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Using hydrogel polymers to mitigate the negative impact of salinity stress on *Calendula officinalis* plants

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Abstract

Calendula officinalis is well-Known for medical, ornamental, and cosmetic uses. This plant has a variety of secondary metabolites (carotenoids, flavonoids, steroids, and terpenoids) that may be a source of antioxidants. These chemical components have various biological effects, such as antioxidant, anti-cancer, and anti-inflammatory activates. However, salinity stress limited the growth and productivity of *Calendula officinalis* as a result of physiological, biochemical, and morphological changes. Hydrogel use is an alternative for reducing the harmful impact of salt stress. So, this study aimed to estimate the effects of hydrogel applied in *Calendula officinalis* grown under salt stress. The experiment design includes three replications, a factorial randomized complete block design with two factors, using three concentrations of salinity (0, 200 and 3000 ppm) and three different levels of hydrogel polymer (0, 0.4, and 0.6% w/w) both added to the soil before planting and the interaction between them. The results observed that the most significant increase in growth and flowering parameters was treatment with hydrogel alone at 0.6% (w/w). Similarity, salt tolerance index (STI), photosynthetic pigments in fresh leaves, lycopene and β-carotene pigments in fresh petals, elements (N, P, and K) in shoot and root, and protein analysis. Whereas the highest content of total sugar, phenol, proline, antioxidant enzymes activity Catalase (CAT),Superoxide dismutase (SOD), and Peroxidase (POD), content of elements (Na and Cl), and decreased of some protein bands in the leaves and total free amino acid content was obtained from plants treated with salinity at 3000 ppm alone without hydrogel. In addition, the interaction between hydrogel at 0.6% (w/w) and 3000 ppm salinity showed a positive impact on most growth parameters and chemical analysis as well as overcoming the negative effect of salinity stress of *Calendula officinalis* L. plant.

Key word: Salinity, *Calendula officinalis*, hydrogel polymer, enzyme activity, protein bands, pigments.

1. Introduction

The major abiotic environmental stresses include salinity, drought, and heavy metal contamination [1]. Salt stress is the most significant stress that affects agricultural areas with arid and semi- arid climates and those with limited irrigation systems. Around 1 billion hectares of arable land are affected by salinity, and the issue is getting worse due to poor drainage and irrigation managing (counting irrigation with salt water), high evaporation, low rainfall, and global warming [2]. One of the most problematic issues in the ornamental green area and coastal gardens is salt stress from sea spray, which harms and damages plants [3, 4]. In this landscape, plant survival and growth are limited by salinity stress [5], according to the physiological and morphological traits of various genotypes. Most species of ornamental and medical plants are not halophytes; therefore, it is essential to evaluate their salt tolerance [6]. Moreover, it may case adverse effects of physiological dysfunctions and decreased yield [7, 8]. In addition, [9] found that phytotoxicity and nutritional imbalance in salt stress can have an impact on plant growth and quality due

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to the ornamental plants market places a premium on the selection of resilient and high quality plants, this characteristic may limit the commercialization of those plants [10].

The annual plant *Calendula officinalis* L. belongs to the Asteraceae family [11].The native region between the regions of the Mediterranean Sea, Egypt, and Europe. *Calendula officinalis* plants are used as potted flowering plants and cut flowers. In addition, it's crucial for human health and has antioxidant properties [12]. This plant has amino acids, triterpenoids, carotenoids, saponins, volatile oil, and flavonoids. It is utilized as an antiviral, antibacterial, anticancer, and anti- inflammatory because it has a variety of secondary metabolites [13, 14]. Additionally, floral carotenoids (yellow color) have long been utilized as a perfume essence and a coloring agent. In recent years, marigold has been employed as an oil seed crop due to conjugated α and γ- tocopherols and fatty acids and are also utilized in the paint and food industries [15, 16]. Under normal irrigation, ornamental cultivars are frequently produced for cut flowers, border plants, and potted plants [17].

Hydrogels are polymers capable of absorbing large amounts of water and fixing it to plant roots, which promote plant establishment and growth by increasing soil water retention [18]. It is a soil conditioner that can maximize the usage of fertilizer by maintaining and making available a higher concentration of macronutrients in the substrate, stimulating greater plant development [19]. Additionally, the hydrogel induced maintenance of moisture in the root zone of plants [20], may encourage the dilution of salt and water absorption and render the water- retention polymer a potential salt stress mitigate [21].

In previous studies, different hydrogel concentrations were used to improve growth and chemical analysis in several plants under drought stress and fertilization for example [22, 23] showed that medium added with hydrogel enhances water use and plant growth. The significant impact of gel-forming on establishment and seed germination according to [24].Numerous studies showed the positive impact of hydrogel in decreasing water irrigation quantity either with a drip or sprinkler irrigation system on the barley [25], on two wheat cultivars [26], and [27] on *Eleusine coracana* L. Additionally, there are few reports on applying hydrogel on ornamental and medical plants, as well as using hydrogel to improve growth under salinity conditions. Therefore, the application of hydrogel in the present study to understand the interaction between hydrogel and salinity stress of *Calendula officinalis*, the plant gives attention not only to enhancing the morphological, flowering parameters and stimulating the chemical composition of *Calendula officinalis* but also to overcoming the harmful and damage effects of salinity stress.

Therefore, this research aims to evaluate the use of hydrogel to mitigate the damage caused by salinity on morphology and productivity, as well as the chemical composition of *Calendula officinalis* plants.

2. Material and Methods:

2.1. Experimental condition and plant material:

Pot experiments were conducted throughout two seasons (2020/2021) in the National Research Centre (NRC) greenhouse; Giza, Egypt. Seedlings of local *Calendula officinalis* were obtained from the Department of Medicinal and Aromatic Plants Horticulture Research Institute, Agricultural Research Centre, Egypt. Seedlings have an average height of 12-15 cm with 5-7 leaves and are grown in plastic pots at 25 cm filled with 10 kg of sandy soil (two seedlings/ pot) in the first week of November in two seasons. The salt and hydrogel concentrations were added to the soil pot experiment before the seedlings were transplanted. According to Jackson [28], as shown in Table (1) physical and chemical properties of the soil experiment.

2.2. Experimental treatments:

Three salinity concentrations at (0, 2000 ppm, and 3000 ppm) were added in the soil as (NaCl: $CaCl_2$: $MgSO_4$ at 2:2:1, respectively). And three levels of hydrogel were added to the soil (at 0, 0.4%, and 0.6% (w/w)) in addition to the interaction between salinity and hydrogel treatments.

Hydrogel (Copolymer is a sodium acrylamide-acrylate) (Fig. 1) is a commercially available soil-improving product (Barbary) that is manufactured by the France company and registered with the France Ministry of Agriculture under number (9010133). It was also, registered by the Egyptian Ministry of Agricultural Research Centre. It Contains macro- and micronutrients (40% Hydrogel polymer, 6.5% N, 4.8% P, 8.2% K, and a holding capacity of 300-500%).

Table. 1. Physical and chemical analysis of pot experimental soil:

Physical properties	Clay	Silt	Sand						Texture	
	4.1	3.90	92.0						sand	
Chemical analysis	pH	EC dS/m	Soluble Cations mmol/L						Soluble Anions(mmol/L)	
			Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Co_3^{-}	HCO_3	Cl^{-}	SO_4^{--}
	7.78	1.36	0.74	3.50	0.21	-	1.15	0.10	1.20	2.30

Electrical conductivity (EC); deciSiemens per meter (dS/m).

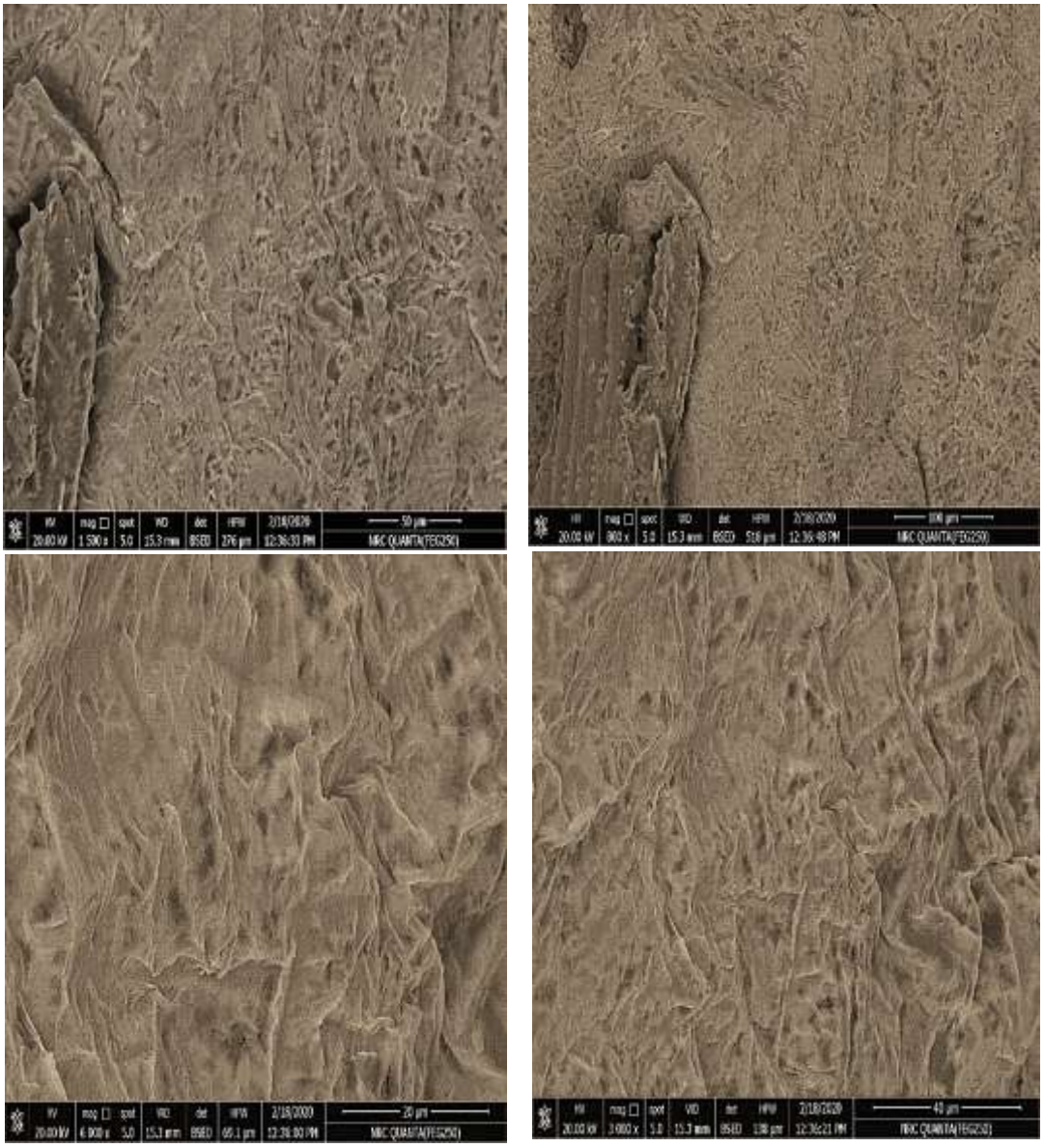

Fig.1. Field emission scanning electron microscopy (FESEM) of acrylamide hydrogel polymer

At the harvest date, the plants from each treatment were taken to determine the following traits:

2.2.1. Growth parameters:
plant height (cm), number of leaves/ plant, stem diameter (cm), number of branches/plant, total leaf area (cm^2), fresh and dry weight of shoot and root (g), dry weight shoot/root ratio.

2.2.2. Salt tolerance index (STI):
It could be used to indicate salinity tolerance in plants according to Sbei *et al*., [29] the following equation:

$$\text{STI} = (\text{TDW } at\ Sx/\text{TDW } S1)$$

TDW= total dry weight, S1= control treatment, Sx= salinity treatment.

2.2.3. Floral parameters:

number of inflorescences/plant, diameter of inflorescence (cm) by using a calliper, fresh and dry weight of inflorescences (g), Flowering time (day): The day of the first flower appearance was documented for each treatment and flowering period (day).

2.3. Chemical constituents:

2.3.1. Photosynthetic pigments:

In fresh leaf, samples were estimated for chlorophyll, a& b, total chlorophyll, and total carotenoids (mg/g F.W.) according to Saric *et al*., [30] as follows: Fresh leaves of the sample (0.5 g) were homogenized with acetone (85%), and the acetone extract was filtered using a glass funnel (G4). The residue was washed several times with acetone until the filtrate became colourless. The extract was completed to a known volume (10 ml), and the extract was taken for the colorimetric determination of pigments, using a spectrophotometer at wavelengths of 660, 640, and 440 nm (for chlorophyll a, b, and carotenoids, respectively). Acetone (85%) was used as a standard blank.

The calculated according to the following:

$$\text{Chlorophyll a} = 9.784 \times \text{E } 660 - 0.99 \times \text{E } 640$$

$$Chlorophyll\ b = 21.426 \times E\ 640 - 4.65 \times E\ 660$$

$$Total\ Carotenoids = 4.695 \times E\ 440 - 0.268 \times (chl\ a + chl\ b)$$

Where E= reading of sample

2.3.2. Lycopene and β- carotene pigments:

Fresh petals of inflorescences were determined to contain lycopene and β- carotene (mg/100 ml extract) according to the method by Nagata and Yamashita [31] as follows: All samples were extracted with acetone- and hexane in the ratio (4:6) at once, then the optical density of the supernatant by spectrophotometer at the same time as the following equation:

Lycopene (mg/100 ml) = -0.0458 A_{663}+ 0.204 A_{645}+ 0.372 A_{505}- 0.0806 A_{453}

β- carotene (mg/100 ml) = 0.216 A_{663}- 1.22 A_{645}- 0.304 A_{505}+ 0.452 A_{453}

(A663, A645, A505 and A453 are absorbance at 663 nm, 645 nm, 505 nm and 453 nm each other).

2.3.3. Determination of total sugars, total free amino acid, and total phenol in fresh leaves:

The ethanol extract was prepared by 2.5 g of fresh shoots, it was crushed in a porcelain mortar using about 25 ml of 80% ethanol, after it was filtrated through sintered glass silica it was filtered, and the residue was adjusted to 50 ml using ethanol (80%).

Total sugars were estimated using a method described by Dubois *et al*., [32] as follows: ethanol extract (1 ml) was mixed with 1 ml phenol (5%) in a test tube, followed by the addition of 5 ml concentrated sulfuric acid, and then the mixture was gently mixed and allowed to cool. The blank contains all reagents+ (1 ml) ethanol (80%). The absorbance was measured at 490 nm by using a spectrophotometer (JENWAY 6315).

Total free amino acids were determined according to the method of Moore and Stein [33] as the follows: (1 ml) of ethanol extract was mixed with (0.5 ml) of buffer solution (36 g sodium acetate +266 ml distilled water + 66 ml glacial acetic acid, the mixture was then replaced 20 ml of it with 20 ml of sodium cyanide), followed by the addition of (0.5 ml) of ninhydrin, the mixture was heated in boiling water for 15 min. After that, cool the reaction at room temperature and add (5 ml) of isopropanol: water (1:1). The absorbance was measured at 570 nm using a spectrophotometer (JENWAY 6315).

Total phenols were measured by the Swain and Hillis [34] method. As the following: (0.5 ml) of foline- Ciocalteau reagent was added (1 ml ethanol extract) + (7.5 ml distilled water) in a test tube, and after 10 minutes added the 4 ml of (2.5 N) sodium carbonate and left the tubes for 20 minutes at room temperature. The absorbance was read at 650 nm by using a spectrophotometer.

2.3.4. Determination of proline content:

Fresh leaves were determined proline by using the method of Bates *et al*., [35] as follows: Reagents: Acid-ninhydrin was prepared by warming (1.25 g) ninhydrin in (30 ml) glacial acid+ (20 ml) 6 M phosphoric acid, with agitation until dissolved (stored at 4° C). Procedure: (0.5g) of leaves were homogenized in (10 ml) of (3%) aqueous sulfosalicylic acid and filtered by filter paper. (2 ml) of the filtrate was reacted with (2 ml) acid ninhydrin + (2 ml) of glacial acid in a test tube for 1 hour (at 100° C), and the reaction terminated in an ice bath. The reaction mixture was extracted with (4 ml) toluene, 15-20 sec of vigorous mixing using a test tube stirrer, and the absorbance was read at 520 nm.

2.3.5. Extraction and determination of the activities of antioxidant enzymes:

In fresh leaves, the extraction of enzymes described by Mukherjee and Choudhuri [36] was prepared by (2 g) plant tissue frozen in liquid nitrogen and finely pestled in a chilled mortar. The frozen powder was extracted in (100 mM) phosphate buffer (pH 6.8) containing KH_2PO_4 and K_2HPO_4, and centrifuged for 20 minutes. The supernatant was made up to a known volume with the same buffer for assaying the activity of certain enzymes. Superoxide dismutase activity (SOD) EC 1.15.1.1 was determined by Marklund and Marklund [37]. Both the activities of Catalase (CAT) EC 1.11.1.6 and peroxidase (POD) EC 1.11.1.7 was determined according to the method of Kar and Mishra [38].

2.3.6. Determination of some elements (N, P, K, Cl , and Na) in shoot and root:

Elements extraction was made by using (0.5 g) of dried samples, followed by adding (10 ml) of concentrated sulphuric acid; the mixture was heated for 10 minutes, and then added (1 ml) of perchloric acid, and heating continued till a clear solution was developed. The digested solution was qualitatively transferred to a (100 ml) volumetric flask using distilled water [39]. The elements Nitrogen, Sodium and chloride were determined by Cottenie *et al*., [40]. Phosphor was determined by Snell and snell, and Potassium was according to Chapman and Pratt.

2.3.7. Determination of Protein analysis:

The protein analysis was performed according to the Laemmli [41] method. Samples preparation and extraction of soluble proteins were performed according to Von-Tersch and Gonzalez [42].

3. Statistical Analysis:

The experiment design included three replications, a factorial randomized complete block design with two factors (salinity stress and hydrogel treatments), according to Sendecor and Cochran [43], an analysis of variance was performed, and all data were analyzed using COSTAT version 6.3.1.1. for windows. A two- way ANOVA analysis followed by the least significant difference LSD test was used p = 0.05.

4. Result:

The main effects of salinity, hydrogel, and the interaction effects between them were significant in all measured growth parameters (Tables 2, 3) of *Calendula officinalis* plants. Salinity reduced the value of most growth parameters in two seasons, especially the high concentration of salinity treatments (3000 ppm), which indicated the lowest values (plant height at 25.66, 23.67 cm, number of leaves at 30.00, 34.78, stem diameter at 0.66, 0.61 cm, number of branches at 3.08, 2,67, total leaf area at 209.22, 257.83 cm^2, fresh weight of shoot at 49.22, 54.15 g, fresh weight of root at 26.33, 29.57 g, dry weight of shoot of at 11.93, 14.72 g, dry weight of root at 9.83, 10.78 and salt tolerance index (STI) at 0.82, 0.81) respectively in two seasons compared with control plants. And the same line is found in the value of the dry weight shoot/root ratio in the second season (at 1.38). Hydrogel added to the soil showed a significant positive correlation with or without treatments of *Calendula officinalis* plants, especially hydrogel at 0.6%, which indicated the highest values of all growth parameters compared with 0.4% concentration or control plants.

4.1. Flowering parameters:

The results of the data analysis in Table 4 showed that the salinity stress condition reduced the values of most flower parameters of *Calendula officinalis* plants. The 3000 ppm concentration of salinity indicated the lowest value of flower (number of inflorescences at 3.92, 4.94, inflorescence diameter at 3.88, 4.06 cm, fresh weight of inflorescences at 1.06, 1.11 g, dry weight of inflorescences at 0.22, 0.18 g, and flowering period at 79.27, 80.51 days) respectively in two seasons compared with other concentration and control. While the same concentration indicated the highest values of flowering time in two seasons (64.97, 66.20 days). Hydrogel added to the soil, especially 0.6% showed the highest values of flower parameters, except flowering time found the lowest values compared with control plants with or without salinity stress.

4.2. Chemical consistent:

4.2.1. Photosynthesis pigments content:

As shown in Table (5) the content of photosynthesis pigments (chlorophyll a, b, total chlorophyll, and total carotenoids) decreased with the salinity stress treatments. The minimum content was observed in the 3000 ppm concentration (0.51, 0.15, 0.66, and 0.19 mg/g F.W.), respectively. Hydrogel at 0.6% added to the soil markedly increased the content of pigments compared with control plants, and the interaction between salinity stress and hydrogel of *calendula officinals* plants was found to decrease the negative effect of salinity stress by hydrogel treatment.

4.2.2. Lycopene and β-carotene content in inflorescences:

The lowest level of lycopene and β-carotene in inflorescences was found with the highest concentration of salinity (3000 ppm) compared with

control plants. Hydrogel applied increased the level of these pigments in *calendula officinalis* inflorescences under normal or salinity stress conditions, especially at 0.6% treatment.

4.2.3. Total sugar, total free amino acid, total phenol and proline:

Total sugar, phenol, and proline content in *calendula officinalis* plants, showed increased content with an increased concentration of salinity (Table 6). The highest content was found at 3000 ppm (19.18, 1.62, and 2.79 mg/g F.W.), respectively. While the lowest content of total free amino acid is shown in the same concentration (3.69 mg/g F.W.). Under hydrogel treatment, showed an increase in total sugar, total free amino acids, phenol, and proline content in plants compared with untreated plants. The interaction between salinity and hydrogel showed an increased the content of thesis chemical consistent with applied hydrogel, especially at 0.6% added compared with untreated plants.

4.2.4. Antioxidant enzyme activity (CAT, POD and SOD Unit/g F.W. min.):

The current study found increased the enzyme activities of *calendula officinalis* leaves with increased concentration of salinity (Tables 6, 7). The highest activity was indicated with 3000 ppm salt concentration compared with control plants. Also, with the increased concentration of hydrogel in different combinations of potting media, there was a simultaneous increase in enzyme activates with or without stress, especially at a concentration of 0.6% compared with 0.4% or control.

4.2.5. Elements content in shoot and root:

Data in Tables (8, 9) showed the harmful effect of salinity stress on elements content in shoot and root (N, P, K %) and the K/Na ratio with increased concentrations of salinity, while increased the content of Cl and Na in both shoot and root. The addition of hydrogel at any concentration under different conditions with or without salinity stress of *Calendula officinalis* plants significantly improved the content of N, P, K% and K/Na ratio in shoot and root compared with the control treatment, while decreased Cl and Na content.

4.2.6. Protein electrophoretic pattern (SDS-PAGE):

In Table (10) and Fig.(2), SDS-PAGA analysis of total protein extracted from the leaves of *Calendula officinalis* plants of different treatments in this study, revealed that in the leaves salt stress was associated with the disappearance or decrease of some protein bands. And it's clear that the hydrogel treatment with salinity stress increased protein bands compared with plants untreated with hydrogel.

5. Discussion:

In current study, the saline soil affects on plant growth, development, and physiological functions. So, the minimal results for all characteristics were observed with different levels of salinity (Tables 2, 3, 4). These results may be due to the effects of salinity stress, which alter the metabolism, producing reactive oxygen species (ROS) in mitochondria and chloroplast, changes in ion balances, mineral nutrition, stomata behavior, photosynthetic and ultimately causing a decline in plant growth [44, 45, 46]. In addition, affecting endogenous growth hormones, changes in water states caused by osmatic stress, usually arise from a decrease in the solute potential of the soil solution, which impacts the hydraulic conductivity and is often observed as reducing water and solute uptake [47]. The excess salinity mediates ion toxicity, which results from the increasing accumulation of toxic ions like Na^+, it stimulates the efflux of cytosolic K^+ and Ca^{2+}, leading to an imbalance in their cellular homeostasis, oxidative stress, nutrient deficiency, retarded growth, and cell death [48, 44]. Furthermore, the cumulative effect of salinity stress on plants leads to a reduction in the quality of flowers (size, color, length, and stem thickness) [49] and significantly delayed flowering [50] . The reduction of flowering parameters may be inaugurated by the inability of the plant to adjust osmotically and counteract toxicities [51]. These results are in harmony with [49] on roses, [52] on *Helianthus annuus*, and [53] on *Calendula officinalis* plant.

Modifying the soil with hydrogel showed an appositive enhancement in seedling development, growth, and quality [54, 18]. The results of the research were similar to those of Kale and Arican [55], who found the highest average in fresh and dry weight of sweet corn plants when the hydrogel was applied. Ljubojević *et al*., [56] on Salvia species plants, the hydrogel effects were most notable in the fresh, dry weight, and root system volume, and the hydrogel provided a sufficient amount of available water and ions, which were usually in competition and limited by immoderate Na^+ ion amounts. Kumar *et al*., [57] and Xu, *et al*., [58] reported that applying hydrogel to the soil, especially at a very low rate, i.e.,

2.5-5 kg/ha, improved the growth and yield of different fields of ornamental and vegetable crops. Hydrogel (super absorbent polymers) can prevent nutrition materials from washing and therefore increase the growth of plants [59].

Table.2. Effect of salinity stress and hydrogel added on plant height, number of leaves, stem diameter, number of branches and total leaf area of *Calendula officinalis* plants in 2020-2021 seasons.

	(A) Salinity (ppm)							
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	1st			**Plant height(cm)**	**2nd**			
Control	21.5	21.0	18.0	**20.17**	30.0	27.0	20.0	**25.67**
Hydrogel 0.4%	38.0	29.0	25.0	**30.67**	35.0	32.0	23.0	**30.00**
Hydrogel 0.6%	43.5	39.0	34.0	**38.83**	40.0	35.0	28.0	**34.33**
Mean(A)	**34.33**	**29.66**	**25.66**		**35.00**	**31.33**	**23.67**	
LSD at 5%	A	B	AB		A	B	AB	
	0.77***	0.77***	1.34***		0.74***	0.74***	1.28*	
				Number of leaves				
Control	35.00	30.06	25.00	**30.02**	42.00	35.50	28.72	**35.41**
Hydrogel 0.4%	45.50	34.50	30.00	**36.67**	54.00	41.11	35.12	**43.42**
Hydrogel 0.6%	51.50	40.00	35.00	**42.17**	60.00	51.60	40.50	**50.70**
Mean(A)	**44.00**	**34.85**	**30.00**		**52.00**	**42.74**	**34.78**	
LSD at 5%	A	B	AB		A	B	AB	
	1.83***	1.83***	3.17*		0.88***	0.88***	1.52***	
				Stem diameter(cm)				
Control	0.60	0.55	0.50	**0.55**	0.51	0.50	0.47	**0.50**
Hydrogel 0.4%	0.80	0.70	0.70	**0.73**	0.90	0.65	0.60	**0.72**
Hydrogel 0.6%	1.00	0.80	0.77	**0.86**	1.06	0.80	0.74	**0.87**
Mean(A)	**0.80**	**0.68**	**0.66**		**0.82**	**0.65**	**0.61**	
LSD at 5%	A	B	AB		A	B	AB	
	0.019***	0.019***	0.033***		0.028***	0.028***	0.048***	
				Number of branches				
Control	3.50	3.05	0.25	**2.93**	3.05	2.50	2.00	**2.52**
Hydrogel 0.4%	5.00	4.06	3.00	**4.02**	4.11	4.15	2.50	**3.59**
Hydrogel 0.6%	7.00	6.00	4.00	**5.67**	6.25	5.00	3.50	**4.92**
Mean(A)	**5.17**	**4.37**	**3.08**		**4.47**	**3.88**	**2.67**	
LSD at 5%	A	B	AB		A	B	AB	
	0.38***	0.38***	0.66*		0.30***	0.30***	0.53***	
				Total leaf area(cm^2)				
Control	250.50	214.65	177.00	**214.05**	274.75	236.01	200.20	**236.98**
Hydrogel 0.4%	372.80	283.20	207.05	**287.68**	494.10	323.95	267.80	**361.95**
Hydrogel 0.6%	318.00	369.25	243.60	**376.95**	668.15	396.00	305.50	**456.55**
Mean(A)	**380.43**	**289.03**	**209.22**		**479.00**	**318.65**	**257.83**	
LSD at 5%	A	B	AB		A	B	AB	
	15.98***	15.98***	27.69***		36.06***	36.06***	62.46***	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table.3. Effect of salinity stress and hydrogel added on fresh and dry weight of shoot and root, dry weight shoot/root ratio and salt tolerance index of *Calendula officinalis* plants in 2020-2021 seasons.

	(A) Salinity (ppm)							
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	1st			**Fresh weight of shoot(g)**	**2nd**			
Control	57.50	50.50	46.90	**51.63**	60.92	54.22	51.25	**55.46**
Hydrogel 0.4%	63.88	52.50	49.19	**55.19**	65.67	56.23	54.16	**58.69**
Hydrogel 0.6%	71.38	53.50	51.57	**58.82**	73.38	58.14	57.04	**62.86**

Mean(A)	**64.25**	**52.17**	**49.22**		**66.66**	**56.20**	**54.15**	
LSD at 5%	A	B	AB		A	B	AB	
	2.75***	2.75***	4.76*		1.23***	1.23***	2.13***	
Fresh weight of root (g)								
Control	28.73	26.13	21.75	**25.54**	35.99	30.38	24.89	**30.42**
Hydrogel 0.4%	30.55	29.71	25.66	**28.64**	37.91	34.38	30.21	**34.17**
Hydrogel 0.6%	38.28	32.81	31.59	**34.23**	42.55	36.24	33.60	**37.46**
Mean(A)	**32.52**	**29.55**	**26.33**		**38.82**	**33.67**	**29.57**	
LSD at 5%	A	B	AB		A	B	AB	
	1.04***	1.04***	1.80*		0.83***	0.83***	1.45**	
Dry weight of shoot(g)								
Control	15.06	12.62	11.16	**12.95**	17.79	15.18	13.73	**15.57**
Hydrogel 0.4%	16.99	13.33	11.90	**14.08**	19.44	15.91	14.68	**16.68**
Hydrogel 0.6%	19.20	13.80	12.73	**15.25**	22.01	16.69	15.74	**18.15**
Mean(A)	**17.08**	**13.25**	**11.93**		**19.75**	**15.93**	**14.72**	
LSD at 5%	A	B	AB		A	B	AB	
	0.70***	0.70***	1.21*		0.35***	0.35***	0.61***	
Dry weight root (g)								
Control	11.29	9.95	8.02	**9.76**	13.71	11.27	8.98	**11.32**
Hydrogel 0.4%	12.10	11.44	9.54	**11.03**	14.63	12.93	10.99	**12.85**
Hydrogel 0.6%	15.31	12.66	11.91	**13.29**	16.59	13.73	12.37	**14.23**
Mean(A)	**12.90**	**11.35**	**9.83**		**14.98**	**12.64**	**10.78**	
LSD at 5%	A	B	AB		A	B	AB	
	0.40***	0.40***	0.70**		0.31***	0.31***	0.53*	
Dry weight shoot/root ratio								
Control	1.34	1.27	1.39	**1.33**	1.30	1.35	1.53	**1.39**
Hydrogel 0.4%	1.40	1.17	1.25	**1.27**	1.32	1.23	1.34	**1.30**
Hydrogel 0.6%	1.25	1.09	1.07	**1.13**	1.33	1.21	1.27	**1.27**
Mean(A)	**1.33**	**1.18**	**1.23**		**1.32**	**1.26**	**1.38**	
LSD at 5%	A	B	AB		A	B	AB	
	0.065***	0.065***	0.113*		0.046***	0.046***	0.080***	
Salt tolerance index (STI)								
Control	1.00	0.86	0.72	**0.86**	1.00	0.84	0.72	**0.85**
Hydrogel 0.4%	1.00	0.94	0.81	**0.91**	1.00	0.91	0.81	**0.90**
Hydrogel 0.6%	1.00	1.00	0.93	**0.98**	1.00	0.97	0.89	**0.95**
Mean(A)	**1.00**	**0.93**	**0.82**		**1.00**	**0.91**	**0.81**	
LSD at 5%	A	B	AB		A	B	AB	
	0.032***	0.032***	0.064***		0.021***	0.021***	0.042***	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table.4. Effect of salinity stress and hydrogel added on number of inflorescences, inflorescence diameter, fresh and dry weight of inflorescences, flowering time and flowering period of *Calendula officinalis* in 2020-2021 seasons.

	(A) Salinity (ppm)							
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	1st Number of inflorescences				**2nd**			
Control	5.50	3.68	2.48	**3.89**	6.25	4.70	3.50	**4.82**
Hydrogel 0.4%	7.20	5.04	4.03	**5.43**	8.22	6.06	5.05	**6.45**
Hydrogel 0.6%	9.00	6.37	5.25	**6.87**	10.03	7.39	6.27	**7.90**
Mean(A)	**7.24**	**5.03**	**3.92**		**8.17**	**6.05**	**4.94**	
LSD at 5%	A	B	AB		A	B	AB	
	0.16***	0.16***	0.27**		0.18***	0.18***	0.31***	
Inflorescence diameter (cm)								
Control	4.89	3.10	2.77	**3.59**	5.18	4.11	3.58	**4.29**

Hydrogel 0.4%	5.42	4.11	3.23	**4.26**	6.49	5.25	3.97	**5.24**
Hydrogel 0.6%	6.54	5.51	5.62	**5.89**	7.41	5.83	4.64	**5.96**
Mean(A)	**5.62**	**4.24**	**3.88**		**6.36**	**5.06**	**4.06**	
LSD at 5%	A	B	AB		A	B	AB	
	0.29***	0.29***	0.50**		0.25***	0.25***	0.43**	
Fresh weight of inflorescences (g)								
Control	1.27	1.10	0.87	**1.08**	1.48	1.17	0.95	**1.20**
Hydrogel 0.4%	1.84	1.14	1.01	**1.33**	1.93	1.44	1.13	**1.50**
Hydrogel 0.6%	2.14	1.45	1.30	**1.63**	2.50	1.64	1.25	**1.80**
Mean(A)	**1.75**	**1.23**	**1.06**		**1.97**	**1.42**	**1.11**	
LSD at 5%	A	B	AB		A	B	AB	
	0.15***	0.15***	0.27*		0.05***	0.05***	0.09***	
Dry weight of inflorescences (g)								
Control	0.26	0.22	0.20	**0.23**	0.27	0.22	0.14	**0.21**
Hydrogel 0.4%	0.36	0.31	0.21	**0.30**	0.35	0.28	0.18	**0.27**
Hydrogel 0.6%	0.48	0.37	0.25	**0.37**	0.50	0.35	0.22	**0.36**
Mean(A)	**0.37**	**0.30**	**0.22**		**0.38**	**0.29**	**0.18**	
LSD at 5%	A	B	AB		A	B	AB	
	0.03***	0.03***	0.05**		0.04***	0.04***	0.06 *	
Flowering time (day)								
Control	62.25	64.00	66.67	**64.31**	64.37	65.23	67.90	**65.84**
Hydrogel 0.4%	58.78	63.19	64.67	**62.21**	60.01	64.42	65.90	**63.44**
Hydrogel 0.6%	57.11	62.06	63.56	**60.91**	58.34	63.29	64.79	**62.14**
Mean(A)	**59.38**	**63.08**	**64.97**		**60.91**	**64.31**	**66.20**	
LSD at 5%	A	B	AB		A	B	AB	
	0.63***	0.63***	1.08**		0.49***	0.49***	0.84***	
Flowering period (day)								
Control	86.67	82.62	77.45	**82.25**	88.89	83.73	78.78	**83.80**
Hydrogel 0.4%	91.12	83.12	79.68	**84.64**	93.12	84.78	80.17	**86.02**
Hydrogel 0.6%	97.23	84.67	80.67	**87.53**	95.73	85.22	82.56	**87.84**
Mean(A)	**91.67**	**83.47**	**79.27**		**92.58**	**84.58**	**80.51**	
LSD at 5%	A	B	AB		A	B	AB	
	1.30***	1.30***	2.25***		0.89***	0.89***	1.55**	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table.5. Effect of salinity stress and hydrogel added on different pigments of *Calendula officinalis* plants, mean two seasons.

	(A) Salinity (ppm)							
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	Chlorophyll a (mg/g F.W.)				**Leaves**	**Chlorophyll b (mg/g F.W.)**		
Control	0.59	0.54	0.49	**0.54**	0.20	0.16	0.12	**0.16**
Hydrogel 0.4%	0.72	0.59	0.50	**0.60**	0.22	0.19	0.15	**0.19**
Hydrogel 0.6%	1.08	0.60	0.54	**0.74**	0.31	0.20	0.17	**0.23**
Mean(A)	**0.80**	**0.58**	**0.51**		**0.24**	**0.18**	**0.15**	
LSD at 5%	A	B	AB		A	B	AB	
	0.026***	0.026***	0.045***		0.015***	0.015***	0.025***	
	Total chlorophyll (mg/g F.W.)				**Leaves**	**Total carotenoids (mg/g F.W.)**		
Control	0.79	0.70	0.61	**0.70**	0.26	0.21	0.16	**0.30**
Hydrogel 0.4%	0.94	0.78	0.65	**0.79**	0.29	0.25	0.19	**0.24**
Hydrogel 0.6%	1.40	0.81	0.71	**0.97**	0.41	0.27	0.22	**0.21**
Mean(A)	**1.04**	**0.76**	**0.66**		**0.32**	**0.24**	**0.19**	
LSD at 5%	A	B	AB		A	B	AB	
	0.027***	0.027***	0.047***		0.019***	0.019***	0.033***	
	Lycopene (mg/100 ml extract)			**Inflorescences**	**β- carotene (mg/100 ml extract)**			
Control	0.71	0.54	0.47	**0.56**	0.34	0.30	0.24	**0.27**

Hydrogel 0.4%	0.72	0.60	0.47	**0.60**	0.39	0.37	0.24	**0.32**
Hydrogel 0.6%	0.86	0.64	0.51	**0.66**	0.51	0.41	0.24	**0.38**
Mean(A)	**0.77**	**0.57**	**0.48**		**0.43**	**0.31**	**0.23**	
LSD at 5%	A	B	AB		A	B	AB	
	0.029***	0.029***	0.050*		0.033***	0.033***	0.057*	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table.6. Effect of salinity stress and hydrogel added on some chemical composition and enzyme activity of *Calendula officinalis* plants, mean two seasons.

	(A) Salinity (ppm)							
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	Total sugar (mg/g F.W.)				**Total free amino acid (mg/g F.W.)**			
Control	9.59	12.70	15.47	**12.58**	4.95	4.06	3.07	**4.03**
Hydrogel 0.4%	11.59	14.73	17.48	**14.60**	5.60	4.71	3.71	**4.67**
Hydrogel 0.6%	13.41	16.03	24.60	**18.01**	7.88	5.13	4.29	**5.77**
Mean(A)	**11.53**	**14.49**	**19.18**		**6.14**	**4.64**	**3.69**	
LSD at 5%	A	B	AB		A	B	AB	
	1.15***	1.15***	1.99***		0.73***	0.73***	0.64***	
	Phenol (mg/g F.W.)				**Proline (mg/g F.W.)**			
Control	0.81	1.07	1.30	**1.06**	0.67	1.12	2.37	**1.39**
Hydrogel 0.4%	0.98	1.24	1.47	**1.23**	0.75	1.67	2.75	**1.72**
Hydrogel 0.6%	1.13	1.35	2.07	**1.52**	0.77	2.11	3.24	**2.04**
Mean(A)	**0.97**	**1.22**	**1.62**		**0.72**	**1.63**	**2.79**	
LSD at 5%	A	B	AB		A	B	AB	
	0.10***	0.10***	0.17***		0.15***	0.15***	0.26***	
	CAT (Unit/g F.W. min)				**POD (Unit/g F.W. min)**			
Control	15.75	18.17	21.12	**18.34**	1.84	2.48	2.90	**2.64**
Hydrogel 0.4%	17.41	18.87	22.65	**19.64**	2.11	2.79	4.64	**3.18**
Hydrogel 0.6%	17.57	20.01	24.68	**20.76**	2.36	3.11	4.46	**3.58**
Mean(A)	**16.91**	**19.02**	**22.82**		**2.29**	**2.98**	**4.13**	
LSD at 5%	A	B	AB		A	B	AB	
	0.51***	0.51***	0.89*		0.23***	0.23***	0.40**	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table.7. Effect of salinity stress and hydrogel added on SOD activity of *calendula officinalis* plants, mean two seasons.

	(A) Salinity (ppm)			
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	SOD (Unit/g F.W. min)			
Control	5.42	6.84	9.79	**7.35**
Hydrogel 0.4%	6.08	7.54	11.32	**8.31**
Hydrogel 0.6%	6.24	8.68	13.35	**9.43**
Mean(A)	**5.91**	**7.69**	**11.49**	
LSD at 5%	A	B	AB	
	0.52***	0.52***	0.90**	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table.8. Effect of salinity stress and hydrogel added on some elements content in shoot of *calendula officinalis* plants , mean two seasons.

	(A) Salinity (ppm)							
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	N%				**Shoot**		**P%**	
Control	2.17	1.81	1.37	**1.78**	0.31	0.24	0.18	**0.24**
Hydrogel 0.4%	2.35	2.04	1.63	**2.00**	0.47	0.33	0.22	**0.34**
Hydrogel 0.6%	3.22	2.22	1.81	**2.42**	0.51	0.38	0.25	**0.38**
Mean(A)	**2.58**	**2.02**	**1.60**		**0.43**	**0.31**	**0.22**	
LSD at 5%	A	B	AB		A	B	AB	

	0.17***	0.17***	0.29**		0.031***	0.031***	0.053*	
	K%				**Na%**			
Control	3.40	3.04	2.57	**3.00**	0.60	0.84	1.05	**0.83**
Hydrogel 0.4%	3.58	3.27	2.86	**3.23**	0.56	0.77	0.90	**0.74**
Hydrogel 0.6%	4.48	3.45	3.04	**3.66**	0.51	0.64	0.83	**0.66**
Mean(A)	**3.82**	**3.25**	**2.82**		**0.56**	**0.75**	**0.93**	
LSD at 5%	A	B	AB		A	B	AB	
	0.174***	0.174***	0.301**		0.035***	0.035***	0.060*	
	Cl%				**K/Na ratio**			
Control	3.89	5.08	6.48	**5.14**	5.67	3.63	2.45	**3.92**
Hydrogel 0.4%	2.98	3.33	4.92	**3.74**	6.45	4.22	3.18	**4.62**
Hydrogel 0.6%	2.80	3.12	3.29	**3.07**	8.75	5.43	3.65	**5.94**
Mean(A)	**3.22**	**3.84**	**4.89**		**6.95**	**4.43**	**3.09**	
LSD at 5%	A	B	AB		A	B	AB	
	0.20***	0.20***	0.35***		0.32***	0.32***	0.56***	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table.9. Effect of salinity stress and hydrogel added on some elements content in root of *calendula officinalis* plants, mean two seasons.

(A) Salinity (ppm)								
(B) Treatment	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**	**0 ppm**	**2000 ppm**	**3000 ppm**	**Mean(B)**
	N%				**Root P%**			
Control	0.89	0.74	0.55	**0.73**	0.15	0.14	0.12	**0.13**
Hydrogel 0.4%	0.96	0.83	0.67	**0.82**	0.16	0.15	0.13	**0.14**
Hydrogel 0.6%	1.33	0.91	0.74	**0.99**	0.20	0.15	0.14	**0.16**
Mean(A)	**1.06**	**0.83**	**0.65**		**0.17**	**0.14**	**0.13**	
LSD at 5%	A	B	AB		A	B	AB	
	0.071***	0.071***	0.123**		0.008***	0.008***	0.013**	
	K%				**Na%**			
Control	1.27	1.17	0.85	**1.10**	0.72	0.82	0.91	**0.82**
Hydrogel 0.4%	1.36	1.19	1.00	**1.18**	0.70	0.79	0.85	**0.78**
Hydrogel 0.6%	1.73	1.21	1.11	**1.35**	0.68	0.74	0.82	**0.75**
Mean(A)	**1.45**	**1.19**	**0.99**		**0.70**	**0.78**	**0.86**	
LSD at 5%	A	B	AB		A	B	AB	
	0.100***	0.100***	0.174*		0.015***	0.015***	0.025*	
	Cl%				**K/Na ratio**			
Control	1.70	1.94	2.15	**1.93**	1.77	1.43	0.94	**1.38**
Hydrogel 0.4%	1.66	1.87	2.00	**1.84**	1.94	1.50	1.18	**1.54**
Hydrogel 0.6%	1.61	1.74	1.93	**1.76**	2.53	1.65	1.35	**1.84**
Mean(A)	**1.66**	**1.85**	**2.03**		**2.08**	**1.52**	**1.16**	
LSD at 5%	A	B	AB		A	B	AB	
	0.035***	0.035***	0.060*		0.15***	0.15***	0.25*	

A=Salinity treatments, B= Hydrogel, A*B= interaction, ****High significantly, **= Moderate significant, ns=non- significant.

Table. 10. Effect of salinity stress and hydrogel added on protein banding pattern of *calendula officinalis* leaves

MW	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
59	+	+	+	+	+	+	+	+	+
32	+	+	+	+	+	+	+	+	+
26	-	+	+	-	+	+	-	-	-
19	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+
12	-	-	-	-	+	+	+	-	-
9	+	+	+	+	+	+	+	+	+
7	-	-	-	-	+	+	-	-	+
6	-	-	-	-	+	+	-	-	-
Total number of protein bands	5	6	6	5	9	9	6	5	6

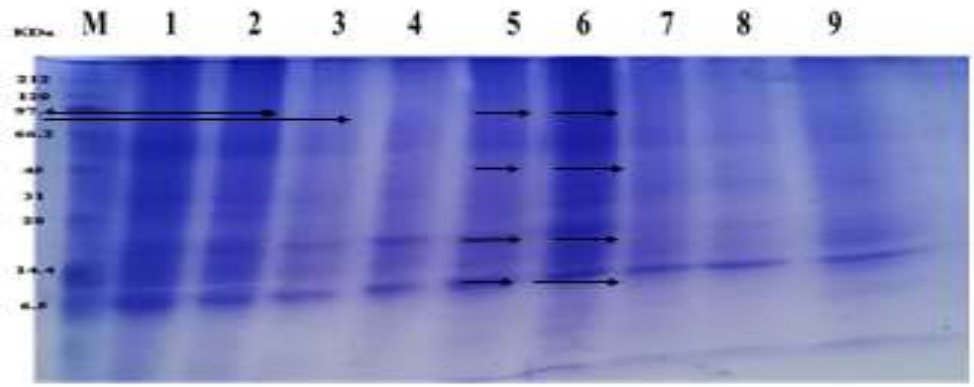

Fig. 2. Protein banding pattern in 9 treatments, 1= control plants, 2= hydrogel at 0.4%, 3= hydrogel at 0.6%, 4= salinity at 200ppm, 5= 200 ppm+ hydrogel at 0.4% , 6= 200 ppm+ hydrogel at 0.6%, 7= salinity at 300 ppm, 8= 300 ppm+ hydrogel at 0.4%, 8= 300 ppm+ hydrogel at 0.6%, M= marker lane

The effect of hydrogel on the growth of plants may be attributed to the provision of sufficient water in the lower layer of the soil added to it, which provides favorable conditions for microorganisms in the roots for an extended period, which has a positive effect on plant activity [60]. On *Dendranthema grandiflora* L. plants, 0.5% hydrogel of pot media produced the best results for flower diameter, days taken to first flower bud appearance, flower weight, duration of flowering, and total yield of flowers [61]. Hydrogel encourages early, dense flowering with a longer flowering period, improves flower quality and production, and delays the onset of the permanent wilting point [62].

The activity of photosynthetic is considered one of the main factors which control the growth of plants [63]. Photosynthesis is directly slowed in plants by salinity stress, and photosynthesis is directly related to stomata conductance, chlorophyll content, transpiration, and water potential [64]. The depressive effects of salinity on chlorophyll leaf content might be recognized by the diminished biosynthesis of chlorophyll, nutrient deficiency, and enhanced chlorophylls activity [65]. The photosynthesis rates were remarkably decreased by high salinity concentrations in maize plants [46]. It's the same results that were found in this study.

Moreover, Vivek *et al*., [66] showed increased chlorophyll a, b, and total chlorophyll content of groundnut plants with 4.0 kg/ha hydrogel. The hydrogel application increases soil moisture and enhances the synthesis of photosynthetic pigments. Increased carotenoids and chlorophyll content with hydrogel polymer's performance may be attributed to decrease chlorophyll degradation or increased biosynthesis of chlorophyll due to adequate water and nutrients supply to the plant [67]. In addition, various stress conditions can mitigate the damage to plants' photosynthetic apparatus by applying a water-retaining polymer (hydrogel) [68].

In plants, the main carotenoids are lutein, lycopene, and β-carotene [69]. β-carotene is a precursor of zeaxanthin, which is a precursor of abscisic acid, these phytohormones may play important roles in the abiotic stress response [70]. β-carotene is created from lycopene by lycopene β-cyclase [71]. Martinez *et al*., [72] found that the salt stress increased the lycopene but decreased the β-carotene concentration in the fruit of tomato plants. Sankari *et al.*, [73] and Leiva- Ampuero *et al*., [74] indicated that the level of β-carotene in *Bixa orellana* L. plant increased with the highest concentration of salt stress treatment, and Leiva- Ampuero *et al.*, [74] on tomato plants showed increased content of lycopene and β-carotene with salt treatment at 120 and 160 mM NaCl. Maurya *et al*., [75] suggested that when plants were exposed to salt, a decrease in β-carotene levels was observed. This may be due to carotenoid degradation under salt stress conditions or decreased gene expression of carotenoid pathway genes. The obtained results in this study are in line with those of Alshallash et al., [76] and Abdelaziz et al., [77], who demonstrated that adding hydrogel to the soil improved the highest average for the accumulation of plant pigments.

To protect the plant cells from the adverse effects of salt stress, the plant produces osmolytes such as soluble sugar and proline, which maintain the

osmotic strength of cytosol with that of vacuole and external environment [78, 79, 80]. The increased enzymatic activity that aids in regulating cellular structures and functions by interacting with macromolecules may be because of the rise in soluble sugars [81, 82, 67].On the other hand, Colak *et al*., [83] on wheat plant and Hussein and Alshammari, [84] on *Linum usitatissimum* plant indicated that salinity stress decreased the level of total soluble sugars compared to control plants. The positive effect of applied hydrogel in the soil on total sugar content may be due to enhanced photosynthetic pigments [77]. This is in harmony with Liu *et al*., [85], who indicated that superabsorbent polymers promoted total dry weight, soluble sugar, and chlorophyll in the leaves of the coffee tree. In addition, in mango trees, increased total sugar values were observed with treated 750g hydrogel tree^{-1} compared to the control plant [76].

Amino acids are a fundamental to all living cells for plant structure and metabolism [86]. The lower level of amino acids in plants under salt stress is due to the down regulation of protein synthesis [84]. In contrast, the increase in the concentration of various amino acids in plants is associated with the accumulation of compatible solutes for increasing salt tolerance [87]. Several studies showed the positive effect of hydrogel on the content of total free amino acids in different plants, such as Nascimento *et al*., [88] the highest amino acid values were obtained with hydrogel applied in soybean cultivation. Vivek *et al*., [89] found that graded levels of hydrogel application raised the content of protein, carbohydrate, and total amino acids in *Arachis hypogaea* plants.

Under salinity stress conditions, the phenolic compounds play a significant role in absorbing and neutralizing free radicals, and decomposing peroxides. Bistgani *et al*., [90] found the total phenolic content of *Thymus vulgris* and *Thymus daenensis* plants, increased by 20% after applying 60 mM NaCl compared with the control plant. Zhou *et al.*, [91] showed increased phenolic content in Schizonepeta tenuifolia plants under mild salinity level (25 mM) but depressed content under harsh treatments (75 and 100 mM). And the same results were found in *Salvia lavandulifolia* plants [92]. In several studies that found different effects of hydrogel treatment on the phenolic compounds content in plants, Besharati *et al*., [93] showed increased total phenolic content and antioxidant activity by hydrogel treatment in *Hibiscus sabdariffa* plants , and the same result agree with Barkia *et al*., [94] in olive plants. In the other study, on *Hibiscus sabdariffa* plants, the hydrogel treatment decreased the amount of anthocyanin and sepal phenolic compounds, which may be due to the modification of stress conditions by the hydrogel [95].

In plants, proline protects enzymes and stabilizes their structure [96, 97]. Additionally, proline content positively correlates with salt stress level [98]. This is consistent with Khalil et al., [99], who recommended that proline accumulation arose due to a disturbance in cell homoeostasis in response to salt stress. These results agree with [100] who found an increase in proline values with a rise in the NaCl concentration of wheat plants [100]. Increased proline is one of the critical advantages of hydrogel treatment [101] and is in harmony with the results obtained in this study. From this point, Yang et al., [102] suggested that there is no correlation with relative water content (RWC) or osmotic potential because the osmotic properties of proline usually explain higher water potential in hydrogel treated plants. And, in Tobacco plants, the levels of osmotic regulators such as proline improved with hydrogel [103].

Protein, lipids, and nucleic acids are harmed by ROS produced as a result of salt stress [104]. So the plant employs an antioxidant defense system to scavenge and detoxify these compounds from the cell surface, which leads to increased plant antioxidant activity [105, 106, 107, 108]. These results are in harmony with Hanifah and Purwestri [109] on *Centella asiatica* and Kumar *et al*., [78] on *Oenanthe javanica*. Superoxide dismutase (SOD) enzyme activity increased up to 75 mM NaCl in *Lepidium draba* plants and then decreased [110, 111]. In asparagus plants, salinity stress enhanced the activity of enzymes [112]. Waqar *et al*., [113] on soybean plants, hydrogel application, showed increased plant antioxidant enzymes SOD and CAT. And this result is consistent with the findings of Besharati *et al*., [93] on *Hibiscus sabdariffa* plants. While these results contrasted with those of Ghobashy *et al*., [114] who found the application of hydrogel decreased the phenol content, catalase, and peroxidase in sunflower plants under drought stress.

Furthermore, asparagus cultivars showed increased Na^+ content in roots, stems, and leaves, while K^+ content and the K^+/ Na^+ ratio decreased with salt stress [112]. Abiotic stresses such as salinity adversely affect nitrogen (N) uptake and assimilation in plants [115]. Salinity in the soil increased the

accumulation of Na^+ and Cl^- in plants while decreasing essential elements such as K^+, Ca, and Mg^{++} [116]. Na^+ competes with K^+ at entry sites and ultimately decreases the absorption of K+, which causes an ionic imbalance. This competition can have negative effects on plants growth and development when the level of Na^+ exceeds that of K^+. Hydrogel provides water and nutrients to the plant when the surrounding soil the root zone of the plants starts to dry up [57]. The use of hydrogel in soil improves phosphatase activity, which makes more P available to the plant and thus increases its uptake. Hydrogel may help plants survive drought or salt stress by increasing the uptake of elements. Assaha *et al*., [117] indicated that K^+ plays an important role in osmotic adjustments, the maintenance of turgor, and thus the mitigation of the harmful effect of saline stress .Additionally, the added hydrogel to the soil enhanced nitrogen, phosphorus, and potassium content [118].

In general, plant proteins play various enzymatic, structural, and functional roles (biosynthesis, photosynthesis, transport, etc.) [119]. Protein act as osmotin, and their accumulation plays a potential role in developing tolerance against salt stress [120]. Different cultivars of *Oenanthe javanica* plants showed increased protein content in both leaves and roots under salt stress conditions [78]. The different types of soluble protein accumulation have evolved as a vital strategy that plays a central regulatory role in the growth and development of plants subjected to salt stress [121]. Furthermore, proteins are a more reliable determinant of salt tolerance than simple gene expression, as they play major roles in shaping physiological traits in salt tolerant phenotypes [121]. Fidelis *et al*., [122] found that the applied 20 kg/ha of hydrogel promoted the content of protein in cowpea bean plants; it is possible to relate this effect to the higher soil water retention promoted by treated hydrogel.

6. Conclusion:

The current study observed that the significant findings on the physiological – biochemical traits were obtained from 0.6% (w/w) alone or in combination with salinity levels (2000 and 3000 ppm) of *Calendula officinalis* plants, considerable increased all growth, biochemical traits, and enzyme activity which are essential traits for salt stress tolerance. In comparison, severe salt stress decreased values in all growth and biochemical traits. Hydrogel application in soil significantly mitigated the negative impacts of salinity stress in *Calendula officinalis*. In addition, hydrogel at 0.6% (w/w) could be an excellent source for improving the morphological and biochemical traits of *Calendula officinalis* due to their properties and mitigating salt stress in floriculture plants to help the floricultural business. Future work will apply hydrogel to other ornamental and medical plants under salt conditions.

7. Conflict of interest:

no conflict

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9. References:

[1]- He M He. C.Q. and Ding N.Z., Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. Front Plant Sci, 9, 1771(2018). https://doi.org/10.3389/fpls.2018.01771

[2]- Ali M., Afzal A., Parveen A., Kamran M., Javed M.R., Abbasi G.H., Malik Z., Riaz M., Ahmad S., Chattha M.S., Ali M., Ali Q., Uddin M.Z., Rizwan M.and Ali S., Silicon mediated improvement in the growth and ion homeostasis by decreasing Na+ uptake in maize (Zea mays L.) cultivars exposed to salinity stress. Plant Physiol. Biochem., 158,208–218(2021). https://doi.org/10.1016/j.plaphy.2020.10.04

[3]- Farieri E., Toscano S., Ferrante A. and Romano D., Identification of ornamental shrubs tolerant to saline aerosol for coastal urban and peri-urban greening. Urban For. Urban Green, 18, 9–18(2016).

[4]- Ferrante A., Trivellini A., Malorgio F., Carmassi G., Vernieri P. and Serra G., Effect of seawater on leaves of six plant species potentially useful for ornamental purposes in coastal areas. Sci. Hortic., 128, 332–341(2011).

[5]- Sánchez-Blanco M.J., Rodríguez P., Morales M.A. and Torrecillas A., Contrasting physiological responses of dwarf sea-lavender and marguerite to simulated sea aerosol deposition. J. Environ. Qual., 32, 2238–2244 (2003).

[6]- Wu S., Sun Y., Niu G., Pantoja G.L.G. and Rocha A.C., Responses of six Lamiaceae landscape species to saline water irrigation. J. Environ. Hortic., 34, 30–35(2016).

[7]- Greaver T.L. and Sternberg L.L.D.S. Linking marine resources to eco-tonal shifts of water uptake by terrestrial dune vegetation. Ecology , 87, 2389–2396 (2006).

[8]- Franco J.A., Martínez-Sánchez J.J., Fernández J.A. and Bañón, S., Selection and nursery production of ornamental plants for landscaping and xerogardening in semi-arid environments. J. Hortic. Sci. Biotechnol., 81, 3–17(2006).

[9]- Braz R. dos S., Lacerda C. F. de; Assis Júnior R. N. de, Ferreira J. F. da S., Oliveira A. C. de and Ribeiro A. A., Growth and physiology of underwater salinity and nitrogen fertilization in two soils. Revista Brasileira de Engenharia Agrícola e Ambiental, 23, 907-913(2019). http://dx.doi.org/10.1590/1807-1929/agriambi. v23n12p907-913

[10]- Bezerra F. M. S., Lacerda C, F. de, Ruppenthal V., Cavalcante E. S. and Oliveira, A. C. de., Salt tolerance during the seedling production stage of Catharanthus roseus, Tagetes patula and Celosia argentea. Revista Ciência Agronômica, 51, 1-9 (2020). https://doi. org/10.5935/1806-6690.20200059

[11]-Massoud H.Y., Abd El-Kafie O.M., Helaly A.A. and Ghanem M.E., Genetic variability studies in Calendula officinalis plant. Journal of Plant Production, Mansoura Univ., 11 (5), 425 – 428(2020).

[12]-Jan N., Andrabi K.I. and John R., Calendula officinalis - an important medicinal plant with potential biological properties. Proceedings of the Indian National Science Academy, 83, 4, 769-787(2017). DOI: 10.16943/ptinsa/2017/49126

[13] –Raal A., Kirsipuu K., Must R. and Tenno S., Content of total carotenoids in Calendula officinalis L. From different countries cultivated in Estonia. Nat. Prod. Commun., 4, 35–38(2009).

[14].- Matic I.Z., Juranic Z., Savikin, K., Zdunic G., Nadvinski N. and Godevac D., Chamomile and marigold tea: Chemical characterization and evaluation of anticancer activity. Phytother. Res., 27, 852–858(2013).

[15]- Joly R., Forcella F., Peterson D. and Eklund J., Planting depth for oilseed calendula. Ind. Crop. Prod., 42, 133–136 (2013).

[16]. Biermann U., Butte W., Holtgrefe R., Feder W. and Metzger J.O., Esters of calendula oil and tung oil as reactive diluents for alkyd resins. Eur. J. Lipid Sci. Technol., 112, 103–109(2010).

[17]. Rezaei Nejad A. and Khosravi Shakib A. Ornamental value of Calendula officinalis "Yellow Gitana" as a result of nitrogen fertilizer and plant density. Int. J. Agric. Crop Sci., 5, 362–365(2013).

[18]-Peyrusson F., Hydrogel improve plant growth in Mars Analog conditions. Frontiers in Astronomy and Space Sciences, 8, 729278(2021). doi: 10.3389/fspas.2021.729278.

[19]- Navroski M. C., Araújo M. M., Reininger L. R. S., Muniz M. F. B., Pereira M. de O. Influência do hidrogel no crescimento e no teor de nutrientes das mudas de Eucalytus dunnii. Revista Floresta, 45,315-328(2015). https://doi.org/10.5380/rf.v45i2.34411

[20]- Neves O. S. C., Avrella E. D., Paim L. P.and Fior C. S. Retenção de água em substratos com hidrogel: influência das características do material e nível de adubação. Ciência Florestal, 31,1751- 1767(2021). https://doi.org/10.5902/1980509843240

[21]- Lessa C. I. N., Oliveira A. C. N. de, Magalhães C. L., Sousa J. T. M. de, Sousa G. G. de. Estresse salino, cobertura morta e turno de rega na cultura do sorgo. Revista Brasileira de Agricultura Irrigada, 13, 3637-36459(2019). https://doi.org/10.7127/rbai. v13n5001122

[22]-Viero P. W. M., Little K. M. and Oscroft D. G., The effect of a soil-amended hydrogel on the establishment of a Eucalyptus grandis x E. camaldulensis clone grown on the sandy soils of Zululand. Southern African Forestry Journal, 188(1), 21-28(2000).

[23]- Mohamed N. N. E., Khalil N., Bakry A., Taha M., and Abdel-Lattif H. M. Maximizing Sesame Productivity and Water Use Efficiency By Using Hydrogel Under Water Stress Conditions In Sandy Soils. Plant Cell Biotechnology And Molecular Biology, 272-284(2021).

[24]-Woodhouse J. M. and Johnson M. S., The effect of gel-forming polymers on seed germination and establishment. Journal of arid environments, 20(3), 375-380(1991).

[25]- El-Karamany M.F., Bakry A.B., Hamed A.A., Omer T.A. Elewa and Waly1 A.I. (Correlation Between Barley Yield and Yield Attributes as

Affected by Hydrogel for Reducing Irrigation Amounts under Drip Irrigation System. International Journal of Water Resources and Environmental Sciences, 11(1),08-14 (2022).

[26]- El-Karamany M. F., Waly A. I., Shaban A. M., and Bakry A. B. , Utilization of hydrogel for reducing water irrigation quantities on two wheat cultivars grown under sandy soil conditions. International Journal of Water Resources and Arid Environments, 8(1), 15-22 (2019).

[27]- Painkra B., Thakur A. K., Kumar M., Chandraker T. and Singh D. P., Effect of mulching and hydrogel in relation to different growth characters, yield and economics of finger millet [Eleusine coracana (L.) Gaertn] under rainfed conditions, The Pharma Innovation Journal, 11(8), 2008-2013 (2022).

[28]-Jackson M.L., Soil chemical analysis. 1st Edn., Prentice Hall Ltd., New Delhi, India, 498(1973).

[29]-Sbei H., Shehzad T., Harrabi M. and Okuno K., Salinity tolerance evaluation of Asian barley accessions (Hordeum vulgare L) at the early vegetative stage. J. Arid Land Stud., 24, 183–186(2014).

[30]-Saric M.R., Kastrori-Cupina T .and Gergis I., Chlorophyll Determination. Univ. Unoven Sadu-Prakitikum is KiziologizeBilika-Beagrad, HaucuaAnjiga., p. 215 (1967).

[31]-Nagata M. and Yamashita I., Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. J. Japan. Soc. Food Sci. Technol. (Nippon Shokuhin Kogyo), 39(10), 925-928(1992).

[32]-Dubois M., Gilles K.A., Hamilton, J.K., Rebers, P.A.and Smith, F., Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28(3), 350-356 (1956).

[33]-Moore S. and Stein W.H., Amodified ninhydrin reagent for photomertric determination of amino acids and related compounds. Journal of Biological Chemistry, 211, 907-913 (1954).

[34]-Swain T. and Hillis ,W.E., The phenolics constituents of Prunus domestica. The quantitative analysis of phenolic constituents. Journal of Science of Food and Agriculture, 10 (2),63-68 (1959).

[35]-Bates L.S., Waldren R.P.and Teare I.D., Rapid determination of free proline for water- stress studies. Plant and Soil , 39, 205-207(1973).

[36]-Mukherjee S.P. and Choudhuri M.A., Implications of water stress- induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in vigna seedlings. Physiologia Plantarum , 58(2), 166-170(1983).

[37]-Marklund S. and Marklund, G., Involvement of the superoxide anion radical in the autoxidation of pyragallol and aconvenient assay for superoxide dismutase. European Journal Biochemistry, 47,467-474 (1974).

[38]-Kar M. and Mishra D., Catalase, peroxidas and polyphenol oxidase activities during rice leaf senescence. Plant Physiology, 57(2), 315-319(1976).

[39]- Piper G.S., Soil and plant analysis. Interscience Publishers In., New York,368 (1947).

[40]-Cottenie A.M., Verloo M., Kiekens L., Velghe G. and Camerlynck, R., Chemical analysis of plant and soil. Laboratory of Analytical and Agrochemistry. State Univ. Ghent, Belgium, 100-129(1982).

[41]-Laemmli U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680-685. https://doi.org/10.1038/227680a0

[42]-Von-Tersch M.A. and Gonzalez T.M., Bacillus thuringiensis cry ET1 toxin and protein toxic to lepidopteran insects, United States patent no.5356623(1994).

[43]-Sendecor G. W. and Cochran W. G., Statistical Methods 7th Ed. Iowa State Univ. Press, Ames. Iowa, USA, PP 305-310 (1981).

[44]-Elkelish A.A., Soliman M.H., Alhaithoul H.A. and EL-Esawi, M.A. (2019) Selenium protects wheat seedlings against salt stress- mediated oxidative damage by up regulating antioxidants and osmolytes metabolism. Plant Physiology and Biochemistry, 137,144-153(2019).

[45]-Ahanger M.A., Tomar N.S., Tittal M., A rgal, S. and Agarwal, R.M., Plant growth under water/ salt stress. ROS production; antioxidant and significance of added potassium under such condition. Physiol Mol Biol Plants, 23(4), 731-744(2017).

[46]-Zahra N., Raza Z.A. and Mahmood S., Effect of salinity stress on various growth and physiological attributes of two contrasting maize genotypes. Brazilian Archives of Biology and Technology, 63, e202000072 (2020).

[47]-Uçarlı C. , Effects of salinity and seed germination and early seedling stage. Abiotic Stress in Plants. Edited by Shah Fahad, Shah Saud, Yajun Chen, Chao Wu , Depeng, Wang(2020). DOI: 10.5772/intechopen.93647

[48]-Ahanger M.A. and Agarwal R.M., Salinity stress induced alterations in antioxidant metabolism and nitrogen assimilation in wheat (Triticum aestivum L) as influenced by potassium supplementation. Plant Physiology and Biochemistry, 115, 449-460 (2017).

[49]-Bilal H.M., Islam H., Adnan M., Tahir R., Zulfiqar R., Umer M.S. and Kaleem M.M. , Effect of salinity on growth, yield and quality of roses: A review. International Journal of Environmental Sciences &Natural Resources, 25(1), 0046-050(2020).

[50]- El- Nashar Y.I., Salicylic Acid's Role in Reducing the Impact of Salinity on Plant Calendula (Calendula officinalis L.). Egyptian Journal of Horticulture, 49(1),73-86(2022).

[51]- Abdel-Mola M.A.M. and Ayyat A.M., Interactive effects of water salinity stress and chitosan foliar-spray application on vegetative and flowering growth aspects and chemical constituents of pot marigold (Calendula officinalis L.) plant. Scientific Journal of Agricultural Sciences, 2 (2), 80-89 (2020).

[52]- Salem E.M.M., Effect of ascorbic acid and zinc on the productivity of Sunflower (Helianthus annuus L.) under saline stress conditions. Egyptian Journal of Agronomy, 43(1),45-54(2021).

[53]- Elhindi K.M., Al-Mana F.A., Algahtani A.M. and Alotaibi M.A., Effect of irrigation with saline magnetized water and different soil amendments on growth and flower production of Calendula officinalis L. plant. Saudi Journal of Biological Sciences, 27, 3072-3078(2020).

[54]-El-Asmar J., Jaafar H., Bashour I., Farran, M.T. and Saoud, I. P., Hydrogel banding improves plant growth, survival, and water use efficiency in two calcareous soils. CLEAN–Soil, Air, Water, 45, 1700251(2017).

[55]-Kale S. and Arican, B., Salinity effects on sweet corn yield and water use efficiency under different hydrogel doses. Scientific papers-Series A. Agronomy, 61(1): 263-266(2018).

[56]-Ljubojević M. , Ognjanov V. , Maksimović I. , Čukanović J. , Dulić J. , Szabò Z. and Szabò, E., Effects of hydrogel on growth and visual damage of ornamental Salvia Species exposed to salinity. Clean- Soil Air Water, 45(2), 1600128 (2017). https://doi.org/10.1002/clen.201600128.

[57]-Kumar R., Yadav S., Singh V., Kumar M. and Kumar M., Hydrogel and its effect on soil moisture status and plant growth: A review. Journal of Pharmacognosy and Phytochemistry, 9(3), 1746-1753(2020).

[58]-Xu H.,Yeum K.J., Yoon Y.H. and Ju J.H., Effect of hydrogels in three substrates on growth and ornamental quality of apple mint (Mentha suaveolens) in unirrigated green roofs. Journal of Horticulture, 6, 260 (2019). Doi: 10.35248/2376-0354.19.06.260.

[59]-Abobatta W.F. and Khalifa S.M., Influence of hydrogel composites soil conditioner on navel orange growth and productivity. Journal of Agriculture and Horticulture Research, 2 (2), 1-6(2019).

[60]-Paris D. and Guerrini G., Hydrogel application in the germination and growth of Zea mays and Solanum lycopersicum seedlings. GSC Advanced Research and Reviews, 12(03), 026–032(2022).

[61]-Kumar A.T., Kameswari P.L. and Girwani A., Impact of pusa hydrogel incorporated growing media on floral characters and yield of pot mums (Dendranthema grandiflora L.) under various irrigation regimes. International Journal of Agricultural Science and Research (IJASR), 6, 195-200(2016).

[62]- Changela Y., Dipal S. B., Tandel B.M., Bhatt S.T. and Patel H.M., Prospective of hydrogel for ornamental plants. The Pharma Innovation Journal, 11(10), 1343-1347(2022).

[63]-Siddiqui H., Hayat S. and Bajguz, A, Regulation of photosynthesis by brassinosteroids in plants. Acta Physiol Plant, 40(3), 59(2018).

[64]-Chandrasekaran M., Chanratana M., Kim K., Seshadri S. and Sa, T. , Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress–a meta-analysis. Front Plant Sci., 10,457(2019).

[65]-Altuntaş C., Demiralay M., Muslu A.S.and Terzi R., Proline-stimulated signaling primarily targets the chlorophyll degradation pathway and photosynthesis associated processes to cope with short-term water deficit in maize. Photosyn Res., 28, 1-4 (2020).

[66]-Vivek M.S., Chandravanshi P., Nataraju S.P., Salimath S. and Naik A.H. K., Effect of hydrogel on chlorophyll content and chlorophyll stability index of groundnut (Arachis hypoaea L.) under rained condition. International Journal of Chemical Studies, 8(3), 2211-2215 (2020 a).

[67]-Abd El-Aziz G. H., Ibrahim A. S. and Fahmy A. H., Using environmentally friendly hydrogels to alleviate the negative impact of drought on plant. Open Journal of Applied Sciences, 12, 111-133 (2022). https://doi.org/10.4236/ojapps.2022.121009.

[68]-Beltramin F.A. , Silva W. C. , Santos C. C. , Scalon, S. de P. Q. and Vieira M. do C., Water-retaining polymer mitigates the water deficit in Schinus terebinthifolia: photosynthetic metabolism and initial growth. Engenharia Agrícola, 40(6), 684-691 (2020). Doi: http://dx.doi.org/10.1590/1809.

[69]-Babaei M. , Shabani L. and Hashemi Shahraki S., Improving the effects of salt stress by β-carotene and gallic acid using increasing antioxidant activity and regulating ion uptake in Lepidium sativum L., Botanical Studies, 63, 22 (2022). https://doi.org/10.1186/s40529-022-00352-x

[70]-Gharbi E., Martínez J.P., Benahmed E., Dailly H. and Lutts S.(2017) The salicylic acid analog 2,5-dichloroisonicotinic acid has specific impact on the response of the halophyte plant species Solanum chilense to salinity. Plant Growth Regul., 82, 517–525(2017).

[71]- Kössle S. ,Kössler T., Armarego-Marriott D. Tarkowská V. Turečková S., Agrawal J. Mi, L. P. de Souza M. A., Schöttler A. ,Schadach A. Fröhlich, et al., Lycopene β-cyclase expression influences plant physiology, development, and metabolism in tobacco plants. Journal of Experimental Botany, 72, 2544–2569 (2021). https://doi.org/10.1093/jxb/erab029

[72]-Martinez J.P., Fuentes R., Farias K., Lizana C., Al faro J.F., Fuentes L. et al., Effects of salt stress on fruit antioxidant capacity of Wild (Solanum chilense) and Domesticated (Solanum lycopersicum var. cerasiforme) Tomatoes. Agronomy, 10 (10), 1481(2020). https://doi.org/10.3390/agronomy10101481

[73]-Sankari M. , Hridya H. Sneha P. , George Priya Doss C. , Godwin Christopher J. , Mathew J. , Zayed H. and Ramamoorthy S., Implication of salt stress induces changes in pigment production, antioxidant enzyme activity, and qRT-PCR expression of genes involved in the biosynthetic pathway of Bixa orellana L.. Functional &Integrative Genomics, 19, 565-574 (2019).

[74]-Leiva-Ampuero A. , Agurto M. , Matus J. T. , Hoppe G. , Huidobro C. , Inostroza-Blancheteau C., Reyes-Díaz M. , Stange C. , Canessa P. and Vega A., Salinity impairs photosynthetic capacity and enhances carotenoid-related gene expression and biosynthesis in tomato (Solanum lycopersicum L. cv. Micro-Tom). PeerJ , 8, e9742 (2020). doi: 10.7717/peerj.9742

[75]-Maurya V. K., Srinvasan R., Ramesh N., Anbalagan M. and Gothandam K.M.(2015) Expression of carotenoid pathway genes in three capsicum varieties under salt stress. Asian Journal of Crop Science, 7 (4), 286-294(2015). DOI: 10.3923/ajcs.2015.286.294.

[76]-Alshallash K. S. , Sharaf M., Hmdy A. E. , Khalifa S. M. , Abdel-Aziz H. F., Sharaf A. , Ibrahim M.T. S. , Alharbi K. and Elkelish A., Hydrogel improved growth and productive performance of mango trees under semi-arid condition. Gels, 8, 602 (2022). https://doi.org/ 10.3390/gels8100602

[77]-Abdelaziz A.M., Dacrory S., Hashem A.H., Attia M.S., Hasanin M., Fouda H.M., Kamel S. and ElSaied H., Protective role of zinc oxide nanoparticles based hydrogel against wilt disease of pepper plant. Biocatal. Agric. Biotechnol. 35, 102083 (2021).

[78]-Kumar S., Li G., Yang J., Huang X., Ji Q. , Liu Z. , Ke W. and Hou H., Effect of salt stress on growth, physiological parameters, and ionic concentration of water dropwort (Oenanthe javanica) Cultivars. Frontiers in Plant Science, 12, 660409(2021). doi: 10.3389/fpls.2021.660409

[79]-Soliman D.M., Mazhar A.A.M., Eid R.A. and Abd El Aziz, N.G., Biostimulation effects of linseed and citrus oils on growth, antioxidant enzymes activity, metabolic changes and water relations of Khaya senegalensis seedlings under drought stress, Journal of Pharmaceutical Negative Results, 13, 2790-2801 (2022a).

[80]-Soliman D.M., Ahmed A.M.A. and EL-Sayed I.M., Lead toxicity and spermine as affecting the chemical composition and growth of

Solidago canadensis L. cv. Tara plant. Egyptian Journal of Chemistry, 65(2), 471-485.(2022b).

[81]-Sharif P., Seyedsalehi M., Paladino O., Damme P. Van, Sillanpa M. and Sharifi A. A., Effect of drought and salinity stresses on morphological and physiological characteristics of canola. Int. J. Environ. Sci. Technol., 15, 1859–1866 (2017). doi: 10.1007/s13762-017-1508-7

[82]-Ibrahimova U. F., Mammadov A. C. and Feyziyev, Y. M., The effect of NaCl on some physiological and biochemical parameters in Triticum aestivum L. genotypes. Plant Physiol. Rep., 24, 370–375(2019). doi: 10.1007/s40502-019-00461-z

[83]-Colak N., Tarkowski P. and Ayaz, F. A., Effect of N-acetyl-L-cysteine (NAC) on soluble sugar and polyamine content in wheat seedlings exposed to heavy metal stress (Cd, Hg and Pb). Bot. Serbica, 44, 191–201(2020).

[84]-Hussein H.A.A. and Alshammari, S.O., Cysteine mitigates the effect of NaCl salt toxicity in fax (Linum usitatissimum L) plants by modulating antioxidant systems. Scientific Reports, 12, 11359 (2022). https://doi.org/10.1038/s41598-022-14689-7

[85]-Liu X., Li F., Yang Q. and Wang, X., Effects of alternate drip irrigation and superabsorbent polymers on growth and water use of young coffee tree. Journal of Environmental Biology, 37, 485-491(2016).

[86]-Xie E., Wei X., Ding A., Zhang L., Wu X. and Anderson B. , Short-term effects of salt stress on the amino acids of Phragmites australis root exudates in constructed wetlands. Water, 12(2), 569(2020). https://doi.org/10.3390/w12020569

[87]-Haque S.I. and Matsubara, Y.I., Relationship between salt tolerance and free amino acid content in mycorrhized strawberry plants. Journal of Japanese Society of Agricultural Technology Management, 25, 2, 43-50(2018).

[88]-Nascimento V. L., Rauber W. A., Silva G. S., Siebeneichler S. C. and Fidelis R. R., Hydrogel effects in biochemical composition of soybean grains cultivated under water deficit in Brazilian Cerrado. Communications in Plant Sciences, 9, 13-19(2019). DOI: http://dx.doi.org/10.26814/cps2019003

[89]-Vivek M.S., Chandravanshi P., Nataraju S.P., Salimath S. and Naik A.H.K., Improvement in growth parameters, yield attributes and economics of groundnut (Arachis hypogaea L.) through graded levels of hydrogel application. International Journal of Current Microbiology and Applied Sciences, 9, 4, 3047-3057(2020b). https://doi.org/10.20546/ijcmas.2020.903.xx

[90]-Bistgani Z. E., Hashemi M., DaCosta M., Craker L., Maggi, F. and Morshedloo, M. R., Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of Thymus vulgaris L. and Thymus daenensis Celak, Industrial Crop and Products, 135,311-320 (2019). https://doi.org/10.1016/j.indcrop.2019.04.055

[91]-Zhou Y. , Tang N. , Huang L. , Zhao Y. , Tang X. and Wang K., Effects of salt stress on plant growth, antioxidant capacity, glandular trichome density, and volatile exudates of Schizonepeta tenuifolia Briq.. International Journal of Molecular Sciences, 19, 1, 252 (2018). doi: 10.3390/ijms19010252

[92]-Bayat H., Shafie F.and Shahraki B., Salinity effects on growth, chlorophyll content, total phenols, and antioxidant activity in Salvia lavandulifolia Vahl.. Advances in Horticultural Science, 36(2), 145-153(2022). DOI: 10.36253/ahsc¬12015

[93]-Besharati J., Shirmardi M., Meftahizadeh H., Ardakani M. D.and Ghorbanpour M., Changes in growth and quality performance of Roselle (Hibiscus sabdariffa L.) in response to soil amendments with hydrogel and compost under drought stress. South African Journal of Botany, 145, 334-347(2022). https://doi.org/10.1016/j.sajb.2021.03.018

[94]-Barkia N. M., Aissaoui F., Chehab H., Dabbaghi O., del Giudice T., Boujnah D. and Mechri B., Cultivar dependent impact of soil amendment with water retaining polymer on olive (Olea europaea L.) under two water regimes. Agricultural Water Management, 216, 70-75(2019).

[95]-Sajjadi S.G., Tavassoli A. and Dadmehr M., Effects of hydrogel on drought resistance of medicinal plant roselle (Hibiscus sabdariffa L.). Iranian Journal of Medicinal and Aromatic Plants, 37, 584-595 (2021). https://doi.10.22092/IJMAPR.2021.353010.2924

[96]-Badawy E. M. , Ahmed A. H. H. , Habba E. E. , Soliman D. M., Effect of mycorrhizal fungi (AMF), brassinosteroids and sodium silicate on vegetative growth, flower production

and Pb concentration of zinnia (Zinnia elegans) plant under Pb stress. Journal of Environmental Science and Technology, 10 (4), 157-174(2017). DOI : 10.3923/jest.2017.157.174

[97]-Alzahrani S. M., Alaraidh I. A., Migdadi H., Alghamdi S., Altaf Khan M. and Ahmad P. Physiological, biochemical, and antioxidant properties of two genotypes of Vicia faba grown under salinity stress. Pak. J. Bot., 51, 786–798(2019). doi: 10.30848/PJB2019-3(3)

[98]-Chen F., Fang P., Peng Y., Zeng W., Zhao X., Ding Y., Zhuang Z., Gao Q. and Ren B., Comparative proteomics of salt-tolerant and salt-sensitive maize inbred lines to reveal the molecular mechanism of salt tolerance. Int. J. Mol. Sci., 20, 4725(2019). [Google Scholar] [CrossRef]

[99]-Khalil C., Abdelmajid H., Mohammed El. H. and El Houssein B.(2016) Growth and proline content in NaCl stressed plants of annual medic species. International Journal of Advanced Research in Biological Sciences, 3(9), 82-90(2016). DOI: http://dx.doi.org/10.22192/ijarbs.2016.03.09.012

[100]-Goharrizi K. J. , Baghizadeh A. , Afroushteh M. , Amirmahani F. and Kermani S. G., Effects of salinity stress on proline content and expression of Δ1-pyrroline-5-carboxylate synthase and vacuolar-type H+ subunit E genes in wheat. Plant Genetic Resources, 18, 334-342(2021). DOI: https://doi.org/10.1017/S1479262120000350

[101]-Tomášková I. , Svatoš M., Macků J., Vanická H., Resnerová K., Čepl J. , Holuša J., Hosseini S. M. and Dohrenbusch A. ,(2020) Effect of different soil treatments with hydrogel on the performance of drought-sensitive and tolerant tree species in a semi-arid region. Forests, 11(2), 211. https://doi.org/10.3390/f11020211

[102]-Yang W., Li P., Guo S., Song R.and Yu J., Co-application of soil superabsorbent polymer and foliar fulvic acid to increase tolerance to water deficit maize: Photosynthesis, water parameters, and proline. Chil. J. Agric. Res., 79, 435–446(2019).

[103]-Song B., Liang H., Sun R., Peng P., Jiang Y. and She, D., Hydrogel synthesis based on lignin/sodium alginate and application in agriculture, International Journal of Biological Macromolecules, 144, 219-230(2020). https://doi.org/10.1016/j.ijbiomac.2019.12.082

[104]-Foyer C.H., Reactive oxygen species, oxidative signaling and the regulation of photosynthesis. ¬ Environ. Exp. Bot., 154, 134¬142 (2018).

[105]-Rezayian M., Niknam V. and Ebrahimzadeh H., ¬ Effects of drought stress on the seedling growth, development, and metabolic activity in different cultivars of canola. ¬ J. Soil Sci. Plant Nutr., 64, 360¬369 (2018).

[106]-Bayat H. and Moghadam A.N., ¬ Drought effects on growth, water status, proline content and antioxidant system in three Salvia nemorosa L. cultivars. ¬ Acta. Physiol. Plant, 41(9), 149 (2019).

[107]-El-Sayed I.M., Salim R.G., El-haggar E.F., El-Ziat R.A., Soliman D.M., Molecular characterization and positive impact of brassinosteroids and chitosan on Solidago canadensis cv. Tara Characteristics. Horticulturae, 6(4).1-18(2020).

[108]-Soliman D.M. and EL-Sayed I.M., Study postharvest characteristics, chemical composition and antimicrobial activity of Dianthus caryophyllus L., cut flowers using some essential oils. Ornamental Horticulture, 29, 37-47(2023).

[109]-Hanifah N. and Purwestri, Y.A., The effect of NaCl salinity stress to phenolic compound, total flavonoid and antioxidant activity of pegan (Centella asiatica (L.) Urban) leaves, The 4th International Conference on Bioinformatics, Biotechnology, and Biomedical Engineering (BioMIC 2021), 41, 5(2021). https://doi.org/10.1051/bioconf/20214106004

[110]-Mansour R.B., Fattoum R.B., Gouia H. and Haouari C.C., Salt stress effects on enzymes activities involved in carbon metabolism and nitrogen availability of two Tunisian durum wheat varieties. Journal of Plant Nutrition, 42, 1142-1151(2018). https://doi.org/10.1080/01904167.2019.1604749

[111]-Waqar A., Bano A. and Ajmal M., Effects of PGPR bioinoculants, hydrogel and biochar on growth and physiology of soybean under drought stress. Communication in Soil Science and Plant Analysis, 35, 826-847(2022). https://doi.org/10.1080/00103624.2022.2028818

[112]-Guo X., Ahmad N., Zhao S., Zhao c., Zhong S., Zhong W., Wang X. and Li, G., Effect of salt stress on growth and physiological properties of Asparagus seedlings. Plants, 11(21), 2836(2022).

[113]-Ghobashy M. M. , Amin M. A. , Nady N. , Meganid A. S. , Alkhursani S. A. , Alshangiti D. M. , Madani M. , Al-Gahtany S. A. and Zaher, A. A. , Improving impact of poly(Starch/Acrylic Acid) superabsorbent hydrogel on growth and biochemical traits of Sunflower under drought stress. Journal of Polymers and the Environment , 30, 1973–1983(2022).

[114]-Goharrizi K.J., Riahi-Madvar A., Rezaee F., Pakzad R., Bonyad F.J. and Ahsaei, M. G. , Effect of salinity stress on enzymes' activity, ions concentration, oxidative stress parameters, biochemical traits, content of Sulforaphane, and CYP79F1 Gene Expression Level in Lepidium draba Plant. Journal of Plant Growth Regulation, 39, 1075-1094 (2020).

[115]-Tian J., Pang Y.and Zhao Z. , Drought, salinity, and low nitrogen differentially affect the growth and nitrogen metabolism of Sophora japonica (L.) in a Semi-Hydroponic phenotyping platform. Frontiers in Plant Science, 12, 715456(2021). https://doi.org/10.3389/fpls.2021.715456.

[116]-Mohamed A., Noredine Y. and Amin G., Influence of salinity soil on the sodium, potassium and calcium content of tomato Lycopersicum esculentum Mill. (Solanales Solanaceae) at 5-leaf stage. Biodiversity Journal, 11 (4), 1037–1044 (2020). https://doi.org/10.31396/Biodiv.Jour.2020.11.4.1037.1044

[117]-Assaha D.V. M. , Ueda A. , Saneoka H. , Al-Yahyai R. and Yaish M.W., The role of Na+ and K+ transporters in salt stress adaptation in glycophytes. Frontiers in Physiology, 8, 509(2017). doi: 10.3389/fphys.2017.00509

[118]-Satriani A., Catalano M. and Scalcione E., The role of superabsorbent hydrogel in bean crop cultivation under deficit irrigation conditions: A case-study in southern Italy. Agric.Water Manag, 195, 114–119(2018).

[119]-Rasheed F., Markgren J., Hedenqvist M.and Johansson E., Modeling to understand plant protein structure- function relationships-implications for seed storage proteins. Molecules, 25(4):873(2020).

[120]-Sarker U. and Oba S., Drought stress effects on growth, ROS markers, compatible solutes, phenolics, flavonoids, and antioxidant activity in Amaranthus tricolor. Appl. Biochem. Biotechnol,. 186, 999–1016(2018). doi: 10.1007/ s12010-018-2784-5

[121]-Rehman Athar H.U., Zulfiqar F., Moosa A., Ashraf M., Zafar Z.U., Zhang, L. et al., Salt stress proteins in plants : An overview. Frontiers in Plant Science, 13, 999058 (2022). https://doi.org/10.3389/fpls.2022.999058

[122]- Fidelis R.R., da Silva G.S., Rauber W.A., Rodrigues L.U., Nascimento V.L. and Siebeneichler S.C., Biochemical response of cowpea beans grown under water-deficit and hydrogel. Revista de Ciencias Agrícolas. 37(2), 38 – 50 (2020). doi: https://doi.org/10.22267/rcia.203701.123