

## EFFECT OF SPRAYING SOME SAFE GROWTH STIMULANTS ON GROWTH AND FLOWERING OF *PETUNIA AXILLARIS* UNDER DROUGHT STRESS

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**ABSTRACT:** The current investigation has been accomplished in a climate of the greenhouse at the farm of the Fac. of Agri, Damanhour Univ., El-Beheira Gov., Egypt, during two consecutive seasons of 2020/2021 and 2021/2022. The aim of this experiment was to evaluate the impact of two safe growth stimulants: seaweed extract of Oligo-x (SWE) and chitosan (CH) each at 0, 3 and 6 ml/l on vegetative growth, flowering growth, and the chemical composition of the leaves of *Petunia axillaris* plants cultivated in drought stress conditions. The acquired results for the two seasons showed that drought stress caused serious negative consequences on vegetative growth, flowering growth, and the chemical composition of leaves, while it increased the proline content and electrolyte leakage. Generally, seaweed extract and chitosan had a profound impact on the studied characteristics. For instance, the growth parameters including plant height, number of branches per plant, leaf area, shoot fresh and dry weights per plant, as well as root length and root fresh and dry weights per plant showed the highest values via the application of seaweed extract at 6 ml/l compared to the other treatments under study. In the same line, flowering parameters such as flowering duration, flower diameter, the number of flowers per plant, flower fresh and dry weights, and leaves chemical composition including total leaf carbohydrate exhibited the most significant improvements by the application of seaweed extract at 6 ml/l. On the contrary, the seaweed extract at 6 ml/l resulted in the lowest value of proline content, and electrolytic leakage. Regarding the chitosan treatment, the 6 ml/l concentration of the solution exhibited the highest values of the number of days to flowering, flower longevity and SPAD index in relative to the other treatments. All the studied traits were expressed using cross correlation analysis.

**Keywords:** Seaweed extract, Oligo-x, algae extract, chitosan, *Petunia axillaris*, drought stress.

### INTRODUCTION

*Petunia* is an annual ornamental plant with significant commercial value in worldwide horticulture because it has the potential to produce great economic returns and is an ideal way for the flower industry to generate

more revenue (Zhang *et al.*, 2012). It is a Solanaceae family plant (Gerats and Vandebussche, 2005). *Petunia* leaves are ovate, and the flowers are trumpet-shaped and may be single or double, and wide; the calyx is deeply 5-parted, and the corolla is funnel-shaped with five rounded petals (white,

yellow, red, pink, purple, or variegated) (Ali and Ali, 2022). Petunia plants are produced from seeds as an annual plant for outdoor decorative purposes. Petunia plant growth necessitates strict environmental requirements; drought and other abiotic stresses severely limit petunia growth and can result in plant death (Jundan *et al.*, 2004). Therefore, it is essential to reduce its water usage by enhancing its drought resilience.

Water is the basic element for agriculture and life in general and it becomes an increasingly limited resource. Drought stress is one of the world's most urgent issues in the world, where it is regarded as one of the most significant obstacles to agricultural productivity that has a major effect on crop production (Khan *et al.*, 2013). In dry and semi-arid climates, lack of water is the main factor restricting plant growth and development, leading the plant to react in several ways across the molecular, cellular, and physiological levels (Ahmad and Haddad, 2011). Drought can prevent plant respiration, tissue water absorption, stomatal movement, and photosynthesis, affecting physiological processes metabolism and plant growth (Yang *et al.*, 2021). Climate change is anticipated to intensify droughts in the future, making the scarcity of water supplies worse. In addition, plants are unable to move, so adaptation mechanisms have great importance in dealing with various environmental stresses. Exogenous chemical application is one strategy for reducing the negative impacts of abiotic stressors. (Yuan and Lin, 2008).

Plant biostimulants are substances that can improve the growth and productivity of plants. Biostimulants come naturally from many economically and environmentally credible sources including microbes, chitin and chitosan derivatives, humic compounds, amino acids, and seaweed extracts. For many years, plant biostimulants like seaweed extracts have been employed in agriculture to increase antioxidant levels and defense against harmful environmental conditions (Sakr and Metwally, 2009). Seaweed contains

many growth-promoting hormones like auxins (Verkleij, 1992), gibberellins (Strik and Staden, 1997), cytokinins (Durand *et al.*, 2003), trace elements, amino acids, vitamins, micro and macronutrients, polysaccharides, polyphenols, proteins, osmolytes and polyunsaturated fatty acids. The usage of seaweed aids in fostering the growth and development of beneficial soil microorganisms (Khan *et al.*, 2009), improving soil nutrient absorption (Turan and Kose, 2004) and increasing the productivity and development of plants (Kumari *et al.*, 2011).

Chitosan (CH) is a biocompatible, eco-friendly polymer, not harmful, allergic-causing, or poisonous and reasonably priced material with several uses in agriculture, feed industries and biomedical (Asgari-Targhi *et al.*, 2018). It is derived from chitin by an alkaline deacetylation process, obtained from fish, crustacean shells including those of shrimp, crab, and prawns, insect exoskeletons, and fungus cell walls. It has several uses in both biotic and abiotic stress management techniques, therefore it may be used to alleviate the water stress in petunias. Chitosan foliar application lowers stomatal conductance, decreases transpiration, and maximizes water usage by acting as an antitranspirant compound via encouraging the synthesis of jasmonic acid via affecting how much water plants utilize since abscisic acid has been shown to cause stomatal closure in plants (Bittelli *et al.*, 2001 and Iriti *et al.*, 2009).

The main objective of this study was to determine how applying osmoprotective substances like seaweed extract and chitosan topically affect the vegetative development, flowering growth and chemical composition of *Petunia axillaris* drought-stressed plants.

## MATERIALS AND METHODS

### Greenhouse experimental design:

There were 15 treatments in this study, covering all combinations of the three irrigation interval levels and 5 stimulants (two seaweed extract of Oligo-x treatments and two chitosan treatments, besides the control).

The treatments were set up in a split-plot experiment in a randomized complete plot design with three replicates (experimental units) and three blocks. The different irrigation treatments were randomly dispersed in the main plots, while sub-plots were dedicated to the various seaweed extract and chitosan treatments.

**Treatments:**

**Irrigation intervals:**

Three irrigation intervals were applied throughout the period of plant life, at 3, 6 and 9 days (designate as D3, D6 and D9, respectively) between irrigations.

**Seaweed extract and chitosan foliar treatments:**

The seaweed extract of Oligo-x was used at 3 and 6 ml/l (designate as SWE1 and SWE2, respectively) and chitosan was used at 3 and 6 ml/l (designate as CH1 and CH2, respectively), besides the control.

**Preparation of seaweed extract and chitosan:**

The extract of seaweed represented as algae extract (Oligo-x) was utilized in this research. It contains *Sargassum* spp., *Laminaria* spp, *Ascophyllum* spp. and *Fucus* spp. It was bought from AGAS (Arabian Group for Agricultural Service, Co.), having the following chemical composition: oligosaccharide (3%), glutamic acid (0.0019%), algenic acid (5%), alanine (0.026%), menthol (0.001%), phytin (0.003%), natural growth regulators like cytokines (0.001 %), pepsin (0.02%) and

indole acetic acid (0.0002%) and minerals (phosphorus oxide 0.5%, potassium oxide 12%, N 1%, Fe 0.2%, Zn 0.3%, and Mn 0.1%).

Chitosan was acquired from the commercial commodity Chitosan Powder, produced by Chitosan Egypt. To administer dosages of chitosan, a solution was made in accordance with (Dzung *et al.*, 2011) by dissolving 1 g of chitosan powder in 100 ml of 0.5% acetic acid for 12 hours. This solution was then diluted by the addition of distilled water to get the corresponding concentrations.

**Planting and growth conditions:**

Two pot trials were performed in a climate of the greenhouse at the farm of the Fac. of Agri, Damanhour Univ., El-Beheira Gov., Egypt, during the two successive winter growing seasons, 2020/2021 and 2021/2022. The *Petunia axillaris* species was employed in these experiments. High-quality seeds of this species were bought from Ontario Seeds Company Ltd., (located in Waterloo, Ontario, Canada).

On November 18<sup>th</sup>, the seeds were sown in 20 cm black plastic pots, filled with sandy soil, for both seasons. On January 1<sup>st</sup>, after 43 days from seed sowing, plants were thinned to one plant per pot for both seasons. The mechanical and chemical examinations of the soil were carried out according to the conventional procedure outlined by Jackson (1958), and the soil was examined at the Natural Resources and Engineering Soil Dept., Fac. of Agric., Damanhour Univ. (Table, 1).

**Table 1. Some physical and chemical analyses of the experiment's soil samples during 2020/2021 and 2021/2022 seasons.**

Seasons	Chemical properties					
	pH	EC (dSm <sup>-1</sup> )	Ca (meq/l)	Mg (meq/l)	SO <sub>4</sub> (meq/l)	K (meq/l)
2020/2021	7.7	0.78	20.21	6.21	8.21	5.31
2021/2022	7.9	0.81	20.30	6.78	7.95	5.33
	Physical properties					
	Sand	Silt	Clay	Texture class		
2020/2021	91.00	6.25	2.75	Sand		
2021/2022	92.20	6.03	1.77	Sand		

### **Foliar application of seaweed and chitosan:**

After 76 days from seed sowing for both seasons, plants were sprayed four times early in the morning, once a week with seaweed extract and chitosan each at (0, 3 and 6 ml/l) on the leaves of each plant, and then irrigation was stopped.

Treatments with chitosan and seaweed extract were always followed by drought stress. Before applying seaweed extract and chitosan, the pot surface was covered with polyethylene to stop spray droplets from dripping onto the growth media, using a hand sprayer. Tween 80 (a non-ionic surfactant) was added to all treatments at 0.05% (v/v) to increase the contact angle of sprayed droplets and reduce surface tension. Each plant had its own unique spraying, ensuring that the foliage was evenly wet to the point of runoff. The spraying volume was 17 ml per plant, and the amount of water that was added to the pot to irrigate plants was 460 ml per plant.

To prevent mineral precipitation, similar amounts of soluble N, P, and K fertilizers were applied to all treatments. All other cultural customs were modified as required and in accordance with accepted techniques for petunia commercial production.

### **Data recorded:**

#### **Plant growth characteristics:**

Three plants from each treatment in each replication were used for the experiment's final analysis to collect data on vegetative growth characteristics as plant height (cm), number of branches/plant, leaf area (cm<sup>2</sup>), and shoot fresh and dry weight per plant (g) were measured without the inflorescences and roots. The dry weights of the plant samples were determined by drying them in an oven at 70 °C until they reached a constant weight. Likewise, root growth characteristics were measured, such as root length (cm), root fresh and dry weight per plant (g).

#### **Flowering characteristics:**

were also measured, such as the number of days to flowering, flower longevity, flowering duration (day), flower diameter

(cm), number of flowers per plant and flower fresh and dry weights (g).

### **Leaves chemical analyses:**

According to Yadava (1986), a SPAD-502 (Single-Photon Avalanche Detector), chlorophyll meter (Konica Minolta, Kearney, NE, USA) was used to measure the total leaf chlorophyll content (SPAD index). Petunia leaves were examined for total leaf carbohydrate (% of D.W.) using the techniques outlined in (Herbert *et al.*, 1971). Using Bates *et al.* (1973) methodology, the free proline content of leaf fresh weight was calculated. To determine if the cell membranes were stable or not, electrolyte leakage was utilized. It was assessed utilizing the approach outlined by Lutts *et al.* (1999), and the following equation was used to calculate the Electrolyte leakage (EL):

$$EL = [(EC0/EC1) \times 100] \%$$

EC0 and EC1 refer to primary and secondary electrical conductivity.

### **Statistical Analysis:**

Utilizing CoStat's Statistical Analysis Systems (CoStat, 1989), all data were statistically analyzed, and the Tukey test was utilized to compare significant means with a 0.05 probability.

## **RESULTS AND DISCUSSION**

### **Vegetative growth characters:**

Table (2) highlights the primary impacts of the two factors that were examined (different irrigation intervals and different levels of seaweed extract and chitosan) on plant growth of *Petunia axillaris*, while Table (3) shows their interactions throughout the two growing seasons of (2020/2021) and (2021/2022).

According to data in Table (2), the primary impact of drought stress on plant growth parameters resulted in a considerable decline in plant height, number of branches per plant, leaf area, shoot fresh and dry weights of shoots and roots, with prolonging the period between irrigation in both seasons. However, the length of the roots increased

**Table 2. The main effect of different irrigation intervals and different levels of seaweed extract and chitosan on plant growth parameters of *Petunia axillaris* plants during the 2020/2021 (1<sup>st</sup>) and 2021/2022 (2<sup>nd</sup>) seasons.**

Irrigation intervals (days)	Plant height (cm)		Number of branches/plant		Leaf area (cm <sup>2</sup> )		Shoot fresh weight (g)		Shoot dry weight (g)		Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
<b>D3</b>	52.87 a	53.53 a	6.53 a	7.53 a	7.05 a	6.97 a	31.71 a	30.79 a	7.04 a	6.70 a	13.54 c	13.31 c	17.57 a	17.00 a	5.71 a	5.29 a
<b>D6</b>	39.63 b	44.00 b	4.73 b	4.67 b	3.97 b	3.71 b	22.48 b	21.66 b	4.10 b	4.01 b	21.04 b	20.02 b	15.83 b	15.74 b	3.56 b	3.49 b
<b>D9</b>	27.60 c	31.10 c	3.13 c	2.67 c	2.59 c	2.53 c	12.88 c	12.25 c	2.78 c	2.75 c	25.96 a	23.67 a	7.90 c	7.39 c	2.12 c	2.03 c
<b>Irrigation intervals (days)</b>																
<b>Seaweed extract and chitosan</b>																
<b>Control</b>	33.81 e	38.56 e	4.00 c	4.00 c	3.74 e	3.51 e	18.88 e	18.04 e	3.83 e	3.66 e	18.11 d	16.28 e	10.98 e	10.49 e	2.41 e	2.28 e
<b>SWE 1</b>	43.33 b	45.28 b	5.22 ab	5.56 a	5.07 b	4.94 b	24.15 b	23.45 b	5.06 b	4.93 b	20.44 bc	20.26 b	14.95 b	14.78 b	4.53 b	4.36 b
<b>SWE 2</b>	44.89 a	47.00 a	5.56 a	5.89 a	5.56 a	5.34 a	26.32 a	25.26 a	5.72 a	5.48 a	21.47 a	22.06 a	16.32 a	15.96 a	4.99 a	4.66 a
<b>CH 1</b>	37.57 d	41.11 d	4.33 c	4.33 c	3.95 d	3.95 d	20.43 d	19.79 d	4.05 d	4.05 d	19.87 c	17.79 d	12.59 d	12.20 d	3.20 d	3.01 d
<b>CH 2</b>	40.56 c	42.44 c	4.89 b	5.00 b	4.37 c	4.27 c	22.00 c	21.29 c	4.52 c	4.33 c	21.02 ab	18.62 c	14.00 c	13.45 c	3.85 c	3.70 c

Means were examined using Tukey's Honest Significant Difference test ( $P \leq 0.05$ ); n = 3

Means with the same letters do not differ significantly between different irrigation intervals or between different amounts of seaweed extract and chitosan

**Table 3. The interaction effect between different irrigation intervals and different levels of seaweed extract and chitosan on plant growth parameters of *Petunia axillaris* plants during the 2020/2021 (1<sup>st</sup>) and 2021/2022 (2<sup>nd</sup>) seasons.**

Irrigation intervals (days)	Treatments	Plant height (cm)		Number of branches/plant		Leaf area (cm <sup>2</sup> )		Shoot fresh weight (g)		Shoot dry weight (g)		Root length (cm)		Root fresh weight (g)		Root dry weight (g)	
		1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
	<b>Control</b>	45.67 e	50.67 d	5.33 cd	6.67 c	5.69 e	5.26 e	27.44 e	27.04 e	5.53 e	5.13 e	12.32 h	11.10 n	14.23 g	14.06 e	1.70 n	1.49 m
	<b>SWE 1</b>	56.67 b	55.33 b	7.33 a	8.33 a	7.88 b	7.88 b	34.33 b	33.33 b	7.91 b	7.74 b	13.03 h	14.33 k	19.57 b	18.21 b	7.32 b	7.04 b
<b>D3</b>	<b>SWE 2</b>	58.33 a	57.33 a	7.67 a	8.67 a	8.65 a	8.44 a	37.00 a	35.33 a	9.01 a	8.51 a	13.46 h	15.57 j	20.53 a	20.12 a	8.50 a	7.60 a
	<b>CH 1</b>	50.00 d	52.00 c	5.67 c	6.67 c	6.19 d	6.34 d	28.89 d	28.15 d	5.83 d	5.82 d	13.71 gh	12.27 m	16.06 f	15.60 d	4.69 d	4.30 d
	<b>CH 2</b>	53.67 c	52.33 c	6.67 b	7.33 b	6.85 c	6.94 c	30.89 c	30.07 c	6.90 c	6.32 c	15.19 g	13.30 l	17.44 d	17.03 c	6.33 c	6.03 c
	<b>Control</b>	33.10 j	39.33 i	4.33 f	3.33 g	3.37 ij	3.15 ij	19.33 j	17.82 j	3.57 j	3.49 j	19.00 f	17.40 i	12.40 h	12.13 f	3.79 g	3.75 f
	<b>SWE 1</b>	43.67 g	46.67 f	5.00 de	5.33 de	4.31 g	4.18 g	23.92 g	23.19 g	4.32 g	4.15 g	21.67 de	21.43 e	16.43 ef	17.43 c	3.91 f	3.77 f
<b>D6</b>	<b>SWE 2</b>	44.67 f	48.00 e	5.33 cd	5.67 d	4.88 f	4.48 f	26.19 f	25.59 f	4.89 f	4.72 f	22.00 de	23.30 c	18.67 c	18.67 b	4.01 e	3.98 e
	<b>CH 1</b>	36.37 i	42.33 h	4.33 f	4.00 f	3.42 i	3.24 i	20.67 i	20.15 i	3.74 i	3.76 i	21.33 e	18.63 h	14.83 g	14.40 e	2.97 i	2.89 h
	<b>CH 2</b>	40.33 h	43.67 g	4.67 ef	5.00 e	3.85 h	3.50 h	22.30 h	21.55 h	3.97 h	3.94 h	21.20 e	19.33 g	16.83 de	16.07 d	3.13 h	3.04 g
	<b>Control</b>	22.67 o	25.67 m	2.33 i	2.00 j	2.15 m	2.11 m	9.87 o	9.25 o	2.40 o	2.35 n	23.00 cd	20.33 f	6.32 l	5.28 j	1.75 n	1.58 m
	<b>SWE 1</b>	29.67 l	33.83 k	3.33 gh	3.00 gh	3.01 k	2.77 k	14.18 l	13.82 l	2.96 l	2.89 l	26.63 b	25.00 b	8.86 j	8.70 g	2.35 k	2.28 j
<b>D9</b>	<b>SWE 2</b>	31.67 k	35.67 j	3.67 g	3.33 g	3.14 jk	3.11 j	15.78 k	14.85 k	3.27 k	3.21 k	28.93 a	27.30 a	9.75 i	9.10 g	2.45 j	2.41 i
	<b>CH 1</b>	26.33 n	29.00 n	3.00 h	2.33 ij	2.25 lm	2.29 l	11.74 n	11.08 n	2.57 n	2.6 m	24.57 c	22.47 d	6.87 i	6.60 i	1.96 m	1.83 l
	<b>CH 2</b>	27.7 m	31.33 l	3.33 gh	2.67 hi	2.40 l	2.37 l	12.8 m	12.3 m	2.69 m	2.7 m	26.67 b	23.23 c	7.72 k	7.27 h	2.10 l	2.04 k

Means were examined using Tukey's Honest Significant Difference test ( $P \leq 0.05$ ); n = 3

Means with the same letters do not differ significantly between different irrigation intervals or between different amounts of seaweed extract and chitosan

significantly. In both seasons, severe drought stress resulted in the highest increase in root length and the greatest decrease in the previously listed plant growth traits. The estimated percentages of decrement in plant height, number of branches per plant, leaf area, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were 47.79 and 41.91%, 52.04 and 64.61%, 63.28 and 63.69%, 59.39 and 60.20%, 60.53 and 59.02%, 55.01 and 56.54% and 62.84 and 61.66% and increment of root length under severe drought stress was 91.71 and 77.77% in contrast to the control treatment and for the first and second season, respectively.

Water shortage led to a decline in plant growth and development, which was shown as a reduction in cell volume, turgor, elongation, and division, and eventually cell growth (Banon *et al.*, 2006 and Shao *et al.* 2008); or it could be caused by a decrease in photosynthesis because a smaller leaf area makes it harder to trap light, causing an imbalance between light capture and use (Shao *et al.*, 2008). One of the plants' adaptation mechanisms for avoiding drought stress is assumed to reduce the leaf area and this is done by restricting evapotranspiration and reducing water use (Toscano *et al.*, 2014). Additionally, the impact of water deficit on plant development may result from insufficient moisture in the rhizosphere, which reduces nutrient absorption (Singh *et al.*, 1997). Additionally, the lack of water results in an excessive accumulation of ROS, which in turn damages proteins, lipids, and deoxyribonucleic acid through oxidative processes, eventually impairing growth (Ahmad and Haddad, 2011). As is known, when the soil is dry, the roots create more in-depth profiles of the soil, which results in an increase in root length. Yin *et al.*, (2005) stated that when water is scarce, fine root mass decreases significantly. Because the distribution of roots is comparable to the distribution of moisture, extending the watering interval causes a decrease in the fresh and dry weight of the roots (Kramer and Boyer, 1995). The obtained results were consistent with the results of El-Sabagh *et al.*

(2017) on canola plants, and Wang *et al.* (2019) and Patmi and Pitoyo (2020) on rice.

In terms of the main effect of different rates of seaweed extract and chitosan on plant growth traits, the data in both seasons (Table, 2) demonstrated that treating petunia plants with seaweed extract and chitosan had a favorable impact on increasing and improving plant growth parameters in comparison to the control. It is obvious that the high concentration of seaweed extract (6 ml/l) recorded the highest values of plant height, leaf area, shoot fresh and dry weights, root length, and root fresh and dry weights, compared to other treatments, in both seasons.

Generally, seaweed extract's ability to promote growth may be credited to its high content of growth-promoting hormones such as cytokinins (Durand *et al.*, 2003), auxins (Stirk *et al.*, 2004), gibberellins (Jennings, 1968), amino acids, micro and macronutrients, polysaccharides and osmolytes that may work synergistically at different concentrations in enhancing growth under abiotic stress (Khan *et al.*, 2009).

The extract of seaweeds contains auxins which promote cell growth and differentiation due to their impact on the release of hydrogen ions and the softening of cell walls, which facilitate cell expansion and the production of proteins and nucleic acids that promote cell division and increase cell density (Krikorian, 1970). Moreover, using seaweed extract under drought stress could increase the activity of antioxidant enzymes like peroxidase enzyme (POD), superoxide dismutase enzyme (SOD), and catalase enzyme (CAT), reduce malondialdehyde content (MDA), conductivity, and the rate of leaf dehydration, significantly increase relative water content, and relieve drought stress damage, leading to an increase in plant height and biomass (Mansori *et al.*, 2015). Besides this, seaweed promotes plant development by boosting nutrient uptake, carbohydrates, proteins, free amino acids, and polyphenols (Hernández-Herrera *et al.*, 2022). The aforementioned findings are much in line with those postulated by Li and

Mattson (2015) on petunia and tomato, Shehata and Walid (2019) on basil and Wally *et al.* (2020) on *Thymus vulgaris* L.

Chitosan is a natural polymer, has a broad range of uses in biotic and abiotic stress management strategies. Foliar application of chitosan lowers the stomatal conductance, reduces transpiration, and improves the effectiveness of water uptake by acting as an antitranspirant compound and promoting the production of jasmonic acid by influencing the water use of plants as abscisic acid results in stomatal closure, as has been reported (Bittelli *et al.*, 2001 and Iriti *et al.*, 2009).

Additionally, the stimulating effect of chitosan on plant development under water deficit may also be attributed to its capability to encourage nutrient and water uptake by changing cell osmotic pressure and decreasing free radicals by promoting antioxidant activity (Guan *et al.*, 2009). The obtained results of chitosan are in harmony with Kamal and Ghanem (2011) on *Physalis peruviana* L., Malekpoor *et al.* (2016) on basil and Waly *et al.* (2020) on *Thymus vulgaris* L.

The interaction between various irrigation intervals and various concentrations of seaweed extract and chitosan on plant growth parameters was significant during both seasons (2020/2021 and 2021/2022) (Table, 3). The statistical analysis, generally, showed that spraying petunia plants with 6 ml/l seaweed extract under 3 days of irrigation interval resulted in the highest mean values of plant height, number of branches per plant, leaf area, shoot fresh and dry weights, root fresh and dry weights. In contrast, the longest root was produced by the combination of irrigation every nine days and 6 ml/l seaweed extract. The estimated percentages increase in plant height, number of branches per plant, leaf area, shoot fresh and dry weights, root length, root fresh and dry weights were 27.72 and 13.14 %, 43.90 and 29.99%, 52.02 and 60.46%, 34.84 and 30.66%, 62.93 and 65.89%, 134.82 and 145.95%, 44.27 and 43.10% and 400 and 410.07% compared to the control treatment

and for the first and second seasons, respectively.

#### **Flowering characteristics:**

Regarding the primary impact of drought stress on flowering traits, the results in Table (4) revealed a negative correlation between flowering parameters and prolonging the interval between irrigations. Flowering parameters such as the number of days to flowering, flower longevity, flowering duration, flower diameter, the number of flowers per plant, flower fresh and dry weight decreased as the irrigation period increased. So, watering of plants every nine days produced the lowest mean values for flowering parameters, while irrigation every three days produced the highest mean values for these flowering parameters, in both seasons. The estimated percentages decreased in number of days to flowering, flower longevity, flowering duration, flower diameter, the number of flowers per plant, flower fresh and dry weight, were 19.71% and 19.98%, 50.59 and 53.91%, 55.56 and 57.24%, 38.83 and 39.20%, 66.25 and 67.29%, 37.5 and 45.57% and 70.83 and 73.91% compared to the control treatment and for the first and second seasons, respectively.

Flowers are very sensitive to water shortage, so the inhibition of flower growth traits under drought stress treatments would most likely result from exposure to harmful levels of drought, which would cause a drop in turgor, causing a decrease in growth and a reduction in cell division and elongation (Kareem *et al.*, 2017), which then leading to a decrease in flower diameter flower fresh and dry weights. Often stressed plants tend to shorten their life span and strive to finish their life cycles more quickly, which reduces the number of days needed to be flowering. Additionally, a lack of water causes plants to grow smaller, resulting in fewer places for bloom initiation and development (Guilioni *et al.*, 2003) which leads to a reduction in the number of flowers per plant and then shortens the flowering duration. Our results were

**Table 4. The main effect of different irrigation intervals and different levels of seaweed extract and chitosan on flowering growth parameters of *Petunia axillaris* plants during the 2020/2021 (1<sup>st</sup>) and 2021/2022 (2<sup>nd</sup>) seasons.**

Irrigation interval (days)	Number of days to flowering		Flower longevity (days)		Flowering duration (days)		Flower diameter (cm)		Flowers number per plant		Flower fresh weight (g)		Flower dry weight (g)	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
<b>D3</b>	100.80 a	101.73 a	11.07 a	11.13 a	106.67 a	106.33 a	6.67 a	6.53 a	21.33 a	21.00 a	0.80 a	0.79 a	0.24 a	0.23 a
<b>D6</b>	91.67 b	92.13 b	7.27 b	7.60 b	74.00 b	73.60 b	5.36 b	5.22 b	13.20 b	12.80 b	0.68 b	0.67 b	0.13 b	0.12 b
<b>D9</b>	80.93 c	81.40 c	5.47 c	5.13 c	47.40 c	45.47 c	4.08 c	3.97 c	7.20 c	6.87 c	0.50 c	0.43 c	0.07 c	0.06 c
<b>Irrigation intervals (days)</b>														
<b>Control</b>	86.89 e	87.22 e	7.00 d	7.11 d	72.44 e	71.44 e	4.86 e	4.72 e	10.89 e	11.22 e	0.59 e	0.53 c	0.12 e	0.10 e
<b>SWE 1</b>	89.22 d	89.78 d	7.56 c	7.44 cd	77.33 b	76.11 b	5.63 b	5.53 b	15.44 b	14.56 b	0.69 b	0.66 ab	0.16 b	0.15 b
<b>SWE 2</b>	90.89 c	91.44 c	7.78 c	7.89 bc	80.22 a	79.33 a	5.85 a	5.74 a	16.33 a	16.11 a	0.72 a	0.70 a	0.18 a	0.17 a
<b>CH 1</b>	93.11 b	93.56 b	8.33 b	8.22 b	74.11 d	73.67 d	5.11 d	5.00 d	12.89 d	12.44 d	0.63 d	0.61 b	0.13 d	0.12 d
<b>CH 2</b>	95.56 a	96.78 a	9.00 a	9.11 a	76.00 c	75.11 c	5.40 c	5.21 c	14.00 c	13.44 c	0.67 c	0.65 ab	0.14 c	0.13 c
<b>Seaweed extract and chitosan</b>														

Means were examined using Tukey's Honest Significant Difference test ( $P \leq 0.05$ ); n = 3

Means with the same letters do not differ significantly between different irrigation intervals or between different amounts of seaweed extract and chitosan

consistent with those of ˇCerekovi'c *et al.* (2013) on *Ribes nigrum* L., Al-Ubaydi *et al.* (2017) on okra and Salama *et al.* (2021) on quinoa plants.

Regarding the primary effect of different rates of seaweed extract and chitosan on flower parameters, data in Table (4) showed that foliar spraying petunia plants with any of the tested seaweed extract and chitosan levels, significantly enhanced the number of days to flowering, flower longevity, flowering duration, flower diameter, the number of flowers per plant, flower fresh and dry weight compared to control treatment during both seasons. Moreover, the treatment of seaweed extract (6 ml/l) and chitosan (6 ml/l) recorded the highest mean values for the aforementioned flowering characteristics. The estimated percentage of increase for the number of days to flowering and flower longevity were 9.98 and 10.96% and 28.57 and 28.13% for the treatment of 6 ml/l chitosan in both seasons, respectively. Likewise, the estimated percentage of increasing flowering duration, flower diameter, the number of flowers per plant, and flowers fresh and dry weights were 10.7 and 11.04%, 20.37 and 21.61%, 49.95 and 43.58%, 22.03 and 32.07% and 50 and 70% and it was recorded by 6 ml/l seaweed extract for the first and second seasons, respectively.

Generally, plants treated with seaweed extract exhibited an enhancement in flowering traits and this could be attributed to nutritional and hormonal components (zinc, Mg, K, and polyphenols ...) of seaweed extract, which serve as a catalyst for oxidative stress in plant cells, regulates sugar of intake, boosts the plant's energy, aid in the production of starch and carbohydrates (Jyung *et al.*, 1975). As the trigger and development of flowering and the number of flowers produced are related to the stage of plant development, seaweed extracts probably enhance flowering through the initiation of plant growth (Sarhan and Ismael, 2014).

Seaweed extract supplies plants with the required nutrients they need, such as potassium, phosphorus, and nitrogen, which

increases the level of amino acids and the creation of important proteins, improves plant readiness, and stimulates cell division and elongation, which increases flower diameter (Jyung *et al.*, 1975). The increase in flowering duration may be related to the increase in flower longevity and flower number. The cause behind increasing flower fresh and dry weight of flowers may be related to the presence of key growth-promoting chemicals that boost photosynthetic efficiency, promote vegetative and radical development, and are reflected favorably in an increase in carbohydrate intake (Al-Khuzayy and Al-Asadi, 2019). Current results of seaweed extract were consistent with those described by Emam *et al.* (2016) on *Calendula officinalis* L.; Al-Khuzayy and Al-Asadi (2019) on narcissus; Al-Shatri *et al.* (2020) on strawberry; Ayyat and Abdel-Mola (2020) on *Tagetes patula*; Alhasan *et al.* (2021) on gerbera and Salama *et al.* (2021) on quinoa.

The beneficial impacts of chitosan on flowering in petunia plants can be linked to its involvement in boosting nutrient availability, and water absorption by altering cell osmotic pressure, protein synthesis, cell development and enzymes (Kisvarga *et al.*, 2022) which resulted in vigorous plants by enhancing vegetative development and then actively transporting photosynthetic products from the source to flowering organs, which leads to a reduction in the C/N ratio, then producing more flowers and enhancing flower diameter, flower longevity and flower fresh and dry weights (Limpanavech *et al.*, 2008). Also, chitosan treatment encourages the enzymatic mechanisms that control many essential physiological and biochemical procedures which accelerate flowering (Hadwiger, 2013 and Sharma *et al.*, 2019). The increase in flower longevity and flowers number may be responsible for the increase in flowering duration. These results agree with those reported by Ramos-García *et al.* (2009) on gladiolus; Sultana *et al.* (2017) and Parvin *et al.* (2019) on tomato; Ayyat and Abdel-Mola (2020) on *Tagetes patula* and Akhtar *et al.* (2022) on *Calendula officinalis* L.

During both seasons, there was a significant interaction among the various treatments on flowering characteristics (Table, 5). According to the statistical analysis, the highest mean values of number of days to flowering and flower longevity were achieved at the combined treatment between 3-days irrigation and 6 ml/l chitosan. Also, the highest mean values of flowering duration, flower diameter, the number of flowers per plant, flower fresh and dry weights of petunia were obtained with this combined treatment in both seasons. The anticipated percentage increase in number of days to flowering, flower longevity, flowering duration, flower diameter, the number of flowers per plant, and flower fresh and dry weights were 11.80 and 13.89%, 16.17 and 23.3%, 7.742 and 8.43%, 15.24 and 16.58%, 39.62 and 37.07%, 10.53 and 10.67% and 40 and 44.44% respectively, compared to the control treatment. However, the lowest mean values of flowering parameters of petunia were achieved in plants irrigated every 9-days without any treatment of chitosan or seaweed in both seasons.

#### **Leaf chemical contents:**

#### **SPAD index, total leaf carbohydrate, proline, and electrolyte leakage:**

Regarding the primary impact of water deficit on the SPAD index, total leaf carbohydrate, proline content, and electrolytic leakage of petunia plants, the data in (Table, 6) revealed that SPAD index and total leaf carbohydrate content were significantly decreased with decreasing levels of irrigation up to the lowest one in both seasons. As opposed to that, both proline content and electrolytic leakage (%) greatly increased as drought got worse in both spring and summer. The estimated percentage decreases of the SPAD index and total leaf carbohydrate content were 20.83 and 19.56% and 58.91 and 59.34%, for the first and second seasons respectively. The projected percentages of increase in proline content and electrolytic leakage % were 84.27 and 91.8% and 93.82 and 92.92% in comparison to the control

treatment for the first and second seasons, respectively.

Under drought stress, the presence of active forms of oxygen causes oxidative stress, which damages numerous cellular constituents like photosynthesis, proteins, and carbohydrates (Jaleel *et al.*, 2007). The amount of chlorophyll may decrease under drought stress, because of decreased cell division and elongation, increased leaf senescence because of decreased turgor pressure, and decreased leaf area (Shao *et al.*, 2008). Smaller leaf areas have a lower light-trapping ability, so there was an imbalance in how much light was captured and used, which caused the rate of photosynthesis to decrease (Shao *et al.*, 2008). Additionally, a lack of water damages metabolism and closes stomata, which has a bad impact on photosynthesis. The excessive photosynthetic electron chain reduction caused by stomatal closure might lead to an increase in the formation of reactive oxygen species (ROS), like superoxide anion ( $O_2^-$ ), which forms  $H_2O_2$ ,  $OH^-$ , and other ROS (Iannone *et al.*, 2009). Moreover, stomatal closure may result in a decline in leaf  $CO_2$  concentration, which may then cause a decrease in the concentration of  $NADP^+$  available to accept electrons from PSI and/or PSII and so generation of ROS, such as  $H_2O_2$ . The stomata of leaves with a modest water deficit open more slowly in the light and close more quickly in the dark (Nuruddin *et al.*, 2003). The results obtained are consistent with the findings of Almohisen (2015) on tomato plants, and Khatiby *et al.* (2016) on sesame.

Drought stress lowers total leaf carbohydrate content owing to its inhibitory influence on the concentrations of photosynthetic pigments and photosynthetic rate, which led to a decline in the content of photo-assimilates consequently causing a decrease in carbohydrates (Neslihan-Ozturk *et al.*, 2002 and Liu *et al.*, 2004). Under water deficit conditions, the disintegration of polysaccharides resulted in the buildup of osmolytes like soluble sugars, which assisted the plants in maintaining cell turgor (Nazarli

**Table 5. The interaction effect between different irrigation intervals and different levels of seaweed extract and chitosan on flowering growth parameters of *Petunia axillaris* plants during the 2020/2021 (1<sup>st</sup>) and 2021/2022 (2<sup>nd</sup>) seasons.**

Irrigation interval (days)	Number of days to flowering		Flowering duration (days)		Flower diameter (cm)		Flowers number per plant		Flower fresh weight (g)		Flower dry weight (g)			
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>		
<b>Control</b>	96.00 e	96.00 e	10.33 c	10.00 c	103.33 d	102.67 e	6.17 e	6.03 e	17.67 e	18.00 e	0.76 d	0.75 abc	0.20 abc	0.18 d
<b>SWE 1</b>	97.33 d	98.00 d	10.33 c	10.67 bc	108.67 b	107.67 b	6.93 b	6.83 b	23.33 b	22.00 b	0.82 b	0.82 a	0.26 a	0.25 a
<b>SWE 2</b>	99.67 c	101.00 c	11.00 bc	11.33 b	111.33 a	111.33 a	7.11 a	7.03 a	24.67 a	24.67 a	0.84 a	0.83 a	0.28 a	0.26 a
<b>CH 1</b>	103.67 b	104.33 b	11.67 ab	11.33 b	103.67 d	104.33 d	6.43 d	6.28 d	19.67 d	19.33 d	0.79 c	0.78 ab	0.21 ab	0.21 c
<b>CH 2</b>	107.33 a	109.33 a	12.00 a	12.33 a	106.33 c	105.67 c	6.70 c	6.47 c	21.33 c	21.00 c	0.81 b	0.80 ab	0.23 ab	0.22 b
<b>Control</b>	87.33 i	87.67 i	6.33 fg	6.67 fg	71.33 h	71.00 h	4.77 j	4.65 j	9.67 i	10.00 j	0.64 i	0.63 de	0.10 def	0.08 i
<b>SWE 1</b>	90.67 h	91.00 h	6.67 f	7.00 f	74.67 f	74.67 f	5.68 g	5.57 g	15.00 f	14.33 g	0.70 f	0.69 cd	0.15 bcde	0.14 f
<b>SWE 2</b>	91.67 g	92.00 g	7.00 ef	7.33 ef	76.67 e	75.33 f	5.88 f	5.77 f	15.67 f	15.33 f	0.73 e	0.71 bcd	0.16 bcd	0.16 e
<b>CH 1</b>	93.33 f	93.67 f	7.67 e	8.00 e	73.33 g	72.67 g	5.07 i	4.99 i	12.33 h	11.67 i	0.67 h	0.66 d	0.11 cdef	0.10 h
<b>CH 2</b>	95.33 e	96.33 e	8.67 d	9.00 d	74.00 fg	74.33 f	5.41 h	5.13 h	13.33 g	12.67 h	0.68 g	0.67 cd	0.12 cdef	0.12 g
<b>Control</b>	77.33 n	78.00 n	4.33 i	4.67 i	42.67 l	40.67 l	3.63 o	3.47 o	5.33 m	5.67 n	0.38 n	0.47 g	0.05 f	0.04 m
<b>SWE 1</b>	79.67 m	80.33 m	5.67 gh	4.67 i	48.67 j	46.00 j	4.29 l	4.18 l	8.00 jk	7.33 l	0.55 k	0.57 ef	0.08 def	0.066 jk
<b>SWE 2</b>	81.33 l	81.33 l	5.33 h	5.00 i	52.67 i	51.33 i	4.55 k	4.43 k	8.67 j	8.33 k	0.59 j	0.41 g	0.09 def	0.07 ij
<b>CH 1</b>	82.33 k	82.67 k	5.67 gh	5.33 hi	45.33 k	44.00 k	3.83 n	3.73 n	6.67 l	6.33 mn	0.44 m	0.49 fg	0.06 ef	0.05 lm
<b>CH 2</b>	84.00 j	84.67 j	6.33 fg	6.00 gh	47.67 j	45.33 j	4.10 m	4.04 m	7.33 kl	6.67 lm	0.51 l	1.19 m	0.07 ef	0.06 kl

Means were examined using Tukey's Honest Significant Difference test ( $P \leq 0.05$ );  $n = 3$

Means with the same letters do not differ significantly between different irrigation intervals or between different amounts of seaweed extract and chitosan

**Table 6. The main effect of different irrigation intervals and different levels of seaweed extract and chitosan on chemical characters of *Petunia axillaris* plants during the 2020/2021 (1<sup>st</sup>) and 2021/2022 (2<sup>nd</sup>) seasons.**

Irrigation intervals (days)	Total leaf chlorophyll content (SPAD index)		Total leaf carbohydrate (% of dry weight)		Proline (mg/g f.w.)		Electrolyte leakage (%)	
	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
<b>D3</b>	49.63 a	48.47 a	4.94 a	4.82 a	39.09 c	37.07 c	21.36 c	20.91 c
<b>D6</b>	42.48 b	42.40 b	3.46 b	3.40 b	61.30 b	60.58 b	30.65 b	29.74 b
<b>D9</b>	39.29 c	38.99 c	2.03 c	1.96 c	72.03 a	71.10 a	41.40 a	40.34 a
<b>Irrigation intervals (days)</b>								
<b>Control</b>	41.09 e	40.79 e	2.87 e	2.79 e	63.63 a	62.70 a	35.51 a	34.40 a
<b>SWE 1</b>	42.25 d	41.98 d	3.69 b	3.62 b	54.36 d	52.46 d	28.76 d	28.36 d
<b>SWE 2</b>	43.43 c	42.81 c	3.99 a	3.87 a	50.55 e	49.55 e	26.84 e	26.36 e
<b>CH 1</b>	44.65 b	44.21 b	3.33 d	3.26 d	60.41 b	59.45 b	33.13 b	32.45 b
<b>CH 2</b>	47.58 a	46.64 a	3.51 c	3.42 c	58.40 c	57.09 c	31.44 c	30.08 c
<b>Seaweed extract and chitosan</b>								

Means were examined using Tukey's Honest Significant Difference test ( $P \leq 0.05$ ); n = 3

Means with the same letters do not differ significantly between different irrigation intervals or between different amounts of seaweed extract and chitosan

*et al.*, 2011), and this is believed to be an adaptive reaction to drought stress circumstances. The obtained results are consistent with Ali and Ashraf (2011) on maize and Khalil and Badr Eldin (2021) on grapevines.

By accumulating osmolytes, plants may partially protect themselves against modest drought stress. Proline is one of the most common appropriate osmolytes in drought-stressed plants. Its buildup may represent a plant's defense against water stress. Additionally, it assists in stabilizing subcellular structures (such as proteins and membranes) and neutralizing free radicals (Hayat *et al.*, 2012 and Huang *et al.*, 2014). Our findings concurred with those of Khatiby *et al.* (2016) on sesame and Ali *et al.* (2022) on okra.

Because the cell membrane is the first part of a cell to be damaged by drought stress, the electrolyte leakage is increased (Inze and Van Montagu, 1995) and cell membranes may become more porous, leading to increase electrolyte leakage (Almeselmani *et al.*, 2015). Due to oxidative stress brought on by drought stress, plant cells produce and store more reactive oxygen species. As a result, the fatty acids in cell walls oxidize, making the cell wall less stable (Inze and Van Montague, 1995). The outcomes from the present study are consistent with those reported by Khatiby *et al.* (2016) on sesame and Mogazy *et al.* (2020) on *Lupinus albus* L.

Regarding the primary impacts of the varying concentrations of seaweed extract and chitosan on SPAD index, total leaf carbohydrate, proline content, and electrolytic leakage % of petunia plants, data in Table (6) indicated that treating petunia plants with seaweed extract and chitosan exhibited a considerably higher SPAD index and total leaf carbohydrate, and significantly reduced proline and electrolyte leakage compared to the control in both seasons. The SPAD index's highest mean value (47.58 and 46.64) was observed at 6 ml/l chitosan for the first and second seasons, respectively. The highest mean value of total leaf carbohydrates (3.99

and 3.87%) was shown at a seaweed extract concentration of 6 ml/l for the first and second seasons, respectively, in comparison to other treatments. Also, the highest mean value of proline content and electrolyte leakage (63.63 and 62.70 mg g<sup>-1</sup>fw) and (35.51 and 34.40%) was estimated in control plants for both seasons. However, 6 ml/l seaweed extract was shown to have the lowest mean proline concentration and electrolyte leakage in both seasons.

Generally, the higher effect of seaweed extract treatments in promoting plant photosynthetic pigments may be as a result of seaweed extract supplies plants with a variety of elements, including phytohormones, nutrients, polymers, and betaines, many of which may work synergistically (Jannin *et al.*, 2013). Furthermore, seaweed extracts not only provide cytokinins but also promote their endogenous production. (Wally *et al.*, 2013). Also, Chloroplasts are protected by cytokinins. (Zavaleta-Mancera *et al.*, 2007) and consequently, they affect chlorophyll content. Also, seaweed extract is rich in glycine betaine which prevents chlorophyll breakdown and delays the loss of photosynthetic activity (Shankar *et al.*, 2015). The outcomes are consistent with Seif *et al.* (2016) on snap bean and Mostafaei *et al.* (2018) on Indian mustard.

The advantageous impact of seaweed extract on carbohydrate content could be attributed to the higher nutrient content of seaweed extract, particularly magnesium, as well as amino acids and vitamins, which enhanced the production of plant pigments and total carbohydrates (Deolu-Ajayi *et al.*, 2022). The obtained results are in harmony with those reported by El-Alsayed *et al.* (2018) on dahlia plants and Mogazy *et al.* (2020) on *Lupinus albus* L.

The substantial concentration of antioxidant chemicals in seaweed extract may be the cause of the drop in proline levels caused by foliar application of seaweed extract (Corsetto *et al.*, 2020). Additionally, systems and associates discussed a potential mechanism of action for seaweed extracts that

entailed the modification of genes involved in the stress response, including those in charge of pigment synthesis and plant antioxidant response (EL Boukhari *et al.*, 2020). Also, the reduction of proline content after seaweed extract application may be due to the increment of soluble sugars which serves as osmo-protectants. The obtained results are in agreement with Campobenedetto *et al.* (2021) on tomato and Jafarlou *et al.* (2023) on *Calotropis procera*.

The application of seaweed extract considerably reduced the electrolyte leakage caused by water stress, demonstrating that seaweed has a crucial function in keeping the membrane integrity of cells of petunia plants. the reduction in electrolyte leakage due to seaweed extract treatments can be attributed to the role of seaweed extract in improving water use efficiency, increasing leaf water content, and improving drought stress tolerance (Rasul *et al.*, 2021). Also, the decrease in electrolyte leakage supported the reactive oxygen species (ROS) scavenging mechanism being activated in petunia plants because of the seaweed extract treatment. These outcomes appeared to be in alignment with those reported by Esmailpour *et al.* (2020) on basil and Mogazy *et al.* (2020) on *Lupinus albus* L.

The greater impact of chitosan treatments in encouraging plant photosynthetic pigments results from enhancing endogenous levels of cytokinins, which encourage chlorophyll production and development, or by making the amino compounds that chitosan releases more readily available (Chibu *et al.*, 2002). Furthermore, it might be brought on by the N content of chitosan, which is crucial for the formation of the chlorophyll tetrapyrrole ring (Behboudi *et al.*, 2019). The results are in agreement with Liaqat *et al.* (2019) on eggplant and Waly *et al.* (2020) on *Thymus vulgaris* L.

The increment of carbohydrates caused by the use of chitosan treatments may be attributable to chitosan's ability to increase photosynthetic pigments, which in turn stimulates photosynthetic activity and

carbohydrate accumulation (Bahloul, 2021). Current results are in harmony with those reported by Waly *et al.* (2020) on *Thymus vulgaris* L. and Khalil and Eldin (2021) on grapevines.

The positive effect of chitosan in reducing proline content and electrolyte leakage because of chitosan could be crucial in preserving plasma membrane integrity, controlling water pressure, and increasing the relative water content, further lessening oxidative stress, which reduces lipid peroxidation (Boyer, 1988 and Ahmed *et al.*, 2016). These results agreed with the findings of Hafez *et al.* (2020) on barley and Mulaudzi *et al.* (2022) on sorghum.

The effect of interaction between the varied irrigation intervals and varying levels of seaweed extract and chitosan on SPAD index, total leaf carbohydrate, proline content, and electrolytic leakage of petunia plants, were significant during both seasons (Table, 7). The results indicated that the highest mean values of SPAD index (58.59 and 56.02) were attained with the treatment of 3-day irrigation and 6 ml/l chitosan. The highest content of total leaf carbohydrate (5.75 and 5.46%) was achieved in plants irrigated every three days and treated with 6 ml/l seaweed extract for the first and second seasons, respectively. Conversely, the highest proline content and electrolytic leakage % for the first and second seasons (78.95 and 77.30 mg/g f.w. and (47.17 and 44.90%, respectively) were achieved in plants irrigated every 9-days without any growth stimulants but the lowest mean values of proline content and electrolytic leakage % (27.30 and 25.85 mg/g f.w. and 16.76 and 16.42%) were achieved at the combined treatment between irrigation every three days and the application with 6 ml/l seaweed extract for the first and second seasons, respectively.

#### **Cross-correlation analysis between petunia traits:**

To elucidate the association among the estimated nineteen traits, Pearson's correlation coefficients were analyzed (Fig., 1). Traits

**Table 7. The interaction effect between different irrigation intervals and of seaweed extract and chitosan on chemical characters of *Petunia axillaris* plants during the 2020/2021 (1<sup>st</sup>) and 2021/2022 (2<sup>nd</sup>) seasons.**

Irrigation intervals (days)	Treatments	Total leaf chlorophyll content (SPAD index)		Total leaf carbohydrate (% of dry weight)		Proline (mg/g f.w.)		Electrolyte leakage (%)	
		1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>	1 <sup>st</sup>	2 <sup>nd</sup>
<b>D3</b>	Control	44.81 e	44.24 e	4.31 e	4.26 e	46.86 k	45.96 k	24.75 k	24.31 k
	SWE 1	45.82 d	45.60 d	5.15 b	5.07 b	35.93 n	31.13 n	19.36 n	19.16 n
	SWE 2	48.27 c	46.79 c	5.75 a	5.46 a	27.30 o	25.85 o	16.76 o	16.42 o
	CH 1	50.65 b	49.70 b	4.63 d	4.60 d	43.98 l	42.98 l	23.54 l	22.86 l
	CH 2	58.59 a	56.02 a	4.87 c	4.70 c	41.35 m	39.42 m	22.39 m	21.81 m
	Control	41.50 ij	41.67 i	2.69 j	2.61 j	65.10 f	64.83 f	34.60 f	33.98 f
<b>D6</b>	SWE 1	42.13 hi	41.90 i	3.72 g	3.68 g	58.14 i	57.68 i	28.08 i	27.40 i
	SWE 2	42.50 gh	42.45 h	3.89 f	3.86 f	56.80 j	55.80 j	26.84 j	26.41 j
	CH 1	42.97 fg	42.83 g	3.41 i	3.31 i	63.84 g	63.06 g	32.61 g	32.07 g
	CH 2	43.30 f	43.17 f	3.62 h	3.56 h	62.61 h	61.53 h	31.10 h	28.83 h
	Control	36.95 n	36.47 n	1.61 o	1.49 o	78.95 a	77.30 a	47.17 a	44.90 a
	SWE 1	38.79 m	38.43 m	2.20 l	2.12 l	69.01 d	68.56 d	38.84 d	38.52 d
<b>D9</b>	SWE 2	39.52 l	39.21 l	2.33 k	2.30 k	67.54 e	67.01 e	36.93 e	36.25 e
	CH 1	40.34 k	40.10 k	1.96 n	1.87 n	73.40 b	72.30 b	43.25 b	42.43 b
	CH 2	40.87 jk	40.73 j	2.04 m	2.01 m	71.23 c	70.32 c	40.83 c	39.61 c
	Control	36.95 n	36.47 n	1.61 o	1.49 o	78.95 a	77.30 a	47.17 a	44.90 a

Means were examined using Tukey's Honest Significant Difference test ( $P \leq 0.05$ ); n = 3

Means with the same letters do not differ significantly between different irrigation intervals or between different amounts of seaweed extract and chitosan

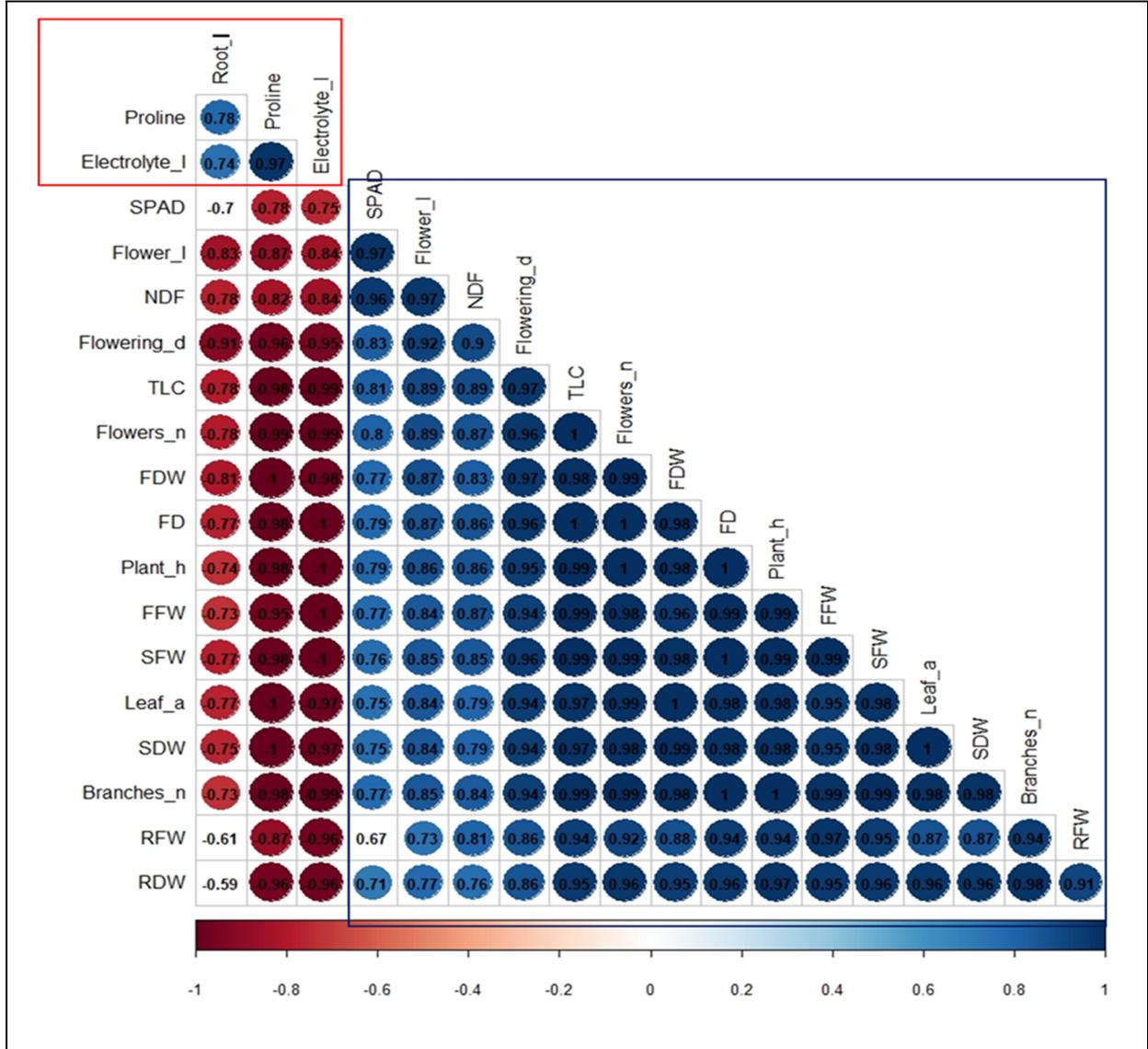


Fig. 1. Pairwise Pearson correlation matrix of the nineteen estimated traits under drought, chitosan, and seaweed extract treatments. The measured traits are vegetative growth traits [plant height (Plant\_h), number of branches per plant (Branches\_n), leaf area (Leaf\_a), shoot fresh weight per plant (SFW) and shoot dry weight per plant (SDW), root length (Root\_l), root fresh weight per plant (RFW) and root dry weight per plant (RDW)], flowering growth traits [ flowering duration (Flowering\_d), flower diameter (FD), the number of flowers per plant (Flowers\_n), flower fresh weight (FFW) and flower dry weight (FDW)], and leaves chemical traits [ SPAD index (SPAD), total leaf carbohydrate (TLC), proline content (Proline), and electrolytic leakage (Electrolyte\_l)].

were separated into two negatively correlated groups based on their significant correlation. The first group including root length (Root\_1), electrolyte leakage (Electrolyte\_1), and proline (Proline). However, the second group encompasses the rest of estimated traits e.g. vegetative growth traits [plant height (Plant\_h), number of branches/plant (Branches\_n), leaf area (Leaf\_a), shoot fresh weight per plant (SFW) and shoot dry weight per plant (SDW), root length (Root\_1), root fresh weight per plant (RFW) and root dry weight per plant (RDW)], flowering growth traits [flowering duration (Flowering\_d), flower diameter (FD), the number of flowers per plant (Flowers\_n), flower fresh weight (FFW) and flower dry weight (FDW)], and leaf chemical traits [SPAD index (SPAD) and total leaf carbohydrate (TLC)]. Clearly, the traits of the first group were positively linked with drought. On contrary, the second group traits represent morphological readout of drought adaptation, and hence positively increased by chitosan and seaweed extract. For instance, proline was positively associated with Electrolyte\_1 ( $r = 0.97$ ) and Root\_1 ( $r = 0.78$ ), but negatively correlated with SPAD ( $r = 0.78$ ), Leaf\_a ( $r = 0.98$ ), as well as the other traits in the second group (Fig., 1). The data revealed that petunia upon drought stress prioritizes defense overgrowth and development, and chitosan and seaweed extract alleviate the negative impacts of stress.

## CONCLUSION

Considering what the current study's findings show, water stress caused by prolonging the irrigation intervals had a negative effect on the growth and development of petunia plants. The growth characteristics and chemical composition of petunia plants significantly increased when seaweed extract and chitosan were applied during drought stress. Consequently, this study offered some proof of the possibility of utilizing seaweed extract and chitosan, especially at 6 ml/l for enhancing the growth and quality of *P. axillaris* plants under drought stress. So, the treatments used in the

current study will increase the economic value of petunia plants and will pave the way for the expansion of petunia usage for cultivation in coastal areas and new cities in light of the scarcity of water.

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### تأثير الرش ببعض محفزات النمو الآمنة على نمو وإزهار نبات البيتونيا (*Petunia axillaris*) تحت ظروف إجهاد الجفاف

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أجريت الدراسة الحالية تحت ظروف الصوب البلاستيكية بمزرعة كلية الزراعة، جامعة دمنهور، محافظة البحيرة، جمهورية مصر العربية خلال الموسمين المتعاقبين ٢٠٢٠/٢٠٢١ و ٢٠٢١/٢٠٢٢. كان الهدف من هذا العمل هو تقييم تأثير محفزين آمنين للنمو: مستخلص الطحالب البحرية والشيتوزان؛ كلاهما بتركيزات ٠ و ٣ و ٦ مل/لتر على النمو الخضري

والنمو الزهري والمحتوى الكيماوي لأوراق نبات البيتونيا النامية تحت ظروف إجهاد الجفاف. أظهرت النتائج المتحصل عليها للموسمين أن إجهاد الجفاف كان له آثار ضارة معنوية على النمو الخضري والنمو الزهري والمحتوى الكيماوي للأوراق، بينما زاد من محتوى البرولين والتسرب الإلكتروليتي. بشكل عام، كان لمستخلص الطحالب البحرية والشيتوزان تأثيرات معنوية على الصفات المدروسة. على سبيل المثال، أظهرت معاملات النمو والتي شملت ارتفاع النبات وعدد الأفرع النباتية لكل نبات ومساحة الورقة والوزن الرطب والجاف للنبات وكذلك طول الجذر ووزن الجذر الطازج والجاف للنبات أعلى القيم من خلال الرش الورقي لمستخلص الطحالب البحرية بمعدل ٦ مل/لتر مقارنة بالمعاملات الأخرى قيد الدراسة. وفي نفس الإتجاه كان هناك تحسن في صفات الإزهار مثل طول مدة الإزهار وقطر الزهرة وعدد الأزهار لكل نبات وأوزان الزهرة الرطب والجاف، وكذلك التركيب الكيميائي للأوراق بما في ذلك الكربوهيدرات الكلية للأوراق من خلال استخدام مستخلص الطحالب البحرية بمعدل ٦ مل/لتر. على العكس من ذلك، فقد نتج عن استخدام مستخلص الطحالب البحرية أقل قيم لمحتوى البرولين والتسرب الإلكتروليتي. فيما يتعلق بمعامله الشيتوزان، فقد أظهر تركيز ٦ مل/لتر من المحلول أعلى قيم لعدد الأيام حتى تزهر النباتات وطول عمر الزهرة ومحتوى النبات من الكلورفيل (SPAD index) مقارنة بالمعاملات الأخرى. تم التعبير عن جميع الصفات المدروسة باستخدام تحليل الارتباط المتبادل.