

## Investigating the Ability of Olive Leaf Extract to Enhance Growth and Physio-Biochemical Performance of Faba Bean Plants Under Salt Stress Conditions

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### ARTICLE

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### Abstract:

A preliminary germination trial determined that 5000 ppm NaCl is the threshold above which germination percentage and performance drop below 50% of control values as the maximum salinity tolerable for faba bean plants. Subsequently, two pot experiments were conducted during the winter seasons of 2020/21 and 2021/22 to assess the negative effects of 5000 ppm NaCl on the growth and physio-biochemical performance of faba bean plants, as well as the efficacy of OLE in mitigating these negative effects in NaCl-stressed plants. The results showed that salinity at 5000 ppm NaCl adversely affected faba bean growth, resulting in significant decreases in root system size, stem length, leaf and branch numbers, total leaf area, and the fresh and dry weights of roots, stems, and leaves. It also reduced concentrations of photosynthetic pigments, total carbohydrates, crude protein, and minerals (N, P, K, Ca, Mg), as well as the K<sup>+</sup>/Na<sup>+</sup> ratio. Conversely, leaf area ratio (LAR), free amino acids, proline, polyphenols, and Na<sup>+</sup> concentrations increased compared to control plants. Treating seeds with 0.1% and 0.2% ALE before NaCl stress exposure effectively mitigated salinity's negative effects, resulting in improved growth parameters, significantly increased levels of various bioconstituents and mineral nutrients, and reduced Na<sup>+</sup> levels along with an enhanced K<sup>+</sup>/Na<sup>+</sup> ratio compared to the control in both seasons, with 0.2% OLE being the most effective. This study recommends the application of 0.2% OLE as a strong biostimulant to enhance the growth and physiological performance of faba beans under stressful conditions.

### INTRODUCTION

Globally, sustainable agriculture and human nutrition face many challenges due to growing environmental stresses and climate change. Legumes can significantly address these issues. As a key protein source in human diets, they offer health benefits and account for about 50% of the total seed legumes consumed (Broughton *et al.*, 2003). However, legumes' economic, nutritional, and ecological benefits are often hindered by their sensitivity to environmental stresses, which can significantly reduce crop production by more than half (Wang *et al.*, 2003).

Faba bean (*Vicia faba* L.) is one of the most important legume crops, cultivated for human

consumption, especially in the Middle East, including Egypt (Zhou *et al.*, 2018). It is a valuable source of protein for both human consumption and animal feed, providing essential amino acids, although it has a low concentration of sulfur-amino acids. Therefore, increasing faba bean production is one of the most important targets of agricultural policy in Egypt (Taie *et al.*, 2013).

In Egypt, cultivated lands are mainly located in the Nile Valley, accounting for approximately 4% of the total land area. Many cultivated areas are affected by salt accumulation due to the seawater intrusion which salinizes water resources and lands (Filipović *et al.*, 2020). Due

to limited freshwater resources (relying on the Nile River) and inadequate cultivated lands to meet the food needs of Egypt's growing population, it is necessary to expand the cultivation of various crops such as faba bean to newly reclaimed lands, which are mainly irrigated with saline underground water. Consequently, salinity poses a remarkable challenge for both traditional agricultural regions and newly reclaimed lands (Ahmed, 2005).

Salinity adversely affects plants at all growth stages, from seed germination to maturity, causing substantial yield losses (Ashraf *et al.*, 2008; Sakr *et al.*, 2014). It impacts plants through osmotic stress, toxic ion accumulation, and reactive oxygen species (ROS) generation (Khan *et al.*, 2013). Osmotic stress causes water deficiency in plant cells, leading to smaller leaves and stomatal closure, thus hindering photosynthesis and plant growth (Roy *et al.*, 2014). Ionic imbalance leads to excessive  $\text{Na}^+$  and  $\text{Cl}^-$  accumulation in leaves, resulting in premature senescence (Munns and Tester, 2008; Roy *et al.*, 2014) and hindering the absorption of essential ions like  $\text{K}^+$ ,  $\text{Ca}^{2+}$ , and  $\text{Mn}^{2+}$ , which further impairs photosynthesis and enzyme activities (Muchate *et al.*, 2016). Salinity-induced stress increases ROS production, which damages cell and organelle membranes and can ultimately lead to cell and plant death under severe conditions (Hasanuzzaman *et al.*, 2021). Additionally, plant growth rates are influenced by essential processes such as cell division, enlargement, differentiation, and various genetic, morphological, physiological, and ecological factors, all of which are significantly affected by abiotic stressors (Taize *et al.*, 2014).

Despite this, plants possess effective antioxidant defense mechanisms, including both enzymatic and non-enzymatic antioxidants, to confront oxidative stress and mitigate the harmful effects of abiotic stresses (Apel and Hirt, 2004). The efficacy of these mechanisms influences the level of damage caused by abiotic stress (Sevengor *et al.*, 2011; Rady *et al.*, 2018). However, these internal antioxidant systems are often inadequate for healthy plant growth, requiring external support, such as plant extracts, to withstand abiotic stress (Wanas, 2006; Desoky *et al.*, 2018; Wanas *et al.*, 2018).

Many plant extracts are nowadays used as biostimulants in agriculture not only to improve plant growth and productivity but also to enhance plant tolerance to abiotic stresses due to their richness in osmoprotectants, antioxidants, and essential nutrients (Wanas *et al.*, 2018; Desoky *et al.*, 2018 & 2019).

In this regard, olive tree (*Olea europaea* L.) leaves are a renewable and rich source of bioactive compounds that offer antioxidant properties, particularly phenolic compounds (Bouaziz *et al.*, 2008). The main polyphenol found in olive leaves is Oleuropein, consisting of oleonic acid and hydroxytyrosol. It can convert to hydroxytyrosol, known for its powerful bioactive properties due to the catechol group (Ranalli *et al.*, 2006; Kourti *et al.*, 2024). Olive leaves also contain tyrosol, hydroxytyrosol, caffeic acid, gallic acid, syringic acid, coumaric acid, and luteolin (Soler-Rivas *et al.*, 2000; Korukluoglu *et al.*, 2004). Moreover, they are rich in phytohormones like  $\text{GA}_3$ ,  $\text{GA}_4$ , and zeatin, as well as essential elements like Ca and Fe (Ulgar *et al.*, 2004). The presence of these bioactive substances in olive leaves, especially phenolic compounds and zeatin, make their extract a powerful biostimulant for enhancing plant growth and productivity, even under stressful conditions. To our knowledge, the use of olive leaf extract (OLE) to alleviate abiotic stress on plants is scarce. Thus, this study aims to evaluate the potential effects of OLE application on the growth, physiological, anatomical, and yield characteristics of faba beans under salt-stress conditions.

## Materials and Methods

Natural substitutes are progressively being incorporated into current agricultural systems, often known as green agri-technology, to boost plant yield and diminish contamination of edible plant parts. This study aimed to examine the efficacy of olive leaf extract (OLE) in promoting the growth and productivity of faba bean plants growing under salt-stress conditions. Faba bean (*Vicia faba* L.) cultivar Sakha-1 was used as a botanical material in this investigation, with seeds obtained from the Directorate of Agriculture in Damietta, Egypt. To accomplish the aim of this study, it includes the following experiments:

### a) A preliminary experiment:

In this experiment, faba bean seeds were exposed to a succession of NaCl salinity levels from 1000 to 7000 ppm in 1000 ppm increments, with tap water as a control, to determine the level beyond which the germination percentage declines below 50% of the control value. This threshold is considered the maximum salinity tolerable level for faba bean plants, according to the proposition Wanas (1996), and will be employed in the main experiment. To achieve the purpose of this experiment, seeds were washed with distilled water, disinfected for 2 minutes in a 1% sodium hypochlorite solution, and then completely rinsed with distilled water

before sowing. Thirty seeds were allocated to each salinity level and tap water (control). Seeds of each treatment were planted in 15 plastic pots, 7 cm in diameter, filled with a mixture (1:1 v/v) of sand and peat moss (2 seeds pot<sup>-1</sup>). The experiment was set up in a completely randomized block design, with eight treatments and three duplicates, each containing five pots. Irrigation was done in equal amounts for all pots with the specified salinity level. On October 15th, 2020, germination was conducted at a laboratory temperature of 25± 2 °C at the Botany Department, Faculty of Agriculture, Damietta University.

Counts of germinated seeds were recorded every day from day 3 to day 10 of the experiment; a seed was considered germinated when the plumule emerged above the soil surface. The formulae to calculate the germination percentage (GP) and mean rate of germination (MRG) are as follows:

- a) Germination percentage (GP) (Tanaka-Oda, *et al.*, 2009) =  $\frac{\text{Number of germinated seeds}}{\text{Total number of sown seeds}} \times 100$   
 b) Mean rate of Germination (MRG) GR (Edwards and Sundstrom, 1987) =  $\frac{\sum T_n N_n}{\sum N_n}$ .

MGR is expressed as the mean number of days needed for germination, where  $T_1$  represents the number of days passed from sowing to the first count,  $T_2$  represents the number of days passed from sowing to the second count, and  $N_1$  represents the number of germinated seeds at the first count,  $N_2$  represents the number of germinated seeds at the second count, and so on.

- c) Germination performance index (GPI) (Edwards and Sundstrom, 1987) =  $\frac{\text{Germination percentage}}{\text{MRG}}$

#### MRG

The preliminary experiment showed that faba bean cv. Sakha-1 can tolerate salinity levels up to 5,000 ppm which was utilized in the main experiments.

#### b) Main experiments:

Two pot experiments were executed on an outdoor experimental farm next to the Ras El-Bar power station in Damietta Governorate (31°28'16" N and 31°47'34" E), Egypt during two subsequent winter seasons (2020/21 and 2021/22) to investigate the effects of olive leaf extract (OLE) on growth and biochemical characteristics of faba bean plants grown under

salt-stress conditions. The experiment included the following four treatments to fulfill the study's objective:

- T<sub>1</sub>: Control (tap water)  
 T<sub>2</sub>: Salinity (NaCl at a concentration of 5000 ppm).  
 T<sub>3</sub>: OLE at a concentration of 0.1 % + NaCl at a concentration of 5000 ppm.  
 T<sub>4</sub>: OLE at a concentration of 0.2 % + NaCl at a concentration of 5000 ppm.

The salinity level (5000 ppm NaCl) was selected based on the preliminary experiment findings and was used as an irrigation solution for plants of T<sub>2</sub> – T<sub>4</sub>. The specified concentrations of OLE were used as soaking materials for faba bean seeds designated for T<sub>3</sub> and T<sub>4</sub> for 12 hours before sowing, whereas seeds allotted for control (T<sub>1</sub>) and salinity (T<sub>2</sub>) were soaked in tap water. These pre-soaked seeds were then planted in 30 cm diameter pots (3 seeds pot<sup>-1</sup>) filled with 10 kg clay and sand mixture (1:1 v/v) on November 20th, in the 2020/21 and 2021/22 seasons. A randomized complete-block design with four replications, each containing five pots, was used for the experiment. Three weeks after planting, seedlings were thinned into one pot<sup>-1</sup>.

Throughout the experiment, control plants (T<sub>1</sub>) received weekly tap water irrigation, while plants of T<sub>2</sub> – T<sub>4</sub> were irrigated with tap water containing 5000 ppm of NaCl beginning from the appearance of the 3rd leaf until the end of the experiment. To prevent salt buildup, the soil was rinsed with running tap water once every three times irrigation with saline water. Before sowing, the soil in each pot was thoroughly mixed with 2 g of granular ammonium sulfate (20.5% N), 1 g of single superphosphate (15% P<sub>2</sub>O<sub>5</sub>), and 1.25 g of potassium sulfate (48% K<sub>2</sub>O).

#### 1. Preparation of the assigned treatments:

##### 1.1. Salinity levels:

A stock saline solution (20,000 ppm) was prepared by dissolving 200 g of sodium chloride (NaCl) in 10 liters of tap water to produce various applied salinity levels. The tap water was analyzed via inductively coupled plasma optical emission spectroscopy to determine its elemental content. Additionally, the electrical conductivity and pH were measured following the methods outlined by Jackson (1973). All these details are shown in Table 1.

**Table 1: Chemical properties of tap water.**

Season	pH	Ec ppm	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>	Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>
			mg l <sup>-1</sup>						
2020/21	7.50	204.80	6.41	78.25	52.40	21.00	6.20	59.10	7.80
2021/22	7.70	206.40	6.11	84.97	53.90	22.20	6.50	58.60	9.40

**1.2. Olive leaves extract (OLE).**

In mid-October, olive leaves were collected from the Picual olive cultivar planted in Ras El-Bar, Damietta, Egypt. The leaves were air-dried at ambient temperature, ground into a fine powder, and stored in a dark environment. A stock olive leaf extract was prepared by macerating 10 grams of olive leaf powder in 100 ml of 80% ethanol for four hours at 40°C. The resulting mixture was filtered using Whatman No. 1 filter paper to eliminate coarse particles.

**Table 2. Concentrations of phytohormones and mineral nutrients in ‘Memecik’ olive leaves (Ulger *et al.*, 2004).**

Total sugars (mg g <sup>-1</sup> DW)					40.18			
Mineral nutrients								
mg g <sup>-1</sup> DW					µg g <sup>-1</sup> DW			
N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
10.40	1.10	5.90	20.60	1.70	93.77	33.82	21.61	29.13
Phytohormones (µg g <sup>-1</sup> DW)								
IAA		GA <sub>3</sub>		GA <sub>4</sub>		Zeatin		ABA
1.29		3.27		33.38		11.76		0.31

**Table 3: The concentrations of total phenolics and total flavonoids in olive leaf extract (M'Rabet *et al.*, 2023).**

Parameters	Phenolic content (g <sup>-1</sup> DE)
Total phenolics “TPC (mg GAE)”	134.73 ± 1.05
Total flavonoids “TFC (mg QE)”	62.48 ± 0.43

DE: dry matter of extract, GAE: Gallic acid equivalent, QE: Quercetin equivalent.

**2. Soil sampling and analysis:**

Soil samples were randomly taken from the soil mixture used in the main experiment before sowing in the 2020/21 and 2021/22 seasons. The samples were then analyzed using the method of Miller and Miller (1987) to identify the physical properties and the methods of Dewis and Freitas (1970) and Jackson (1973) to evaluate the chemical properties, and the results were displayed in Table 4.

**3. Plant sampling and data collection:****3.1. Vegetative growth characteristics:**

Eight plants were randomly chosen from each treatment on the 45th day after planting (DAP) in both seasons to measure various growth parameters, including root size (cm<sup>3</sup>), root fresh and dry weights (g), plant height (cm), number of branches,

The filtrate was then evaporated at 25°C under vacuum using a rotary evaporator. The concentrated extract, with a mud-like consistency, was stored in a refrigerator at 2-4°C until preparing the desired concentrations (0.1% and 0.2%). Ulger *et al.* (2004) determined the levels of phytohormones and mineral nutrients in “Memecik” olive leaves as summarized in Table 2. Additionally, Table 3 displays the concentrations of total phenolics, total flavonoids, according to M'Rabet *et al.* (2023).

shoot fresh and dry weights (g), stem diameter (cm), number of leaves, fresh and dry weights of leaves (g), and total leaf area (cm<sup>2</sup>) plant<sup>-1</sup>.

**Table 4: Physical and chemical properties of the experimental soil for the two growing seasons.**

Soil analysis	2020/2021	2021/2022
Soil particles distribution %		
Sand	51.24	53.03
Silt	29.87	27.91
Clay	18.89	19.06
Textural class	Sandy loam	Sandy loam
Soil pH	7.65	7.88
Ec (ppm)	432.06	433.11
Organic Matter (%)	0.28	0.31
Total CaCO <sub>3</sub> (%)	4.21	3.98
Available nutrients (mg kg <sup>-1</sup> )		
N	6.71	5.98
P	2.45	2.01
K	145	134
Soluble anions and cations (mg 100 g <sup>-1</sup> )		
Cl <sup>-</sup>	19.31	20.87
HCO <sub>3</sub> <sup>-</sup>	22.11	23.91
CO <sub>3</sub> <sup>-2</sup>	-	-
Mg <sup>+2</sup>	2.05	2.01
Ca <sup>+2</sup>	6.58	5.99
Na <sup>+</sup>	15.21	16.84
K <sup>+</sup>	4.63	5.16

To determine the root system size, the plant pot was tilted, and a quiet stream of water was gently poured over the soil surface until all soil was eliminated from the roots, ensuring that the whole root system was collected. The root system was then dried using paper towels before being placed in a volumetric flask holding a known volume of water. The rise in water volume was then quantified to assess the root system's size. Meanwhile, the total leaf area ( $\text{cm}^2$ )  $\text{plant}^{-1}$  was determined using the disk method of Waidyanatha and Goonasekera (1975).

Additionally, the plant's roots, stems, and leaves were separated and cleaned with deionized water to eliminate contaminants, then dried at  $70^\circ\text{C}$  until a constant weight. The dried leaves and stems were finely powdered with a NIMA grinder (model NO: BL-888A, Japan) and stored in paper sachets at room temperature ( $25^\circ\text{C}$ ) for chemical analysis.

The data gained on plant dry matter and total leaf area  $\text{plant}^{-1}$  were employed to compute certain significant growth indices:

- a) **Root/shoot ratio:** It compares the dry matter accumulation in roots to that in shoots, as follows:

$$\text{Root/ Shoot ratio} = \frac{\text{Root dry weight Plant}^{-1}}{\text{Shoot dry weight Plant}^{-1}}$$

- b) **Leaf area ratio (LAR)  $\text{plant}^{-1}$ :** It indicates the leaf area ( $\text{cm}^2$ ) created per unit of plant biomass (g). It was determined using the formula of Radford (1967).

$$\text{LAR} (\text{cm}^2 \text{g}^{-1}) = \frac{\text{Total leaf area} (\text{cm}^2) \text{ Plant}^{-1}}{\text{Plant dry weight}}$$

### 3.2. Determination of Photosynthetic pigments:

The concentrations of chlorophylls "a" & "b" and carotenoids were determined in the 4<sup>th</sup> apical leaf, 45<sup>th</sup> DAP, in both seasons. The method involved extracting the pigments using dimethylformamide (DMF) and then measuring their optical densities with a spectrophotometer at 664, 647, and 480 nm, according to Wellburn (1994). The concentrations were presented as  $\text{mg g}^{-1}$  fresh weight (FW).

### 3.3. Determination of certain biochemical consistent in faba bean shoots:

Dry shoot samples, 45<sup>th</sup> DAP, were utilized to determine specific biochemical components. These include free proline, total soluble sugars, total carbohydrates, total phenolics, and free amino acids. The procedures employed for these determinations were according to the methods of Bates *et al.* (1973), Yemm and Willis (1954), Sadasivam (1996), Stabell *et al.* (1996), and Lee

and Takahashi (1966), respectively. The concentrations were expressed as  $\text{mg g}^{-1}$  DW.

### 3.4. Determination of some mineral nutrients in faba bean shoots:

Samples (0.2 g each) of fine dry shoot powder, 45<sup>th</sup> DAP, were wet-digested using a mixture of sulfuric and perchloric acids. The clear solution was then transferred to a volumetric flask and diluted to 50 ml with distilled water before analysis (Nagorny, 2013). The microKjeldahl method (Horneck and Miller, 1998) was used to quantify total nitrogen. Crude protein was computed by multiplying total nitrogen by 6.25 (A.O.A.C., 2005). Phosphorus was determined according to Jackson (1973). A flame emission spectrophotometer (Jenway PFP 7) was used to measure potassium, sodium, and calcium concentrations, as described by Horneck and Hanson (1997). Additionally, magnesium was analyzed using an atomic absorption spectrophotometer following the method of Wright and Stuczynski (1996). Concentrations were shown as  $\text{mg g}^{-1}$  DW.

### 4. Statistical Analysis:

Germination, morphological, chemical and yield data were subjected to analysis of variance (ANOVA) using a one-way analysis for a completely randomized block design by the IBM SPSS Statistics program version 29.0.1.0. To compare the treatment means with those of the control, the least significant difference (L.S.D.) test at  $P \leq 0.05$  was employed following Snedecor and Cochran (1989).

### Result and Discussion

#### 1. The preliminary germination experiment:

Table 5 demonstrates that increasing salinity (NaCl) level from 3000 to 7000 ppm reduced proportionally the germination percentage (GP) and prolonged the germination period (mean rate of germination; MRG). As a result, the germination performance index (GPI) declined in proportion to these salinity levels compared to the control (tap water). In contrast, lower NaCl levels (1000 and 2000 ppm) had no effect on the germination parameters relative to the control.

The results indicate that a salinity level of 5000 ppm is the threshold beyond which the germination percentage and germination performance index drop below 50% of control values. Thus, this level was considered the maximum tolerable salinity for faba bean cv. Sakha-1, following the suggestion of Wanas (1996). It was subsequently employed in the main experiment to investigate the effects of salinity on the growth, physiological, anatomical, and yield properties of faba bean plants.

**Table 8: Effect of various salinity levels on germination characteristics.**

Measurements		GP	MRG	GPI
Salinity levels				
Control (tap water)	$\bar{X}$	100.00	8.20	12.20
	$\pm\%$	0.00	0.00	0.00
1000 ppm	$\bar{X}$	100.00	8.20	12.20
	$\pm\%$	0.00	0.00	0.00
2000 ppm	$\bar{X}$	100	8.20	12.20
	$\pm\%$	0.00	0.00	0.00
3000 ppm	$\bar{X}$	88.87	8.37	10.61
	$\pm\%$	-11.13	+2.07	-13.03
4000 ppm	$\bar{X}$	77.73	8.73	8.86
	$\pm\%$	-22.27	+6.46	-27.38
5000 ppm	$\bar{X}$	66.60	9.33	7.14
	$\pm\%$	-33.40	+13.78	-41.48
6000 ppm	$\bar{X}$	44.40	9.73	4.55
	$\pm\%$	-55.60	+18.66	-62.70
7000 ppm	$\bar{X}$	11.10	4.17	0.88
	$\pm\%$	-88.90	-49.15	-92.79
LSD		23.57	4.43	2.32

Abbreviations: GP= Germination percentage, MRG= Mean rate of germination, GPI= Germination performance index  $\pm\%$  =  $\pm\%$  relative to the control values.

Similar results about the deleterious effect of NaCl salinity on germination properties of faba bean were reported by Bekhiet *et al.* (2022) and Danial and Basset (2024).

NaCl salinity negatively affects seed germination by increasing Na<sup>+</sup> and Cl<sup>-</sup> ion accumulation, leading to cell toxicity that slows or inhibits germination, thereby reducing germination rates (Naseer *et al.*, 2022). Additionally, salinity induces osmotic stress, hindering water absorption and negatively impacting hydrolytic enzyme activity and embryonic cell division and development (Taiz *et al.*, 2014).

## 2. The main experiment:

### 2.1. Vegetative growth characteristics of faba bean plants:

Compared to the control, irrigation with 5000 ppm NaCl significantly reduced various growth indices, including root system size, stem length, the number of leaves and branches, total leaf area plant<sup>-1</sup>, and both fresh and dry weights of roots, stems, and leaves, as well as the root/shoot ratio. Conversely, it significantly increased the leaf area ratio (LAR) of the treated plants compared to control plants irrigated with tap water during both study seasons (Table 6).

Applying the assigned concentrations of OLE as seed-soaking treatments before planting not only mitigated but also overcame the negative

effects of salinity on salt-stressed faba bean plants (irrigated with 5000 ppm NaCl), resulting in improved growth parameters compared to control plants. Since the root system size, both fresh and dry weights of roots, stems, and leaves, the number of leaves and branches, and total plant biomass were significantly increased relative to control values, whereas the root/shoot ratio and leaf area ratio (LAR) were significantly reduced, with OLE outperforming at 0.2%. The results showed the same trend in both growing seasons.

Salinity negatively affected faba bean growth, which is consistent with the findings of Kumar *et al.* (2022) and Abdelfattah *et al.* (2024). This negative effect of salinity may be primarily due to its adverse effect on photosynthetic efficiency, as shown by the reduction in the levels of photosynthetic pigments (Table 7) and total leaf area plant<sup>-1</sup>, which was accompanied by an increased LAR (leaf area in cm<sup>2</sup> required for the creation of one gram of plant biomass) and thus reduced the dry matter in different plant organs (Table 6). Additionally, salinity causes injury through osmotic stress, toxic ion accumulation, and increased reactive oxygen species (ROS) production (Khan *et al.*, 2013). Osmotic stress results in water deficiency in plant cells, reducing leaf area and causing stomatal closure, which hinders photosynthesis and overall growth (Roy *et al.*, 2014). Ionic imbalances lead to excessive Na<sup>+</sup> and Cl<sup>-</sup> accumulation in leaves, causing early aging (Munns and Tester, 2008; Roy *et al.*, 2014) and inhibiting the uptake of essential ions like K<sup>+</sup>, Ca<sup>2+</sup>, and Mn<sup>2+</sup>, which disrupts photosynthesis and enzyme functions (Muchate *et al.*, 2016). High salinity also boost ROS production, resulting in oxidative damage to cell and organelle membranes, which can lead to cell and plant death under harsh conditions (Hasanuzzaman *et al.*, 2021). Furthermore, plant growth rates are influenced by vital processes such as cell division, enlargement, and differentiation, alongside various genetic, morphological, physiological, and ecological factors, all of which are significantly affected by different abiotic stressors (Taize *et al.*, 2014).

Despite this, plants have efficient antioxidant defense systems that contain both enzymatic and non-enzymatic antioxidants to resist oxidative stress and protect them from the negative impacts of abiotic stressors (Apel and Hirt, 2004). Plants' natural antioxidant defense systems are usually inadequate to maintain healthy growth, requiring exogenous enhancers

like plant extracts to withstand abiotic stress (Rehman *et al.*, 2018; Desoky *et al.*, 2019).

The results indicate that olive leaf extract (OLE) is an efficient biostimulant, improving the growth of faba bean plants under salt-stress conditions. This growth-promoting impact of OLE is mainly ascribed to its richness in flavonoids, phenolics (M'Rabet *et al.*, 2023), GAs, and zeatin, as well as important nutrients like Ca and Fe (Ulgar *et al.*, 2004). Flavonoids and phenolic substances have antioxidant capabilities, which neutralize free radicals (Macheix *et al.*, 2005; Prakash *et al.*, 2007). GAs and zeatin are well known to improve plant growth via promoting cell proliferation and enlargement, chlorophyll biosynthesis, and delaying leaf aging. Zeatin also enhances antioxidant enzyme activity and promotes the

formation of lateral roots and branches (Hwang *et al.*, 2012; Taiz *et al.*, 2014). Calcium (Ca<sup>2+</sup>), prevalent in OLE, has structural and signaling roles, including membrane stability, strengthening cell walls, and serving as a secondary messenger for various signals (Marschner, 2012; Cacho *et al.*, 2013). It also stimulates growth-promoting processes such as cell division and assimilate synthesis and translocation during stress (Pereira and Mello, 2002), while also maintaining a balanced hormonal profile with increased GAs and IAA levels and decreased ABA and ethylene levels in various plant organs (Ferguson, 1988). Furthermore, it protects against abiotic stress by acting as an antioxidant and controlling gene expression (Clayton *et al.*, 1999; Sanders *et al.*, 2002).

**Table 6: Effect of OLE treatments on some growth criteria of salt-stressed faba bean plants at 45 DAP during the 2020/2021 and 2021/2022 seasons.**

Treatments	Characters	Root Parameters			Stem Parameters				Leaves				Total plant DW (g)	Root/shoot ratio	LAR	
		Size	FW (g)	DW (g)	length (cm)	No. of Br. plant <sup>-1</sup>	FW (g)	DW (g)	No.	FW (g)	DW (g)	Area (cm <sup>2</sup> )				
Season 2020/21																
Control (Tap water)		$\bar{X}$	31.64	22.99	4.59	53.33	3.00	20.55	2.01	26.33	20.69	1.57	474.27	8.17	1.28	58.05
Salinity (5000 ppm NaCl)	OLE 0.0 %	$\bar{X}$	27.11	19.19	3.76	52.00	2.00	14.21	1.31	20.44	18.72	1.24	387.65	6.31	1.47	61.43
		$\pm\%$	-14.32	-16.53	-18.08	-2.49	-33.33	-30.85	-34.83	-22.37	-9.52	-21.02	-18.26	-19.09	+14.84	+5.82
	OLE 0.1 %	$\bar{X}$	33.27	23.98	4.99	65.00	3.67	34.07	3.48	30.56	21.79	2.83	500.57	11.30	0.79	44.30
		$\pm\%$	+5.15	+4.31	+8.71	+21.88	+22.33	+67.64	+73.13	+16.07	+5.32	+80.25	+5.55	+38.31	-38.28	-23.69
	OLE 0.2 %	$\bar{X}$	33.91	24.32	5.07	68.00	4.00	36.45	3.88	32.22	24.44	2.89	558.11	11.84	0.75	47.14
		$\pm\%$	+7.17	+5.79	+10.46	+27.51	+33.33	+77.35	+93.03	+22.37	+17.06	+84.08	+17.68	+44.92	-41.41	-18.79
LSD at 0.05		1.60	0.93	0.26	1.09	0.28	1.92	0.16	2.11	1.86	0.31	4.75	0.43	0.11	3.29	
Season 2021/22																
Control (Tap water)		$\bar{X}$	32.12	23.86	4.64	52.67	3.00	20.95	2.24	26.56	21.00	1.88	476.92	8.76	1.13	54.44
Salinity (5000 ppm NaCl)	OLE 0.0 %	$\bar{X}$	26.38	18.67	3.65	50.67	2.33	14.07	1.43	21.67	18.16	1.35	385.50	6.43	1.31	59.95
		$\pm\%$	-17.87	-21.75	-21.34	-3.80	-22.33	-32.84	-36.16	-18.41	-13.52	-28.19	-19.17	-26.60	+15.93	+10.12
	OLE 0.1 %	$\bar{X}$	33.10	24.63	4.91	67.33	4.00	35.58	3.69	30.33	23.43	3.03	511.41	11.63	0.73	43.97
		$\pm\%$	+3.05	+3.23	+5.82	+27.83	+33.33	+69.83	+64.73	+14.19	+11.57	+61.17	+7.23	+32.76	-35.40	-19.23
	OLE 0.2 %	$\bar{X}$	33.80	24.70	5.13	69.00	4.00	39.56	4.00	36.67	24.55	3.07	563.87	12.17	0.73	46.33
		$\pm\%$	+5.23	+3.52	+10.56	+31.00	+33.33	+88.83	+78.57	+38.06	+16.90	+63.30	+18.23	+38.93	-35.40	-14.90
LSD at 0.05		1.25	0.96	0.32	1.22	0.33	2.10	0.27	2.03	0.67	0.31	6.06	0.47	0.09	4.24	

Abbreviations: OLE = olive leaf extract, FW= fresh weight, DW= dry weight, No= number, Br. = Branches, LAR= Leaf area ratio and  $\pm\%$  =  $\pm\%$  relative to the control values.

**2.2. Photosynthetic pigments:**

When faba bean plants were exposed to the highest endurable salinity level of 5000 ppm NaCl, they showed significant reductions in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids levels in their leaves. In the first season, these levels decreased by 18.10%, 24.32%, 19.61% and 19.05% below control levels, respectively. The second season showed similar decreases (Table 7).

The seed-pres soaking treatments with OLE at 0.1% and 0.2% effectively mitigated and

eliminated the detrimental effects of salinity on faba bean plants stressed by 5000 ppm NaCl, resulting in increased photosynthetic pigment levels in salt-stressed faba bean leaves compared to the control (tap water). The 0.2% OLE treatment was the most effective, increasing chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids over the control values by 20.69%, 27.03%, 22.22%, and 38.10%, respectively, in the first season. In the second season, these increases were 15.00%, 31.58%, 18.99%, and 45.45%.

The adverse effect of salinity on the levels of photosynthetic pigments in faba bean leaves is consistent with that reported by Abdelfattah *et al.* (2024) and Ahmed and Sattar (2024). According to Munns and Tester (2008) and Roy *et al.* (2014), excessive buildup of Na<sup>+</sup> and Cl<sup>-</sup> might cause rapid leaf aging and chlorophyll breakdown. Furthermore, high salinity triggers the overproduction of ROS, which causes oxidative damage to cell and organelle membranes, including thylakoids that contain photosynthetic pigments (Wanas *et al.*, 2018; Hasanuzzaman *et al.*, 2021).

On the other hand, the increment in photosynthetic pigment levels by OLE

treatments under salt- stress circumstances correlates strongly with higher total plant biomass (Table 6) and justifies the reduction in LAR recorded with OLE. The enhancing influence of OLE on photosynthetic pigment levels is potentially due to its a considerable content of zeatin and gibberellins, which enhance chlorophyll biosynthesis and retention, thus delaying senescence (Hwang *et al.*, 2012; Taiz *et al.*, 2014). Additionally, OLE contains effective antioxidants like phenolic compounds and flavonoids that inhibit chlorophyll breakdown, protect chloroplasts from harmful free radicals, and reduce photo-oxidation of these pigments (Sidduraju and Becker, 2003).

**Table 7: Effect of OLE treatments on the concentration of photosynthetic pigments (mg g<sup>-1</sup> FW) in leaves of salt-stressed faba bean plants at 45 DAP during 2020/21 and 2021/22 seasons.**

Parameters		Season 2020/21				Season 2021/22				
		Chl. a	Chl. b	Total Chl.	Carot.	Chl. a	Chl. b	Total Chl.	Carot.	
Control (Tap water)		$\bar{X}$	1.16	0.37	1.53	0.21	1.20	0.38	1.58	0.22
Salinity (5000 ppm NaCl)	0.0%	$\bar{X}$	0.95	0.28	1.23	0.17	0.93	0.26	1.19	0.16
		$\pm\%$	-18.10	-24.32	-19.61	-19.05	-22.50	-34.29	-24.68	-27.27
	0.1%	$\bar{X}$	1.36	0.45	1.81	0.27	1.34	0.44	1.78	0.30
		$\pm\%$	+17.24	+21.62	+18.30	+28.57	+11.67	+15.79	+12.66	+36.36
	0.2%	$\bar{X}$	1.40	0.47	1.87	0.29	1.38	0.50	1.88	0.32
		$\pm\%$	+20.69	+27.03	+22.22	+38.10	+15.00	+31.58	+18.99	+45.45
LSD at 0.05			0.07	0.04	0.05	0.04	0.09	0.05	0.04	0.06

Abbreviations: Chl. = chlorophyll, Carot, = carotenoids, OLE = olive leaf extract, DAP = Days after planting,  $\pm\%$  =  $\pm\%$  relative to the control value.

### 2.3. Chemical composition of faba bean shoots:

#### 3.2.3.1. Biochemical constituents:

Table 8 shows that applying the maximum tolerable salinity level (5000 ppm NaCl) to faba bean plants significantly decreased total carbohydrates, polysaccharides, and crude protein. Whereas, it substantially increased soluble sugars, free amino acids, proline, and total phenolics concentrations in the shoots compared to the control plants irrigated with tap water during the 2020/21 and 2021/22 growing seasons.

Applying OLE at 0.1% and 0.2% as seed-pressoaking treatments before planting effectively eliminated the negative effects of salinity and significantly increased the concentrations of all determined bioconstituents in salt-stressed faba bean shoots compared to control plants irrigated with tap water, with a superiority of 0.2% OLE. In the first season, increases achieved by 0.2% OLE exceeded the control values by 25.86% for soluble sugars, 122.66% for free amino acids, 34.37% for total phenolics, and 113.95% for proline. While in the second season, the

increases reached 27.11%, 131.10%, 39.89%, and 72.22%, respectively.

Similar results were reported about the effect of NaCl salinity on the bioconstituent levels in faba beans by Boghdady *et al.* (2017) and El-Metwally and Sadak (2019).

The results indicate that salinity reduced total carbohydrate level while increasing soluble sugars in faba bean shoots. This reduction in carbohydrates is mainly due to decreased photosynthetic activity, as evidenced by lower levels of photosynthetic pigments (Table, 7), reduced leaf area plant<sup>-1</sup>, and a higher leaf area ratio (Table, 6), indicating lower biomass production. Salt stress negatively impacts photosynthesis by affecting essential components such as enzymes and structural proteins involved in light absorption, electron transport, and carbon fixation (Hao *et al.*, 2021). In response to stress, plants often convert starch into simple sugars, which provide energy and serve as osmoprotectants and ROS scavenger, thereby supporting growth under stress (Abid *et al.*, 2021). Additionally, sugars act as signaling molecules that engage with the ABA-dependent



**Table 8: Effects of OLE treatments on the concentrations of some bioconstituents (mg g<sup>-1</sup> DW) in the shoots of salt stressed faba bean plants at 45 DAP during 2020/21 and 2021/22 seasons.**

Determinations Treatments			Total Carbs.	Insoluble sugars	Soluble sugars	Crude protein	Free amino acids	Proline	Total phenolics	
			Season 2020/2021							
Control (Tap water)		$\bar{X}$	242.84	214.98	27.86	383.13	12.71	0.43	20.19	
Salinity (5000 ppm NaCl)	OLE	0.0%	$\bar{X}$	202.24	169.10	33.14	318.13	20.70	0.80	25.12
			$\pm\%$	-16.72	-21.34	+18.93	-16.97	+62.86	+88.26	+24.45
		0.1%	$\bar{X}$	254.03	219.92	34.11	463.13	28.17	0.88	26.96
			$\pm\%$	+4.61	+2.30	+22.41	+20.88	+121.64	+106.23	+33.53
		0.2%	$\bar{X}$	255.54	220.47	35.07	486.25	28.20	0.92	27.13
			$\pm\%$	+5.23	+2.55	+25.86	+26.92	+122.66	+113.95	+34.37
LSD at 0.05			2.13	2.14	0.07	0.37	0.68	0.03	0.08	
Season 2020/2021										
Control (Tap water)		$\bar{X}$	243.98	215.98	28.00	416.88	12.22	0.54	19.78	
Salinity (5000 ppm NaCl)	OLE	0.0%	$\bar{X}$	200.12	163.35	36.77	297.50	21.60	0.85	27.56
			$\pm\%$	-17.98	-24.37	+31.32	-28.64	+76.76	+57.41	+39.33
		0.1%	$\bar{X}$	253.98	219.28	34.70	460.63	27.49	0.91	27.24
			$\pm\%$	+4.10	+1.53	+23.93	+10.49	+124.96	+68.52	+37.71
		0.2%	$\bar{X}$	255.07	219.48	35.59	470.63	28.24	0.93	27.67
			$\pm\%$	+4.55	+1.62	+27.11	+12.89	+131.10	+72.22	+39.89
LSD at 0.05			4.48	6.62	3.02	2.61	1.37	0.04	2.13	

Abbreviations: Carbs = carbohydrates, OLE = olive leaf extract,  $\pm\%$  =  $\pm\%$  relative to the control values.

Proline and free amino acids accumulate in plants as a well-known response to water and salt stress. Proline serves as an antioxidant and provides a quick source of carbon and nitrogen, helping the recovery of stressed plants (Per *et al.*, 2017), along with helping protect plant cells by maintaining osmotic balance between the cytosol, vacuole, and external environment (Rahimi *et al.*, 2012). Additionally, proline enhances antioxidant defenses by increasing the activity of peroxidase, glutathione-S-transferase, superoxide dismutase, and catalase, while improving glutathione redox status (Hoque *et al.*, 2008). Higher proline levels limit free radical production, reduce lipid peroxidation, and support osmotic adjustment and subcellular structural stability (El-Metwally and Sadak, 2019).

Moreover, salinity and other abiotic stressors stimulate the phenylpropanoid biosynthesis pathway, leading to an accumulation of phenolic compounds that scavenge harmful free radicals (Chen *et al.*, 2019; Sharma *et al.*, 2019). These compounds promote nutrient mobilization and facilitate signaling between roots and shoots (Sharma *et al.*, 2019). They also enhance nutrient absorption by chelating metal ions, increasing active absorption sites, and aiding the transport

of elements such as Ca, Mg, K, Zn, Fe, and Mn (Seneviratne and Jayasinghearachchi, 2003).

Seed-soaking treatments with OLE significantly improved the bioconstituents in faba bean shoots. This improvement could be due to increased photosynthetic efficiency, as evidenced by higher photosynthetic pigment levels (Table 7) and a greater total leaf area with a reduced LAR, which expresses the leaf area in cm<sup>2</sup> needed to synthesize one gram of dry matter (Table 6). As a result, more necessary raw materials (simple sugars) are produced, which are required for energy and metabolite synthesis. OLE also contains amino acids, vitamins, phenolics, growth hormones (GAs and zeatin), and essential minerals such as Fe and Ca (Ulger *et al.*, 2004), which are made available to plants after OLE is applied. Thus, the extract serves as an effective biostimulant, enhancing the biosynthesis of chlorophyll, sugars, and proteins due to its growth hormone, mineral, and amino acid content. Additionally, its antioxidant properties, supported by amino acids, phenols, and vitamins, help reduce free radical production under stress. Consequently, the extract's positive effects on the biochemical profile of faba bean shoots further demonstrate its potential to mitigate salinity stress by enhancing osmotic regulation and antioxidant activity in the plants.

### 2.3.2. Mineral elements:

Results in Table 9 show that irrigating faba bean plants with the maximum tolerable salinity level (5000 ppm NaCl) significantly decreased concentrations of N, P, K, Ca, Mg, and the K/Na ratio, whereas significantly increasing the Na<sup>+</sup> concentration in shoots of treated plants compared to control plants irrigated with tap water during the 2020/21 and 2021/22 seasons.

In contrast, applying the two assigned concentrations of OLE as seed-presozaking treatments effectively eliminated the negative effects of salinity in faba beans irrigated with

5000 ppm NaCl. This resulted in significant increases in N, P, K, Ca, and Mg levels, along with a notable reduction in Na levels and a considerable rise in the K/Na ratio compared to the control in both growing seasons, with superiority of 0.2% OLE in this respect. Increases obtained with 0.2% OLE exceeded the control values by 26.94% for N, 24.76% for P, 12.55% for K, 28.84% for Ca, 31.28 for Mg, and 24.55 for K/Na ratio in the first season, while these increase were 13.00%, 20.55%, 16.06, 29.18%, 26.60, and 29.73%, respectively in the second season.

**Table 9: Effects of OLE treatments on the concentrations of mineral elements (mg g<sup>-1</sup>) in shoots of salt-stressed faba bean at 45 DAP during 2020/21 and 2021/22 seasons.**

Determinations			N	P	K	Ca	Mg	Na	K/Na	
Treatments										
<b>Season 2020/2021</b>										
Control (Tap water)			$\bar{X}$	61.32	4.12	27.97	12.31	3.90	25.35	1.10
Salinity (5000 ppm NaCl)	OLE	0.0%	$\bar{X}$	51.00	3.14	19.54	8.49	2.14	57.01	0.34
			$\pm\%$	-16.83	-23.79	-30.14	-31.03	-45.13	+124.89	-69.09
		0.1%	$\bar{X}$	74.17	4.96	30.82	14.99	4.82	23.52	1.31
			$\pm\%$	+20.96	+20.39	+10.19	+21.77	+23.59	-7.22	+19.09
		0.2%	$\bar{X}$	77.84	5.14	31.48	15.86	5.12	22.90	1.37
			$\pm\%$	+26.94	+24.76	+12.55	+28.84	+31.28	-9.31	+24.55
LSD at 0.05				4.11	0.27	1.50	1.79	0.83	1.77	0.06
<b>Season 2021/2022</b>										
Control (Tap water)			$\bar{X}$	66.71	4.33	27.27	12.92	3.91	24.52	1.11
Salinity (5000 ppm NaCl)	OLE	0.0%	$\bar{X}$	47.63	3.03	18.70	8.64	2.57	53.92	0.35
			$\pm\%$	-28.60	-30.02	-31.43	-33.13	-34.27	+119.90	-68.47
		0.1%	$\bar{X}$	73.79	5.01	30.45	16.04	4.63	22.64	1.34
			$\pm\%$	+10.61	+15.70	+11.66	+24.15	+18.41	-7.67	+20.72
		0.2%	$\bar{X}$	75.38	5.22	31.65	16.69	4.95	21.92	1.44
			$\pm\%$	+13.00	+20.55	+16.06	+29.18	+26.60	-10.60	+29.73
LSD at 0.05				3.49	0.22	1.42	1.58	0.99	1.24	0.04

**Abbreviations:** OLE = olive leaf extract,  $\pm\%$  =  $\pm\%$  relative to the control values.

Results indicate that NaCl salinity negatively impacts nutrient levels in faba bean shoots, decreasing N, P, K, Mg, and Ca while increasing Na levels, consistent with Bulut *et al.* (2011) and Afzal *et al.* (2022). This effect arises from high Na<sup>+</sup> and Cl<sup>-</sup> concentrations in the soil, leading to their accumulation in the shoots of salt-stressed plants. Such accumulation causes ionic imbalances and nutrient deficiencies due to the competitive inhibition of Na<sup>+</sup> against essential elements like N, K<sup>+</sup>, P, and Ca<sup>2+</sup> (Borromeo *et al.*, 2023). Elevated Na<sup>+</sup> levels also lower membrane potential and enhance Cl<sup>-</sup> absorption due to chemical gradients (Flowers and Colmer, 2015). High Cl<sup>-</sup> concentrations damage cellular

membranes and reduce chlorophyll content, further hindering growth (Munns and Tester, 2008). Additionally, Na<sup>+</sup> competes with K<sup>+</sup> for binding sites, which leads to enzyme deactivation and disruption of critical cellular processes, as many enzymes are activated by K<sup>+</sup> but inhibited by Na<sup>+</sup>. Therefore, high Na<sup>+</sup> levels or a high Na<sup>+</sup>: K<sup>+</sup> ratio interfere with enzymatic activity (Tester and Davenport, 2003). Salt-resistant plants maintain a favorable K<sup>+</sup>: Na<sup>+</sup> ratio in their cytosol (Maathuis and Amtmann, 1999).

On the other hand, the considerable reduction in different nutrient levels due to NaCl treatment may result from the adverse effects of this salt on

the transport of these nutrients through the competitive interactions that impact the ionic selectivity of cell membranes (Stoeva and Kaymakanova 2008). Bulut and Akinci (2010) indicated that phosphorus (P) tended to accumulate in the faba bean plants growing under low and moderate salinity conditions but reduced at higher salinity levels compared to control values. This could be owing to an adaptation mechanism evolved by the plants to overcome osmotic stress caused by salinity, while the subsequent decrease in P could be attributable to the antagonistic relationship between salt ions and P.

The increased mineral nutrient levels in both unstressed and salt-stressed faba bean shoots treated with OLE can primarily be attributed to the organic extract's ability to enhance mineral absorption through enhancing the plants' root systems (Table 6). Additionally, the observed increases in leaf area, photosynthetic pigments (Table 7), and shoot dry matter (Table 6) indicate that OLE treatments boost photosynthetic efficiency, leading to improved mineral absorption and their translocation from roots to shoots. OLE is also rich in essential minerals like Ca and Fe, as well as phytohormones such as GAs and zeatin, which enhance metabolic processes and promote greater mineral absorption while reducing Na<sup>+</sup> uptake (Faraj 2012). Ca<sup>2+</sup>, abundant in olive leaves, acts as an osmotic agent in vacuoles, stabilizes cellular membranes, strengthens cell walls, and transmits various signals (Marschner, 2012; Cacho *et al.*, 2013). It also alleviates abiotic stress by functioning as an antioxidant and regulating gene expression (Clayton *et al.*, 1999; Sanders *et al.*, 2002). Fe activates enzymes involved in chlorophyll biosynthesis and antioxidant enzymes such as APOX and GR, which combat ROS and protect chlorophyll from degradation (Zayed *et al.*, 2011).

## CONCLUSION

Therefore, it is possible to conclude that OLE can be used as an effective, sustainable natural biostimulant to increase salt tolerance in faba bean plants or food crops grown under salt stress due to its high ability to mitigate or eliminate the negative effects of salinity, thereby enhancing plant growth and related physio-biochemical processes, which ultimately leads to increased yield and sanitary quality. The effectiveness of OLE in reducing salt stress in faba bean plants is mostly due to its high content of osmoprotectants, antioxidants, some vital nutrients, and growth-promoting hormones, which nourish plants and improve their internal

defense systems against stress following OLE application.

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## CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

## AUTHORS CONTRIBUTION

Wanas, A.L.; Hamada, M.S. and Mtawea, Shaimaa.S. developed the concept of the manuscript. Wanas wrote the manuscript. All authors checked and confirmed the final revised manuscript.

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## تحري قابلية مستخلص أوراق الزيتون تحسين النمو والأداء الفسيولوجي والكيموحيوي لنباتات الفول البلدي تحت ظروف الإجهاد الملحي

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### الملخص العربي

أظهرت نتائج تجربة الإنبات التمهيدية التي أجريت لاختبار سلسلة من تركيزات ملح كلوريد الصوديوم تتراوح من 1000 إلى 7000 جزء في المليون بفاصل 1000 جزء في المليون على بعد معايير الإنبات لبذور الفول البلدي مقارنة بماء الصنبور ككنترول أن نسبة الإنبات ودليل كفاءة الإنبات إنخفضت إلى أقل من 50% عند مستويات كلوريد الصوديوم الأعلى من 5000 جزء في المليون من كلوريد الصوديوم، وبالتالي اعتبر هذا تركيز 5000 جزء في المليون هو أقصى مستوى ملوحة يمكن للنبات تحمله وتم استخدامه في التجربة الرئيسية. وبناء عليه أجريت تجربتي أصص خلال فصل الشتاء لعامي 21/2020 و 22/2021 لتقييم التأثيرات السلبية للإجهاد الملحي (5000 جزء في المليون من كلوريد الصوديوم) على النمو والاستجابة الكيموحيوية لنباتات الفول البلدي، بالإضافة إلى استكشاف فعالية مستخلص أوراق الزيتون في التخفيف من هذه التأثيرات السلبية على النباتات المجهدة بكلوريد الصوديوم. أظهرت النتائج أن الملوحة عند مستوى 5000 جزء في المليون من كلوريد الصوديوم أثرت سلباً على نمو الفول ممثلاً في حدوث نقص معنوي واضح في حجم المجموع الجذري وطول الساق وعدد الأوراق الأفرع ومساحة الأوراق الكلية/ نبات والأوزان الطازجة والجافة للجذور والسيقان والأوراق. كما أدى نقص واضح في تركيز صبغات البناء الضوئي والكاربوهيدرات الكلية والبروتين الخام والعناصر المغذية (النيتروجين، الفسفور، البوتاسيوم، الكالسيوم، الماغنسيوم) بالإضافة إلى نسبة البوتاسيوم/الصوديوم. وعلى العكس من ذلك، أدى إلى زيادة مؤشر معدل مساحة الأوراق تركيز كل من الأحماض الأمينية الحرة والبرولين والفينولات الكلية الصوديوم في المجموع الخضري للنباتات المعاملة مقارنة بالكنترول. في المقابل أدت معاملة البذور بتركيزي 0.1% و 0.2% من مستخلص أوراق الزيتون قبل الزراعة وتعرض النباتات الناتجة للإجهاد الملحي باستخدام 5000 جزء في المليون من كلوريد الصوديوم تقليل أو إبطال التأثيرات السلبية للملحة بكفاءة عالية، وحدث تحسين معنوي في صفات النمو الخضري مصحوباً بزيادة معنوية في تركيزات جميع المكونات الحيوية والعناصر المغذية المقدره مع انخفاض معنوي في مستوى الصوديوم مما أدى زيادة نسبة البوتاسيوم/ الصوديوم مقارنة بالكنترول في كلا الموسمين، وكان تركيز 0.2% من مستخلص أوراق الزيتون هو الأكثر فعالية في هذا الصدد. وبناء عليه توصي هذه الدراسة باستخدام تركيز 0.2% من مستخلص أوراق الزيتون كمنشط نمو حيوي وفعال ورخيص الثمن لتحسين النمو والأداء الفسيولوجي والكيموحيوي لنباتات الفول البلدي المنزرعة في أراضي تعاني من مشكلة الملوحة أو تلك التي تروى بمياه جوفية.