08i	6.01c	4.36j	5.21i	6.01h	6.11gh	5.42c
12	4.37n	5.01m	5.12l	5.62k	5.03d	3.65l	3.97k	4.08k	4.20jk	3.98d
Mean	6.03d	6.51c	6.87b	7.18a		5.45d	5.94c	6.46b	6.78a	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's multiple range test.

values of proline content (7.18 and 6.78 µg g^{-1} fw) comparing with yeast at 12 g l^{-1} which gave the lowest values of proline content (5.03 and 3.98 µg g^{-1} fw) in the two seasons respectively. As for the interaction between proline and yeast extract, data in presented Table (4) demonstrated that treated plants with proline at 100 mg l^{-1} without yeast extract gave the highest values of proline content (9.62 and 9.51 µg g^{-1} fw) respectively, in the two seasons comparing with the other treatments in the two seasons.

Enzymes activity, relative water content (RWC %) and electrolyte leakage (EL %) in calendula leaves in the second season:

Regarding the effect of salinity on antioxidant enzymes activity, the presented data in Table (5) indicated that application of proline and yeast led to overcome the harmful effects of salinity as well as increase CAT activity, the best results were recorded with 100 mg l^{-1} proline + 12 g l^{-1} yeast followed by 100 mg l^{-1} proline + 8 g l^{-1} yeast respectively, compared with control plants as presented in Table (5). Furthermore, POX and PPO activity significantly increased with most treatments, the best results of POX were obtained at 100 mg l^{-1} proline + 12 g l^{-1} yeast as well as 75 mg l^{-1} proline + 12 g l^{-1} yeast respectively, compared with control plants and other treatments. Likewise, proline and yeast application significantly increased activity of PPO in the stressed plants compared with control plants and the best treatment was 100 mg l^{-1} proline + 12 g l^{-1} yeast. Under salt stress conditions, the levels of superoxide and hydrogen peroxide (ROS) significantly increased in the salt-stressed plants (56.67 and 41.80). Nevertheless, these levels of ROS were decreased under various treatments of proline and yeast, the minimum level of superoxide and hydrogen peroxide (24.77 and 22.07) was recorded with the best treatment (100 mg l^{-1} proline + 12 g l^{-1} yeast). Data presented in Table (5) indicated that salt stress led to a significant decrease in relative water content, however, proline and yeast application (50, 75 and 100 mg l^{-1} proline as well as 4, 8 and 12 g l^{-1} yeast) led to improve plant growth and increase the relative water content. The best results were recorded with 100 mg l^{-1} proline + 12 g l^{-1} yeast followed by 100 mg l^{-1} proline + 8 g l^{-1} yeast respectively, compared with other treatments and control plants. Under salt stress conditions, electrolyte leakage percentage was increased in calendula plants, nevertheless, application of proline and yeast at various concentrations decreased electrolyte leakage in salt-stressed calendula plants, the best treatment was 100 mg l^{-1} proline + 12 g l^{-1} yeast followed by 75 mg l^{-1} proline + 12 g l^{-1} yeast respectively, compared with other treatments (Table, 5).

3. Mineral elements (%):

It was noticeable that there was a gradual increase in plant of N, P, K, Ca and Mg % combined with a gradual decrease in Na^{+} % in the leaves during the two seasons as shown in Table (6). Proline and yeast application especially at the highest levels (proline at 100 mg l^{-1} and yeast extract at 12 g l^{-1}) alleviated the negative effects of salinity and increased the absorption of the previous elements. The highest values of N% were obtained with proline at 100 mg l^{-1} (1.80 and 1.59 %) and with yeast extract at 12 g l^{-1} (1.78 and 1.57%) in the two seasons, respectively. Also, the same treatments gave the highest values of P, K, Ca^{+2} and Mg^{+2} %. On the other hand, foliar applications of proline at 100 mg l^{-1} or yeast extract at 12 g l^{-1} led to a decrease in the content of Na^{+} in calendula leaves. With regard to the interaction between proline and yeast extract, it is clear from the results listed in Table (6) that the highest N, P, K, Ca^{+2} and Mg^{+2} % were achieved when calendula plants were sprayed with proline at 100 mg l^{-1} and yeast extract at 12 g l^{-1} during the two seasons. On the contrary, the lowest Na^{+} % (0.88 and 1.01%) was obtained when calendula plants were sprayed with the same treatment.

Table 5. Effect of proline, yeast and their interaction on antioxidant enzymes activity, superoxide, hydrogen peroxide, electrolyte leakage (EL %) and relative water content (RWC %) in calendula plants in the second season.

Yeast (g l^{-1})	Proline (mg l^{-1})				
	Control	50 mg	75 mg	100	Mean
	Catalase activity (0.1 min^{-1} mg^{-1} protein)				
Control	42.42h	56.42g	57.00g	70.42f	56.57d
4	77.83ef	96.08d	96.67d	104.92c	93.88c
8	79.92e	112.67bc	106.25c	115.08b	103.48b
12	83.33e	119.33b	112.67bc	140.42a	113.94a
Mean	70.88c	96.13b	93.15b	107.71a	
	Peroxidase activity (U mg^{-1} protein) (U = 1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein)				
Control	0.170h	0.383g	0.383g	0.450e-g	0.347c
4	0.463d-g	0.513c-e	0.557c	0.663b	0.542b
8	0.407fg	0.540cd	0.560c	0.660b	0.549b
12	0.487c-f	0.720ab	0.693ab	0.767a	0.667a
Mean	0.382c	0.539b	0.548b	0.635a	
	Polyphenol oxidase activity (amount of quinon g^{-1} FW min^{-1})				
Control	0.0025j	0.0037ij	0.0037ij	0.0066gh	0.0041d
4	0.0053hi	0.0070f-h	0.0077e-g	0.0080d-g	0.0070c
8	0.0052hi	0.0087c-f	0.0092c-e	0.0135b	0.0092b
12	0.0063gh	0.0096cd	0.0103c	0.0165a	0.0107a
Mean	0.0049c	0.0072b	0.0077b	0.0111a	
	Superoxide (Arbitrary Units)				
Control	56.67a	48.33b	44.53c	38.93de	47.12a
4	45.33bc	35.00f-h	33.83gh	33.03gh	38.33b
8	40.50d	39.00de	37.73d-f	36.07e-g	36.80b
12	39.17de	32.70gh	31.13h	24.77i	31.94c
Mean	45.42a	38.76b	36.81c	33.20d	
	Hydrogen peroxide (Arbitrary Units)				
Control	41.80a	37.33bc	35.37b-e	33.00de	36.88a
4	38.77ab	31.77e-g	32.60d-f	28.30gh	32.86b
8	37.10bc	33.63c-e	31.90e-g	28.33gh	32.74b
12	36.00b-d	28.83f-h	25.60hi	22.07i	28.13c
Mean	38.42a	32.89b	31.37b	27.93c	
	Relative water content (%)				
Control	63.50g	69.63f	71.50ef	73.80de	69.61d
4	73.30de	72.57ef	71.63ef	73.67de	72.79c
8	74.50de	74.10de	76.07cd	81.27ab	76.48b
12	77.80c	78.57bc	78.23c	83.00a	79.40a
Mean	72.28c	73.72b	74.36b	77.93a	
	Electrolyte leakage (%)				
Control	57.33a	41.67b	39.67b	33.60cd	43.07a
4	41.67b	35.00c	33.83cd	33.03cd	35.88b
8	40.50b	32.53cd	30.33d	26.00e	32.34c
12	34.90c	30.67cd	25.60e	22.17e	28.33d
Mean	43.60a	34.97b	32.37c	28.70d	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's multiple range test.

Table 6. Effect of proline, yeast and their interaction on nitrogen, phosphor, potassium, calcium, magnesium and sodium (% dry matter) in calendula leaves in the two seasons.

Yeast (g l^{-1})	Proline (mg l^{-1})					Proline (mg l^{-1})				
	Control	50	75	100	Mean	Control	50	75	100	Mean
	1st season					2nd season				
					N (%)					
Control	1.07j	1.35h	1.48f	1.73cd	1.41d	0.95l	1.16j	1.39g	1.42fg	1.23d
4	1.23i	1.41g	1.61e	1.62e	1.47c	1.04k	1.23i	1.42fg	1.45f	1.29c
8	1.42g	1.60e	1.77c	1.87b	1.67b	1.07k	1.42fg	1.58d	1.70b	1.44b
12	1.58e	1.69d	1.83b	1.97a	1.78a	1.32h	1.51e	1.64c	1.80a	1.57a
Mean	1.32d	1.51c	1.67 b	1.80a		1.10d	1.33c	1.51b	1.59a	
					P (%)					
Control	0.147j	0.195i	0.224gh	0.243fg	0.202c	0.118n	0.181k	0.208i	0.235g	0.186d
4	0.185i	0.214h	0.237g	0.277de	0.228b	0.142m	0.196j	0.225h	0.252f	0.204c
8	0.217h	0.259ef	0.294b-d	0.310b	0.270a	0.158l	0.238g	0.278d	0.295b	0.242b
12	0.189i	0.286cd	0.303bc	0.334a	0.278a	0.205i	0.265e	0.287c	0.316a	0.268a
Mean	0.184d	0.239c	0.265b	0.291a		0.156d	0.220c	0.249b	0.275a	
					K (%)					
Control	1.76k	2.80i	3.45gh	3.73e-g	2.93d	1.34o	2.49l	3.28i	3.69g	2.69d
4	2.03jk	3.10hi	3.63fg	4.02c-e	3.20c	1.92n	2.94k	3.59h	3.87ef	3.08c
8	2.38j	3.83d-f	4.19b-d	4.43ab	3.71b	2.20m	3.78fg	3.97cd	4.34b	3.57b
12	3.15q	4.14b-d	4.28bc	4.72a	4.07a	3.06j	3.88de	4.00c	4.55a	3.87a
Mean	2.33d	3.47c	3.89b	4.22a		2.13d	3.27c	3.71b	4.11a	
					Na (%)					
Control	2.55a	2.25cd	2.06ef	1.90g	2.19a	2.70a	2.36c	2.10e	1.96f	2.28a
4	2.43ab	2.20cde	1.98fg	1.73hi	2.08b	2.48b	2.24d	2.04ef	1.75gh	2.13b
8	2.31bc	1.86gh	1.53j	1.32k	1.75c	2.41bc	1.81g	1.65ij	1.45k	1.83c
12	2.13de	1.67i	1.43jk	0.88l	1.53d	2.22d	1.71hi	1.59j	1.01l	1.63d
Mean	2.36a	2.00b	1.75c	1.46d		2.45a	2.03b	1.85c	1.54d	
					Ca (%)					
Control	1.13k	1.69ij	2.05h	2.67de	1.88d	1.31n	1.73l	2.12i	2.64e	1.95d
4	1.58j	1.97h	2.12gh	2.74cd	2.10c	1.44m	2.05j	2.14h	2.73d	2.09c
8	1.76i	2.43f	2.84c	3.28ab	2.58b	2.01k	2.21f	2.93c	3.07b	2.56b
12	2.27g	2.54ef	3.16b	3.42a	2.84a	2.19g	2.63e	3.06b	3.28a	2.79a
Mean	1.68d	2.16c	2.54b	3.03a		1.74d	2.16c	2.56b	2.93a	
					Mg (%)					
Control	0.166j	0.172j	0.212hi	0.285e	0.209d	0.163i	0.173i	0.208h	0.282e	0.206d
4	0.169j	0.201i	0.222gh	0.296de	0.222c	0.167i	0.205h	0.210h	0.307d	0.222c
8	0.176j	0.264f	0.300d	0.475b	0.304b	0.202h	0.232g	0.315d	0.370b	0.280b
12	0.224g	0.267f	0.345c	0.512a	0.337a	0.213h	0.254f	0.337c	0.406a	0.303a
Mean	0.184d	0.226c	0.270b	0.392a		0.186d	0.216c	0.268b	0.341a	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's multiple range test.

4. Available elements content in the soil:

The presented data in Table (7) indicated that available N, P and K mg kg^{-1} in the soil after harvest significantly decreased with increasing the concentrations of proline and yeast in the two seasons. The highest values of N, P, and K mg kg^{-1} in the soil after harvest were realized with control in the two seasons, respectively. **C**oncerning the interactions between proline and yeast extract on available N, P, and K in the soil data presented in Table (7) showed that all the interaction treatments between proline and yeast extract had significant effects on N, P, and K mg kg^{-1}. The lowest values of N (39.11 and 33.74 mg kg^{-1}), P (8.67 & 8.33 mg kg^{-1}) and K (241.92 and 207.30 mg kg^{-1}) were recorded with the application of proline at 100 mg l^{-1} additional with yeast extract at 12 g l^{-1}, respectively in the two seasons.

Anatomical characters:

The anatomical characters of the leaves were presented in Table (8) and the cross sections are illustrated in Figs. (1 and 2). Our results revealed that the anatomical characters of calendula plants were decreased under salt stress conditions in control treatments (Fig. 1A). It is noted from Table (8) and Figs. (1 and 2) that spraying yeast extract and proline at various levels increased thickness of midvein, leaf lamina, palisade and spongy tissues as well as number of vessels/midvein bundle in salt-stressed calendula plants, the best results were obtained with proline at 100 mg l^{-1} + yeast extract at 12 g l^{-1}.

DISCUSSION

In the present study, spraying *Calendula officinalis* plants with proline, yeast and their interaction improved morphological growth characters, floral parameters, photosynthetic pigments and nutrients %, plant height, number of branches $plant^{-1}$, and plant fresh and dry weights, number of flowers, flower diameter (cm), flowers fresh and dry weights, vase life, chlorophyll, carotene, proline contents, N, P, K, Na, Mg and Ca %. It is clear from our results that the foliar application of proline and yeast extract significantly decreased the deleterious influences of salt stress. However, a higher concentration of proline (100 mg l^{-1}) was more effective than levels of yeast on growth, but the best treatment was the interaction between proline at 100 mg l^{-1} and yeast at 12 g l^{-1}. Several previous studies reported that there was a positive relationship between plant growth and foliar application with proline and yeast under saline soil (Rady *et al.*, 2016).

Application of yeast extract significantly decreased the harmful effects of salinity and enhanced all the vegetative and floral characters as well as chemical compounds in all concentrations especially 12 g l^{-1}. These results are similar to those observed in calendula plants by Nofal *et al.* (2015) who indicated that using yeast extract as a foliar application under saline and non-saline treatments led to improve growth and appeared appropriate variations in the stem and the leaf anatomical structure as affected by salinity. The stimulation influence of dry yeast on plant growth is due to that the active dry yeast is wealthy with vitamins, amino acids and proteins. There was a gradual increment in all morphological growth characters (plant height, number of branches, leaf number, leaf area and leaves dry weight) as well as chemical characters by adding active dry yeast (Matter and Abou-Sreea, 2016) in fenugreek plants. Foliar application of yeast extract significantly increased all morphological characters and yield as well as improved the anatomical characters of the major stem and leaves particularly the phloem and xylem tissues (Nassar *et al.,* 2015) on *Ocimum basilicum*.

In the present investigation, it was observed that the exogenous application of proline, particularly the level of 100 mg l^{-1} significantly enhanced endogenous proline levels in calendula plants, reduced the harmful effects of salinity stress and consequently improved calendula growth, flowering and related characters. This result is due to that proline has been absorbed by

Table 7. Effect of proline, yeast and their interaction on soil available nitrogen, phosphor and potassium (mg kg^{-1}) in the soil at harvest stage of calendula plants in the two seasons.

Yeast (g l^{-1})	Proline (mg l^{-1})					Proline (mg l^{-1})				
	Control	50	75	100	Mean	Control	50	75	100	Mean
	1st season					2nd season				
	N (mg kg^{-1})									
Control	44.03a	43.67cd	43.83b	39.88k	42.85a	43.26a	39.71b	38.88bc	38.35bc	40.05a
4	43.77bc	43.60d	43.34e	39.68l	42.60b	39.08bc	38.71bc	38.66bc	37.81c	38.56b
8	42.81f	42.30g	42.00h	39.22m	41.58c	38.58bc	38.49bc	38.48bc	35.39d	37.74c
12	41.11i	40.22j	40.17j	39.11m	40.15d	38.44bc	38.41bc	38.37bc	33.74e	37.24c
Mean	42.93a	42.45b	42.34c	39.47d		39.84a	38.83b	38.60b	36.32c	
	P (mg kg^{-1})									
Control	13.83a	13.67a	12.33b	10.83ef	12.42a	12.67a	12.50a	11.83b	9.33ef	11.58a
4	12.00c	12.00c	11.33d	10.67fg	11.50b	11.67b	11.00c	10.33d	9.00f	10.50b
8	11.17de	10.67fg	10.33g	9.33i	10.38c	10.17d	9.50e	9.33ef	8.83g	9.33c
12	10.50fg	9.83h	9.33i	8.67j	9.58d	9.33h	9.50i	9.33i	8.33j	9.12d
Mean	12.13a	11.79b	11.08c	9.88d		10.96a	10.62b	10.20c	8.87d	
	K (mg kg^{-1})									
Control	318.68a	310.73ab	281.07c	252.38h-j	290.71a	303.88a	296.75 b	284.33c	227.68hi	278.16a
4	308.58b	306.32b	262.48ef	248.72i-k	285.74b	288.97c	269.85 d	267.90d	226.42hi	263.28b
8	276.47cd	268.83de	261.33e-g	248.13jk	264.60c	245.90e	240.13ef	238.17fg	220.83i	236.26c
12	259.12f-h	257.08f-i	253.52g-j	241.92k	247.79d	236.98fg	235.85fg	232.33gh	207.30j	228.12d
Mean	290.72a	281.53b	263.69c	252.91d		268.93a	260.65a	255.68b	220.56c	

Mean values followed by the same letters are not significantly different at the P<0.05 according to Duncan's multiple range test.

Table 8. Effect of proline and yeast extract on anatomical leaf structure of calendula plants under salt stress conditions in the second season.

Treatments	Anatomical characters of leaves					
	Midvein thickness (µ)	Leaf lamina thickness (µ)	Palisade tissue (µ)	Spongy tissue (µ)	Vessels No/midvein bundle	Vessel diameter (µ)
Control	1336.0	384.5	106.9	241.0	32.6	21.0
Proline 50 mg l^{-1}	1368.2	391.0	111.2	256.7	39.0	25.0
Proline 75 mg l^{-1}	1423.5	417.6	106.8	262.0	41.8	22.6
Proline100 mg l^{-1}	1475.6	429.0	116.0	268.0	42.0	26.0
Yeast 4 g l^{-1}	1384.0	396.0	108.0	259.0	40.0	25.0
Yeast 8 g l^{-1}	1519.0	403.5	109.6	263.6	36.0	24.2
Yeast 12 g l^{-1}	1682.0	449.0	118.0	279.8	39.0	26.0
Proline 50 mg l^{-1} + yeast 4 g l^{-1}	1671.3	431.7	115.0	267.0	41.0	26.0
Proline 75 mg l^{-1} + yeast 4 g l^{-1}	1658.0	427.0	117.0	272.9	38.2	25.5
Proline 100 mg l^{-1} + yeast 4g l^{-1}	1674.5	438.0	116.6	278.4	41.0	24.8
Proline 50 mg l^{-1} + yeast 8 g l^{-1}	1672.7	448.0	119.4	286.0	40.0	23.9
Proline 75 mg l^{-1} + yeast 8 g l^{-1}	1685.0	457.0	121.0	284.0	43.0	26.0
Proline 100 mg l^{-1} + yeast 8 g l^{-1}	1724.0	468.0	125.0	287.0	39.4	25.0
Proline 50 mg l^{-1} + yeast 12 g l^{-1}	1732.4	482.6	126.5	291.3	40.0	26.0
Proline 75 mg l^{-1} + yeast 12 g l^{-1}	1745.0	507.0	137.0	296.0	43.0	25.5
Proline 100 mg l^{-1} + yeast 12 g l^{-1}	1776.2	513.0	135.0	302.0	44.0	26.4

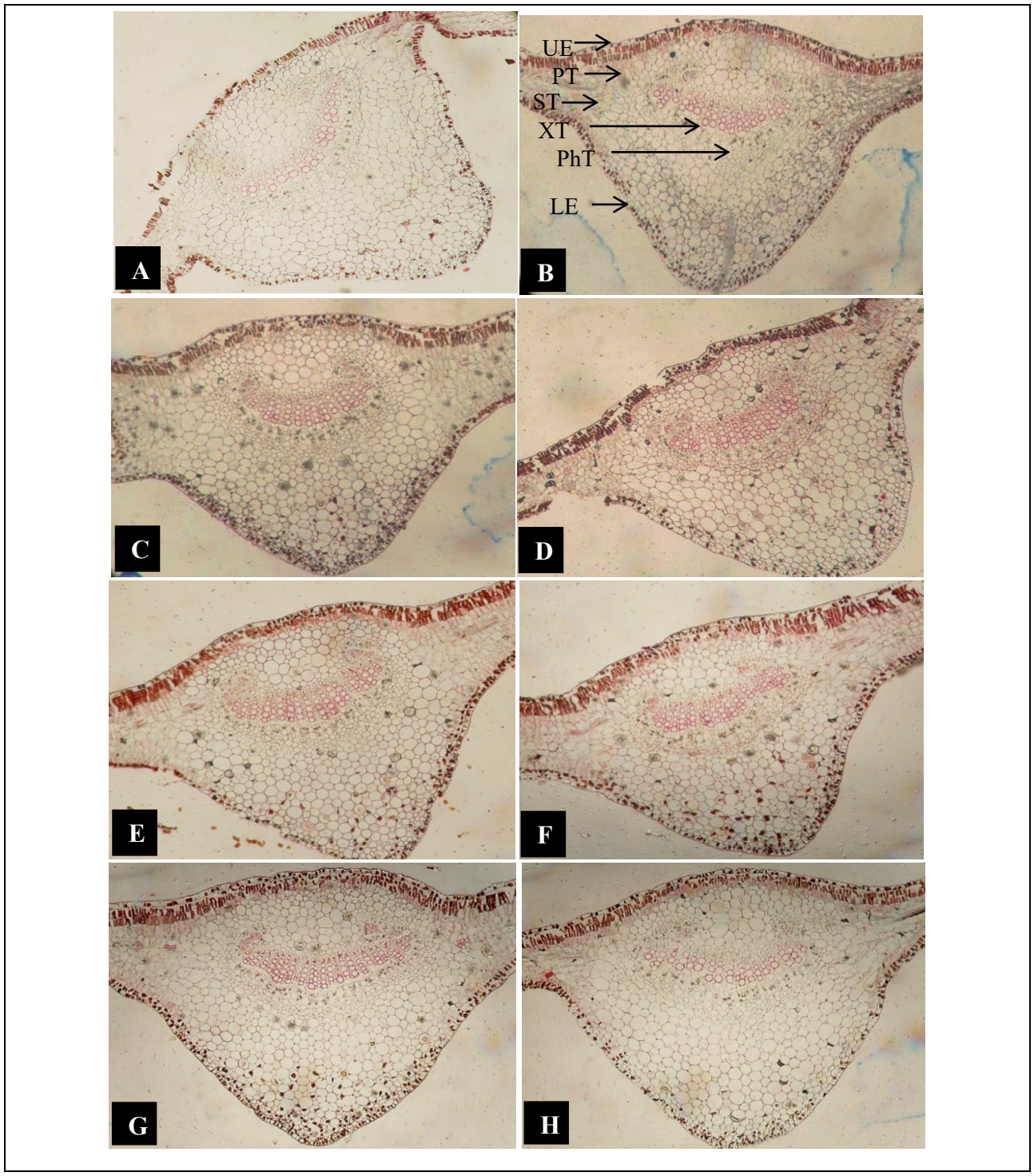

Fig. 1. Transverse sections of calendula leaves.

A. Control (salt stressed untreated plants), B. Proline 50 mg l^{-1}, C. Proline 75 mg l^{-1}, D. Proline100 mg l^{-1}, E. Yeast 4 g l^{-1}, F. Yeast 8 g l^{-1}, G. Yeast 12 g l^{-1}, H. Proline 50 mg l^{-1} + yeast 4 g l^{-1} (X 100), UE. Upper epidermis, PT. Palisade tissue, ST. Spongy tissue, XT. Xylem tissue, PhT. Phloem tissue, LE. Lower epidermis.

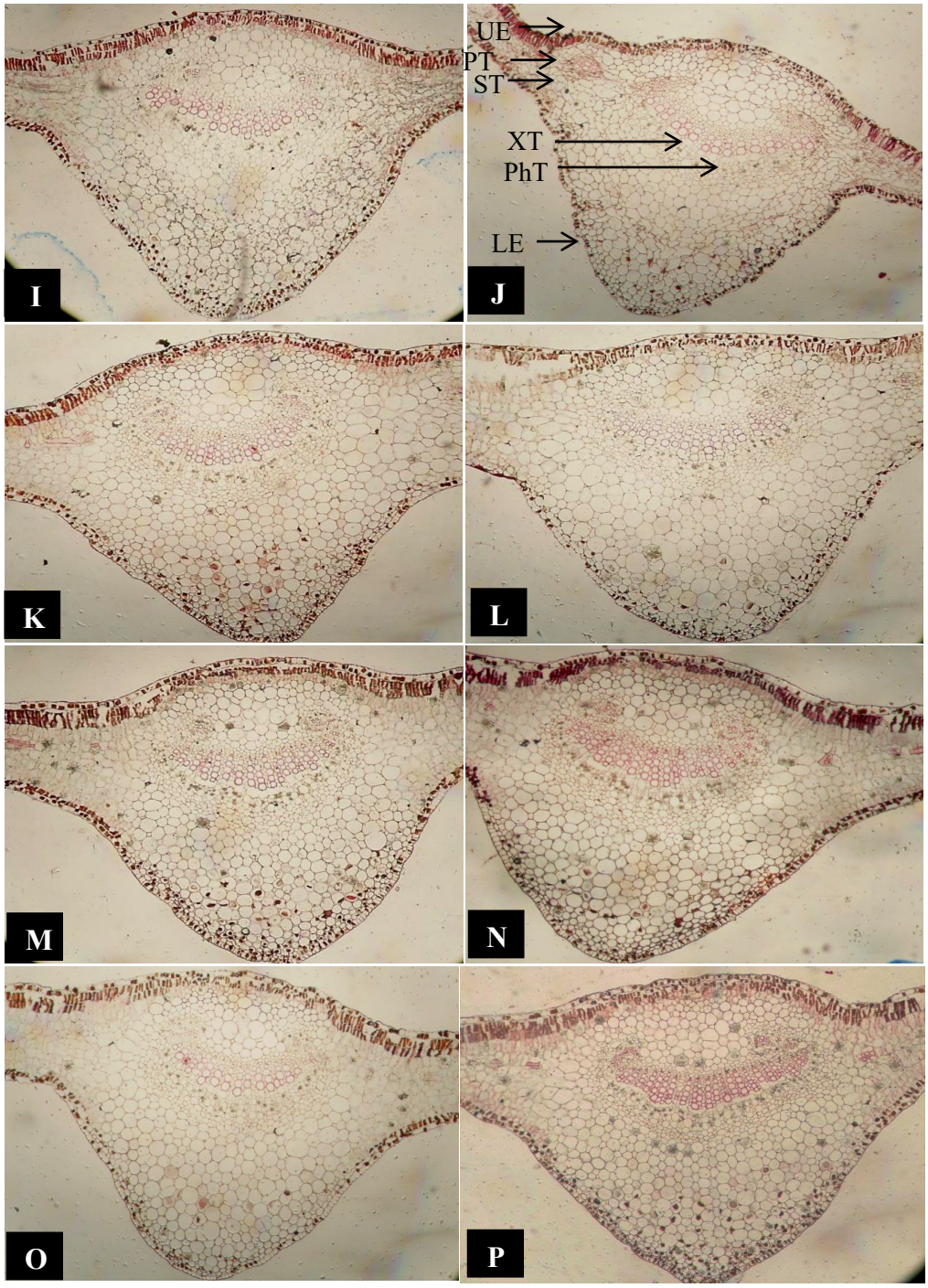

Fig. 2. Transverse sections of calendula leaves.

I. Proline 75 mg l^{-1} + yeast 4 g l^{-1}, J. Proline 100 mg l^{-1} + yeast 4 g l^{-1}, K. Proline 50 mg l^{-1} + yeast 8 g l^{-1}, L. Proline 75 mg l^{-1} + yeast 8 g l^{-1}, M. Proline 100 mg l^{-1} + yeast 8 g l^{-1}, N. Proline 50 mg l^{-1} + yeast 12 g l^{-1}, O. Proline 75 mg l^{-1} + yeast 12 g l^{-1}, P. Proline 100 mg l^{-1} + yeast 12 g l^{-1} (X 100), UE. Upper epidermis, PT. Palisade tissue, ST. Spongy tissue, XT. Xylem tissue, PhT. Phloem tissue, LE. Lower epidermis.

the plant, where it preserved water state by increasing water flow and decreasing the outflow of water under salt stress conditions (Chen and Murata, 2008). Moreover, proline safeguards cell membranes against salt-induced oxidative stress, ion toxicity and increased cellular growth (Banu *et al.*, 2009). The previous studies indicated that the exogenous application of proline significantly ameliorates the deleterious effects of salinity (Siddique *et al.*, 2015). Likewise, proline had a significant role in growth characteristics, yield and physiological characteristics (Rady *et al.*, 2016 on lupine plants). Salt stress induced a significant reduction in chlorophyll and carotenoids concentrations in calendula plants, these results are due to the inhibition of PSII activity and reduction of chlorophylls and CO_2 assimilation in leaves as a result of the toxic ions accumulation (Oyiga *et al.*, 2016). The reduction in chlorophyll concentration was recorded under various stresses (Abdelaal *et al*., 2018).

Results indicated also that total chlorophyll in the leaves and carotene concentration in flowers showed an increase with proline at 100 mg l^{-1}. These increases in chlorophyll are attributed to activating chlorophyll biosynthesis and/or preventing its degradation. Furthermore, these results attributed to the more effective scavenging of ROS with proline and other antioxidant compounds (Abdelhamid *et al.,* 2013). Also, Cirillo *et al.* (2016) reported that ornamental shrubs treated with either glycine betaine or proline led to enhance chlorophyll concentration. This is attributed to an increment in the chloroplast membranes stabilization or the preservation of photosystem using osmoprotectant molecules.

Salt stress is one of the significant abiotic factors affecting physiological and biochemical characters and one of the main limits for plant production. The exposure of calendula plants to salt stress is associated with increasing antioxidant enzymes activity, mainly catalase, peroxidase and polyphenol oxidase (Table, 5). The increase in enzymes activity may point to the efficiency of these antioxidant enzymes in scavenging and detoxification of reactive oxygen species such as superoxide and hydrogen peroxide, consequently protect calendula plant against oxidative stress. These results are similar to the obtained results of Cia *et al*. (2012) in sugarcane plants. With the same trend, the antioxidant enzymes up-regulation improved plant tolerance under salt stress conditions in sweet potato plants (Yan *et al.*, 2016). In the current study, electrolyte leakage (EL) was significantly increased in the salt-stressed plants, this increment of EL can be ascribed to the harmful effects of salt stress on the plasma membrane which resulted in an increase in membrane permeability (Tiwari *et al.*, 2010). The same trend was recorded with El-Banna and Abdelaal (2018) and Faghih *et al.* (2018). This negative effect of salt stress on calendula plasma membrane due to the high accumulation of reactive oxygen species under salt stress conditions led to oxidative damage. Furthermore, salt stress led to a significant decrease in relative water content in stressed plants because of the injurious effect of salinity on root cell wall structure and cell elongation (Byrt *et al.*, 2018). These results are similar to the obtained results with Mickky *et al.* (2019) and Roy *et al.* (2019). Application of proline and yeast extract individually or in combination led to improve the up-regulation of catalase, peroxidase and polyphenol oxidase activities in salt-stressed plants. Additionally, relative water content was increased with the application of these treatments (Table, 5). The interaction between proline and yeast extract was more effective compared with individual foliar applications, the best treatment was proline at 100 mg l^{-1} + yeast at 12 g l^{-1}. With the same trend, Ahmed *et al*. (2010) indicated that proline treatment led to improve activities of antioxidant enzymes, photosynthetic rate and consequently enhanced plant growth in olive trees under salt stress conditions due to the osmoprotective role of proline. The valuable

effects of yeast due its role as a natural source of vitamins and amino acids that encourage protein synthesis, chlorophyll formation (Abdelaal, 2015b) and improve plant growth as well as increase fungal stress tolerance (Bevilacqua *et al.*, 2008). Application of proline and yeast had a significant effect on electrolyte leakage, the obtained results indicated that EL was decreased with all treatments compared with control plants. Electrolyte leakage is an indicator to various stresses in plant tissues. The most reduction in EL was recorded with proline at 100 mg l^{-1} + yeast at 12 g l^{-1} followed by proline at 75 mg l^{-1} + yeast at 12 g l^{-1} (Table, 5). This result is attributed to the active role of proline inactivation of antioxidant enzymes, protect protein and decreased MDA (Chutipaijit *et al.*, 2009) consequently, decreased electrolyte leakage. Results showed also that yeast extract has a pivotal role on the EL due to the various nutrients and vitamins as well as amino acids in yeast extract which improves water status of plant cells and membrane stability.

The increase in ROS levels (superoxide and hydrogen peroxide) under salt conditions in salt-stressed calendula plants (Table, 5) due to the adverse effect of salt stress which, causes oxidative damage to mitochondria, chloroplast and peroxisomes under abiotic stress. This result is agreed with the result of Hasegawa *et al.* (2000). Foliar application of proline and yeast led to improve plant growth and decrease superoxide and hydrogen peroxide levels. This result of proline treatment is due to the role of proline in ROS scavenging and inducing salt tolerance in calendula plants. This result is similar to the results of Khedr *et al.* (2003) and Hoque *et al.* (2008) who reported the role of proline inactivation of the enzymatic antioxidant defense system, consequently, increases salt tolerance in various plants. Likewise, the valuable effect of yeast on ROS scavenging due to its pivotal role in plant growth improvement under stresses. These results are in line with those recorded with Abdelaal *et al.* (2017) under drought stress.

The results in the present study indicated that salinity was associated with decreasing in N, P, K, Mg and Ca %, while increasing Na % in the leaves. These results are in agreement with El-Shawa and Elzohiry, (2018) on roses. This is due to that high Na in the soil caused a deficiency of other nutrients by preventing the uptake of nutrients immediately by interfering with the carrier in the root plasma membrane, like K-selective ion canals, and prevent root growth by the osmotic effects of Na and because of the detrimental effects of Na on soil structure (Tester and Daveport, 2003). The results showed that there is decreasing in Na^+, while increasing N, P, K, Mg and Ca in plant, this is due to the exogenous application of proline which alleviates the negative effect of salinity and protects the plant cells by osmotic adjustment (Hoque *et al.*, 2007). Similar results were obtained by Abdelhamid *et al.* (2013) in *Phaseolus vulgaris* L. Results also indicated that application of proline, yeast and their interaction significantly decreased available (N, P and K mg kg^{-1}) in the soil. This is maybe due to the application of proline, yeast and their interaction, which led to an increase in plant growth, thus the plant absorb more nutrients from the soil more than untreated plants and decrease in the soil's content of elements after harvest.

According to the microscopic measurements in (Table, 8) and micrographs in Figures (1 and 2) the exposed plants to salt stress showed a decrease in anatomical characters of the leaves, as the adverse effect of salt stress due to the negative role of salinity on the growth and physiological characters of calendula plants. Similar results were recorded under various stresses in many plants (Abdelaal, 2015a and Dawood *et al.*, 2014). These results could be mainly attributed to the pivotal role of yeast and proline in improvement the morphological characters consequently enhance the anatomical structure of leaves in salt-stressed calendula plants (Figs. 1 and 2). The effective role of proline and yeast on the anatomical structure of leaves in stressed

plants was recorded by Dawood *et al.* (2014) and Nassar *et al.* (2016).

CONCLUSION

It can be concluded that spraying *Calendula officinalis* plants with proline or yeast extract were highly efficient on mitigating the harmful effect of salty soil. Also, foliar application of proline at 100 mg l^{-1} + yeast extract at 12 g l^{-1} followed by proline at 75 mg l^{-1} + yeast extract at 12 g l^{-1} were the highest treatments to increase the tolerance of calendula to salt stress. So, it can recommend that using proline at 100 mg l^{-1} + yeast extract at 12 g l^{-1} can mitigate the harmful effects of soil salt stress on *Calendula officinalis* and improve the growth and anatomical characters as well as flowers production.

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التخفيف من آثار الإجهاد الملحي على الصفات المورفو-فسيولوجية والبيوكميائية والتشريحية لنبات الأقحوان باستخدام البرولين ومستخلص الخميرة

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أجريت هذه التجربة خلال موسمي الشتاء (٢٠١٧/٢٠١٨ و ٢٠١٨/٢٠١٩) بمحطة بحوث سهل الحسينية بالشرقية بهدف تقييم تأثير الرش بالبرولين بتركيز (٠ و ٥٠ و٧٥ و١٠٠ مليجرام/لتر) والخميرة بتركيز(٠ و ٤ و٨ و ١٢ جرام/لتر) لتخفيف التأثير الضار للملوحة علي نبات الأقحوان. أوضحت النتائج التي تم الحصول عليها أن الرش الورقي بالبرولين والخميرة والتفاعل بينهما أدي إلي تحسين وزيادة صفات النمو الخضري والصفات الزهرية والخصائص الفسيولوجية والكميائية لنبات الأقحوان بالمقارنه بالكنترول مثل طول النبات وعدد الفروع والوزن الطازج والجاف للنبات وعدد الأزهار والوزن الطازج والجاف للأزهار والكلورفيل والكاروتين والمحتوي النسبي للماء ومحتوي البرولين في أوراق النبات ونشاط انزيمات البولي فينول أوكسيديزوالبيروكسيديز والكاتاليز. كما أدت نفس المعاملات إلي تحسين نمو النبات من خلال تقليل نشاط كل من السوبر أوكسيديز والهيدروجين بيروكسيديز ونقص نفاذية الأغشية. أدي الرش بالبرولين والخميرة والتفاعل بينهم إلي زيادة محتوي النبات من النيتروجين والفسفور والبوتاسيوم والكالسيوم والماغسيوم وانخفاض محتوي الصوديوم في أوراق النبات. أظهرت النتائج نقصاً معنويا في النمو الخضري والصفات الزهرية

والخصائص التشريحية تحت ظروف الإجهاد الملحي. كما انخفضت بشكل ملحوظ الصفات الفسيولوجية والكميائية مثل الصباغات النباتية ومحتوي الماء النسبي والعناصر المعدنية (النيتروجين والفسفور والبوتاسيوم والكالسيوم والماغسيوم) على العكس من ذلك أدت لزيادة محتوي الصوديوم وتركيز البرولين ونشاط انزيمات السوبر أوكسيديز والهيدروجين بيروكسيديز ونفاذية الأغشية. علاوة على ذلك تتأثرسلبيا العناصر المتاحه في التربة (النيتروجين والفسفور والبوتاسيوم) بالإجهاد الملحي. أدي الرش بالبرولين بتركيز ١٠٠ مليجرام/لتر بالإضافة للخميرة بتركيز ١٢ جم/لتر يليها معامله البرولين بتركيز ١٠٠ مليجرام/لتر بالإضافة للخميرة بتركيز٨ جم/لتر إلي الحصول علي أعلي قيم لنمو النبات والمكونات البيوكميائية ومحتوي العناصر في النبات وكذلك تحسين الصفات التشريحية وبالتالي زيادة تحمل نبات الأقحوان للإجهاد الملحي تحت ظروف هذه التجربة.