



## Article

# Effect of Some Bio-Stimulants in Mitigating Salt Stress on Sweet Basil (*Ocimum basilicum* L.) Productivity

Wessam M. Serag El- Din, Saleh F.E.M.\* and Reem M. Abd-Elraouf

Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.



\*Corresponding author: [fullsaleh@yahoo.co.uk](mailto:fullsaleh@yahoo.co.uk)

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**Abstract:** Salinity is a significant challenge that adversely affects agricultural activities. Crop growth reduction due to salinity is predominantly associated with the osmotic potential of the root-zone soil solution, which precipitates specific phenological alterations and subsequently reduce productivity. This research studied some management practices (irrigation with saline water using a drip irrigation system at four levels, i.e., 340 (control), 1500, 3000, and 4500 ppm, and some biostimulants, i.e. spermidine, (2.5 mM), licorice root extract (5 g/L), and  $\alpha$ -lipoic acid (0.5 mM) as a foliar application) on reducing salt stress effects and productivity of sweet basil grown in sandy calcareous soil. The experiments were conducted in 2022 and 2023 at the Arab-El-Awamer Agricultural Research Station, Assiut, Egypt. The results showed that increasing water salinity led to a decrease in all growth characteristics, as well as decreases in photosynthetic pigments, nitrogen, and potassium. In contrast, proline, total amino acids, and sodium (Na %) levels rose with increasing salinity. Irrigating with saline water at 4500 ppm and 3000 ppm caused yield losses in dry herbs exceeding 50%. The highest essential oil content (0.92%) and oil yield (0.47 ml/plant) were obtained with water salinity (4500 and 1500 ppm), respectively, with plants sprayed with licorice root extract. Linalool and eugenol were the main components of the essential oil. Control plants contained methyl eugenol (16.28%), but treatment with licorice extract and salinity stress inhibited this substance, enhancing the quality of the essential oil. In summary, applying natural substances like licorice root extract and spermidine helped mitigate the oxidative damage caused by salt stress.

**Key words:** Salinity, basil, licorice, essential oil, spermidine, biostimulant, GC/MS.

## 1. Introduction

In Egypt, saline water is often used for irrigation in newly reclaimed areas. Increased salinity causes stresses (ionic, osmotic, and oxidative stresses) in plants, leading to the production of reactive oxygen species (ROS), reduced water potential, and cell membrane damage,

which negatively impacts photosynthesis and nitrogen assimilation, ultimately harming plant growth (Balasubramaniam *et al.*, 2023). The tolerance levels for salinity and the ability to sustain yield under stress vary among plant species and cultivars (Shannon and Grieve, 1999).

Many studies reported that saline irrigation water accumulate high amounts of salts in the root zone. These soluble salts restrict the roots from withdrawing water from the surrounding soil. Proper management and efficient use of saline water for irrigation is therefore a must to reduce soil salinity buildup and to ensure high crop yields. Drip irrigation system supplies a sufficient quantity of water in the root zone without maximizing salt accumulation. Mixing saline and fresh water resources under drip irrigation has been adopted by several authors (Malash *et al.*, 2005; Hamdy *et al.*, 2005) to improve water quality for irrigation and maintain high crop yields.

Plants have several defense mechanisms against salinity, including antioxidant systems, maintenance of cellular redox state, and higher levels of osmoprotectants like proteins and proline (El-Beltagi *et al.*, 2022 and Erdal *et al.*, 2011). However, prolonged and intense salinity stress can weaken these defenses (Abd Elhady *et al.*, 2021).

While sweet basil plants show a considerable tolerance to salt stress (Barbieri *et al.*, 2012), their endogenous antioxidant system is insufficient for the protection in saline conditions (Desoky *et al.*, 2018). Thus, using exogenous materials like amino acids, antioxidants, plant growth regulators, and natural extracts can effectively counteract salinity in a cost-effective, sustainable way and also environmentally harmless (Desoky *et al.*, 2019 and Turk *et al.*, 2014).

The substances discussed include spermidine, licorice root extract and  $\alpha$ -lipoic acid. Spermidine is one of the organic/ nitrogenous low molecular weight cationic amines known as polyamines, which are important for plant cell differentiation and serve as plant growth regulators (Masson *et al.*, 2017), that is present in animals, plants and bacteria (Sang *et al.*, 2016). They enhance plant growth under salinity stress by increasing photosynthesis and the accumulation of proline and other osmolytes (Peynevandi *et al.*, 2018). Furthermore, polyamines can repair the lamella structure of chloroplasts in stressed plants and protect the photosynthetic organ and alleviated the negative effects on CO<sub>2</sub> assimilation (Drolet, 1986), they act as signaling messengers in response to environmental stressors, regulating antioxidative defense mechanisms (Khoshbakht *et al.*, 2018), (strengthening membranes and reducing free radicals, particularly effective against salinity-alkalinity stress (Hu XH *et al.*, 2012). While, licorice root extract is a potent organic biostimulant rich in antioxidants carbohydrates, vitamins, minerals, and phytohormones, which helps improve resistance to salinity stress due to its high glycyrrhizin, potassium and calcium contents (Desoky *et al.*, 2019). Also,  $\alpha$ -lipoic acid, produced by plants and bacteria, has been found to reduce oxidative damage from salinity by boosting antioxidant defenses and maintaining ionic balance (Bashir *et al.*, 2023). Exogenous alpha lipoic acid also neutralizes and removes reactive oxygen species (Fogacci *et al.*, 2020).

Basil (*Ocimum basilicum* L.), belonging to the Lamiaceae family, is a popular culinary, aromatic, and medicinal herb found in Mediterranean, Asian, and Western countries (Mosadegh *et al.*, 2021). It offers a rich source of proteins, carbohydrates, minerals, and vitamin C, making it valuable for pharmaceutical development. It displays impressive therapeutic properties, including antiviral, antibacterial, antifungal, antioxidant, antidiabetic, and anticancer effects (Incrocci *et al.*, 2019 and Azizah *et al.*, 2023). It is economically significant for its essential volatile oils (El-Saad and El-Saad, 2018) and contains 0.5–1.5% volatile oil, primarily linalool (Hiltunen, 1999). In 2013, Egypt was ranked as the fourth-largest exporter of sweet basil (El-Attar *et al.*, 2019), with increasing demand leading to expanded cultivation (Ciriello *et al.*, 2021).

The objective of this study was to detect the effect of some biostimulants i.e., spermidine, licorice root extract and  $\alpha$ -lipoic acid on mitigating salt stress, growth and productivity of sweet basil grown at sandy calcareous soil.

## 2. Materials and Methods

### 2.1. Experimental layout and treatments

The study was performed in two successive seasons, 2022 and 2023, at the Experimental Farm of the Arab-El-Awamer Agricultural Research Station, Assiut Governorate, Egypt, (lies between 27°, 03 'N (latitude) and 31°, 01 'E (longitude) and the altitude of the area is 71 m<sup>2</sup>). The experiment consisted of four treatments of saline irrigation water levels as first factor (main treatments), and three bio-stimulants as a foliar application as second factor (sub main treatments) as follow:

Saline irrigation water levels: 1- 340 ppm (0.53ds/m) as control, 2- 1500 ppm (2.34ds/m),  
3-3000 ppm (4.7ds/m), 4- 4500 ppm (7.0ds/m).

Bio-stimulants: 1- spermidine (2.5 mM), 2- licorice root extract (5g/L),  
3-  $\alpha$ -lipoic acid (0.5 mM) 4- untreated (control)

Effect of these treatments on the growth, yield and chemical composition of *Ocimum basilicum* L. plants under drip irrigation system in newly reclaimed land of sandy soil were studied. Saline irrigation water levels, were prepared by dilution of the well water (EC 7.1 ds/m= 4544 ppm) by fresh water. The well water analysis (Table1) was carried out according to the methods described by **Fishman and Friedman (1985)**. The experiment soil sample's physical and chemical characteristics were assessed (Table 2) in accordance with **Jackson (1973)**.

**Table (1). Chemical characteristics of well water**

Characters	Value	Characters	Value
pH	8.1	EC	7.1 ds/m
Total dissolved solids	4731.80 (ppm)	Cations	
Anions		Ca <sup>2+</sup>	380.80 (ppm)
Cl <sup>-</sup>	2550 (ppm)	Mg <sup>2+</sup>	343.80 (ppm)
SO <sub>4</sub> <sup>2-</sup>	511.70 (ppm)	K <sup>+</sup>	11.50 (ppm)
HCO <sub>3</sub> <sup>-</sup>	122.00(ppm)	Na <sup>+</sup>	862.00 (ppm)

**Table (2). Soil physico-chemical properties of experimental soil**

Soil properties	Value	Unit	Soil properties	Value	Unit
Sand	91.1	%	Soluble cations and anions		
Silt	5.7	%	Ca <sup>++</sup>	1.69	mmol/kg
Clay	3.2	%	Mg <sup>++</sup>	1.25	mmol/kg
Texture	sandy	sand	Na <sup>+</sup>	0.38	mmol/kg
Water holding capacity	17.8	%	K <sup>+</sup>	0.78	mmol/kg
Organic matter	0.42	%	HCO <sub>3</sub>	0.40	mmol/kg
CaCO <sub>3</sub>	29.8	%	Cl <sup>-</sup>	1.64	mmol/kg
pH	8.31		SO <sub>4</sub> <sup>-</sup>	1.57	mmol/kg
Electric conductivity	0.45	ds/m	Available N	15.0	mg/kg
Available P	7.08	mg/kg	Available K	38.5	mg/kg

## 2.2. Preparation and analysis of licorice root extract (LRE)

In order to extract the active components from licorice root, 10 g of dried root was, soaked in 2L of water at 50°C for 24 hours, filtered, and the final volume was brought down to 2L using distilled water (5 g/L). Table 3 lists some of the chemical components of the licorice roots extract (A.O.A.C, 2005).

**Table (3). Chemical constituents of licorice roots extract (on dry weight basis)**

Components	Value (mg/kg)	Components	Value (mg/kg)	Components	Value (g /kg)
Total auxins	4.2	Total B vitamins	170	P	21.3
Total gibberellins	5.2	Vitamin E	65	(K)	47.2
Zeatin	4.1	Selenium (Se)	0.9	Ca	2.2
Salicylic acid	29.5	DPPH	84.6 %	Mg	3.8
$\alpha$ -Tocopherol	38.4	Total amino acids	172 (g /kg)	S	2.4
Glutathione	30.2	Free proline	36 (g /kg)	Fe	0.94
Ascorbic acid	41	Soluble sugars	148 (g /kg)	Mn	0.62
Vitamin A	154	N	20.2 (g /kg)	Zn	0.21

## 2.3. Experimental design

Uniform seedlings (12-15 cm) of basil (*Ocimum basilicum* L.cv. Genovese) plant supplied by the Horticulture Research Center in Dokki, Giza, Egypt, were transferred to the field soil in 1<sup>st</sup> April. The field experiment was conducted using drip irrigation system for saline irrigation that was described by Dehghanisanij *et al.*, (2006), four water-tanks were used to prepare the control and the saline irrigation water that was pumped into the irrigation system. Each water tank has a main pipeline (irrigate one main plot) was branched into four lateral pipelines, 50 cm apart. Each main plot, 7.6 m<sup>2</sup> (2 x 3.8 meter) divided into 3 subplots, each contain one replicate. Seedlings were transplanted along each lateral pipe, 5.0–7.0 cm away from each of the emitter and spaced 20 cm between the seedlings (48 plant /plot). The drippers deliver 2 liters/ hour for 90 minutes, three times weekly (Yousef *et al.*, 2008). It setup as split plot design with three replicates, the main factor was irrigation water contains Nile River fresh water (control) and three different concentrations of water well, that were applied after transplanting. The sub main one included anti-salinity treatments; spermidine and  $\alpha$ -lipoic acid (purchased from Sigma Chemical, St. Louis, M O, USA), and licorice root extract, in addition to a control (without any treatments) were applied as a foliar spray at the start of the transplanting. All tested treatments were sprayed separately with a hand sprayer in the early morning until run-off. This process was repeated four times following the irrigation treatments: the first one after the transplanting, then every month. For all foliar treatments, 0.05% tween-20 was utilized as a surfactant agent. All cultural practices were carried out as advised for the basil crop in sandy calcareous soils.

## 2.4. Morphological measures and chemical composition

Growth characters and chemical constituent's determinations were carried out at the first and second cuts at the middle of June and at the beginning of August respectively. Five plants were chosen at random from each replication for each treatment and the following data was noted for analysis;

### 2.4.1. Morphological measures

Vegetative growth data included plant height (cm), number of branches per plant, leaf area index (cm<sup>2</sup>), fresh and dry weights (g)/plant, and fresh and dry yield (tons/feddan) were recorded.

### 2.4.2. Chemical composition

Photosynthetic pigments; the amounts of carotenoids, chlorophyll (a), and chlorophyll (b) mg/g, in fresh leaves were measured in accordance with **Moran (1982)**. Proline (mg/g) was determined using the procedure described by **Bates *et al.*, (1973)**. The **Jackson (1973)** method was used to determine the percentages of total nitrogen. The percentages of total amino acids were determined According to **A.O.A.C, (2005)**. **Black (1965)** using a flame photometer to determine the percentages of potassium and sodium in the digested dried leaves.

### 2.4.3. Essential oil extraction

Essential oil percentage was determined in the air dried herb using the procedure according to the **British Pharmacopeia, (1963)**. After oil extraction, water traces were eliminated and basil oil was kept in an airtight vial at 4 °C in a refrigerator for analysis. **Charles and Simon's (1990)** formula was used to determine the oil percentage and yield.

$$\text{Volatile oil \%} = \frac{\text{Oil volume in the graduated tube}}{\text{Weight of dry matter (g)}} \times 100$$

$$\text{Oil yield/plant} = \frac{\text{Oil \%} \times \text{dry weight per plant}}{100}$$

### 2.4.4. GC/MS analysis

Essential oil was subjected to GC/MS analysis using a Hewlett Packard model 5890. Five series mass selective detector 9144 (HP) has been fitted in the gas chromatograph. The SE-54 coloum (30 m X 0.25 mm i.d.) was utilized. After injection, the oven temperature was kept at 60°C for two minutes before being scheduled to rise to 270°C in one minute. The injector temperature was set to 270 °C, and the MS condition was maintained at 280 °C and 42 eV. Compounds were identified by comparing their mass spectra with those recorded in the MS library, and this was further validated by comparing the mass spectra with those of reference compounds or with published data.

### 2.5. Statistical analysis

All obtained data from the field experiments were statistically analyzed as split plot Design ; water salinity levels (340 ppm ( control), 1500 ppm, 3000 ppm, and 4500 ppm) as main plot, and biostimulants [spermidine (2.5 mM), licorice root extract (5g/L), α-lipoic acid (0.5 mM) and untreated as control] foliar application as sub plot, using analysis of variance technique with three replicates for each treatment using the computer software program STATISTIX version 8.1 and the differences between the treatments means compared by using L.S.D. test at 5% probability (**Steel *et al.*, 1997**).

## 3. Results

### 3.1. Vegetative growth and yield characters

The vegetative growth and yield characters of sweet basil plants were significantly affected by different saline irrigation levels, bio-stimulants application and their interaction (Tables 4, 5, 6). Salinity stress significantly decreased all growth parameters by increasing water salinity from 1500 to 4500 ppm compared to non-saline treatment (340 ppm). Water salinity levels at 4500 ppm decreased mean values of plant height, no of branches/plant, leaf area index (cm<sup>2</sup>), fresh weight/plant and dry weight/plant by 48.32%, 53.92%, 31.28%, 67.84% and 58.76%, respectively in the 1<sup>st</sup> cut and by

**Table (4). The effect of water salinity (ppm) and bio-stimulants foliar application on vegetative growth characters of basil plants for two seasons**

Character	Plant height (cm)									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	50.19	40.53	33.33	26.35	37.60	61.21	49.42	40.64	32.14	45.85
Spermidine	61.33	54.29	41.75	31.20	47.14	74.79	66.20	51.24	38.05	57.57
Licorice roots extract	64.58	60.14	52.72	33.76	52.80	78.76	73.34	64.29	41.18	64.39
$\alpha$ -Lipoic acid	56.19	48.80	36.60	28.72	42.58	68.53	59.52	44.64	35.02	51.93
Mean (A)	58.07	50.94	41.10	30.01		70.82	62.12	50.20	36.60	
LSD A	1.67					2.07				
LSD B	2.09					2.56				
LSD A*B	4.19					5.12				
Second season (2023)										
Control	57.08	47.64	38.79	29.94	43.36	61.36	53.50	41.07	31.06	46.75
Spermidine	72.09	63.82	49.08	36.68	55.42	77.75	69.37	53.81	41.58	60.63
Licorice roots extract	75.92	70.70	61.98	39.70	62.08	80.57	75.65	65.46	46.15	66.96
$\alpha$ -Lipoic acid	66.06	57.37	43.03	33.76	50.06	73.96	63.34	45.50	35.39	54.55
Mean (A)	67.79	59.88	48.22	35.02		73.41	65.46	51.46	38.54	
LSD A	1.72					2.14				
LSD B	1.82					1.20				
LSD A*B	3.65					3.15				
Character	No. of branches / plant									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	9.67	7.50	5.67	4.00	6.71	13.33	10.33	7.00	5.00	8.92
Spermidine	13.12	9.76	7.33	6.35	9.14	18.33	13.67	9.33	8.00	12.33
Licorice roots extract	15.64	12.68	8.34	7.00	10.91	21.67	17.67	11.00	9.00	14.83
$\alpha$ -Lipoic acid	11.50	8.30	7.00	5.67	8.12	16.00	11.67	9.00	7.33	11.00
Mean (A)	12.48	9.56	7.09	5.76		17.33	13.34	9.08	7.33	
LSD A	0.95					1.00				
LSD B	0.84					0.81				
LSD A*B	1.68					1.61				
Second season (2023)										
Control	10.00	8.00	6.00	5.33	7.35	14.00	11.00	8.33	7.00	10.08
Spermidine	14.67	10.00	8.00	6.67	9.83	20.00	14.00	11.00	8.67	13.42
Licorice roots extract	16.33	13.67	9.67	8.00	11.92	19.33	19.00	13.33	10.33	15.50
$\alpha$ -Lipoic acid	12.00	9.00	7.33	6.00	8.58	16.33	12.67	10.33	7.67	11.75
Mean (A)	13.25	10.17	7.75	6.50		17.42	14.17	10.75	8.42	
LSD A	0.71					1.22				
LSD B	0.43					1.50				
LSD A*B	0.87					3.01				

- LSD at 5%    -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM)

48.31%, 57.70%, 32.40%, 63.80% and 54.13%, respectively in the 2<sup>nd</sup> cut of the 1<sup>st</sup> season compared to non-saline treatment. Similar results were obtained in the 2<sup>nd</sup> season. Among the bio-stimulants, licorice root extract had maximum mean values of plant height (62.08, 66.96 cm), no. of branches/plant (11.92, 15.50) and leaf area index (15.98, 18.46 cm<sup>2</sup>) in the 1<sup>st</sup> and 2<sup>nd</sup> cuts, respectively in the 2<sup>nd</sup> season, compared to control treatment.

Under saline irrigation treatment (1500 ppm), foliar application of licorice root extract attained the highest mean values and significantly increased fresh weight/plant from 138.80g to 167.50g and 231.50g



to 279.30g in the 1<sup>st</sup> and 2<sup>nd</sup> cuts of the 1<sup>st</sup> season, respectively and from 149.30g to 180.00 g and 247.80 g to 298.90 in the 1<sup>st</sup> and 2<sup>nd</sup> cuts of the 2<sup>nd</sup> season, respectively (Table 6).

**Table (5). The effect of water salinity (ppm) and bio-stimulants foliar application on the leaf area index (cm<sup>2</sup>) of basil plants for two seasons**

Character	Leaf area index (cm <sup>2</sup> )									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	14.76	11.83	10.94	8.90	11.61	16.37	12.96	12.13	10.09	12.89
Spermidine	17.20	14.24	13.47	12.86	14.44	19.06	15.79	15.57	13.64	16.02
Licorice roots extract	18.94	16.11	14.43	12.84	15.58	21.65	18.12	15.99	14.23	17.50
$\alpha$ -Lipoic acid	15.59	13.28	12.41	11.08	13.09	17.23	14.72	13.76	12.28	14.50
Mean (A)	16.62	13.87	12.81	11.42		18.58	15.39	14.36	12.56	
LSD A	1.16					0.30				
LSD B	0.42					0.23				
LSD A*B	0.83					0.45				
Second season (2023)										
Control	15.15	12.15	11.23	9.32	11.96	17.50	14.02	12.98	10.77	13.82
Spermidine	17.64	14.54	13.82	11.81	14.45	20.39	16.88	15.98	13.73	16.75
Licorice roots extract	19.43	16.52	14.80	13.18	15.98	22.14	19.37	17.10	15.23	18.46
$\alpha$ -Lipoic acid	15.99	13.62	12.74	11.37	13.43	18.47	15.74	14.72	13.14	15.52
Mean (A)	17.05	14.21	13.15	11.42		19.63	16.50	15.20	13.22	
LSD A	0.27					0.11				
LSD B	0.18					0.16				
LSD A*B	0.36					0.33				

- LSD at 5%    -Spermidine (2.5 mM), licorice root extract (5 g L-1) and  $\alpha$ -lipoic acid (0.5 mM)

Moreover, the effects of salinity treatments on fresh and dry herb yields (ton)/feddan were significant at all levels (Table 7). Irrigation with saline water at 4500 and 3000 ppm displayed severe yield losses in dry herb that reached up to 55.50 and 40.07%, respectively in the 1<sup>st</sup> season and up to 56.55 and 42.62% in the 2<sup>nd</sup> growing season.

On the other hand, the lowest saline level (1500 ppm) showed a less yield reduction which reached up to 14.97% compared to non-saline treatment. This indicates that sweet basil is a moderately saline crop. Foliar application of licorice root extract enhanced salinity stress tolerance in basil plants and significantly increased dry herb yield and recorded the highest mean values at 1500 ppm saline treatment as 2.27 ton/feddan in the 1<sup>st</sup> season and 2.18 ton/feddan in the 2<sup>nd</sup> season.

### 3.2. Photosynthetic pigments

The study examined the effects of spermidine, licorice extract, and lipoic acid treatments on chlorophyll a and chlorophyll b (mg/g fresh weight) in basil leaves. Results in Table 8 show that salinity levels significantly decreased the contents of chlorophyll a and b by increasing salinity levels from 340 to 4500 ppm in the two cuts in both seasons. The lowest photosynthetic pigment value was noted for the plants irrigated by 4500 ppm water salinity.

In the same line, it was observed that  $\beta$ -carotene (Table 9) in *Ocimum basilicum* plants had been significantly decreased along with water salinity concentrations. Furthermore, foliar application by the tested biostimulants (spermidine, licorice extract, and lipoic acid) ameliorated the salinity effect by

increasing photosynthetic pigments (mg/g) in basil plants over that of untreated plants at the same salinity level. Moreover, the foliar application of licorice root extract produced the highest values of photosynthetic pigments followed by spermidine treatment under the same salinity level in the two cuts during the two cultivated seasons.

Under saline irrigation treatment (1500 ppm), foliar application of licorice root extract recorded the highest mean values of chlorophyll a, b and  $\beta$ -carotene in the 1<sup>st</sup> and 2<sup>nd</sup> cuts for the two seasons.

**Table (6). The effect of water salinity (ppm) and bio-stimulants foliar application on the fresh and dry weights (g)/plant of basil plants for two seasons**

Character	Fresh weight (g) / plant									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	179.80	138.80	73.70	47.78	110.00	290.30	231.50	144.60	79.45	186.50
Spermidine	208.80	157.80	98.17	67.88	133.20	346.30	262.20	185.40	135.80	232.40
Licorice roots extract	226.70	167.50	110.20	82.45	146.70	362.80	279.30	200.90	145.10	247.00
$\alpha$ -Lipoic acid	201.80	147.80	89.52	64.84	126.00	328.50	243.50	171.10	117.80	215.20
Mean (A)	204.3	153.0	92.9	65.74		331.98	254.13	175.5	119.54	
LSD A	8.31					9.13				
LSD B	3.94					7.30				
LSD A*B	7.88					14.6				
Second season(2023)										
Control	193.30	149.30	79.23	51.37	118.30	303.80	247.80	154.80	85.05	197.90
Spermidine	224.40	169.60	105.50	72.97	143.10	370.60	280.60	198.50	145.40	248.80
Licorice roots extract	243.70	180.00	118.50	88.64	157.70	388.30	298.90	215.10	155.30	264.40
$\alpha$ -Lipoic acid	216.90	158.90	96.23	69.70	135.40	351.60	260.70	183.20	126.10	230.40
Mean (A)	219.6	164.4	99.9	70.7		353.6	272.0	187.9	128.00	
LSD A	2.82					5.19				
LSD B	3.19					3.06				
LSD A*B	6.38					6.12				
Character	Dry weight / plant (g)									
First season(2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	26.39	22.77	13.23	9.12	17.87	51.26	42.34	30.05	17.53	35.29
Spermidine	33.42	26.07	18.73	13.80	23.01	61.14	53.34	38.13	29.93	45.63
Licorice roots extract	36.43	30.73	21.31	17.53	26.50	64.05	60.62	42.75	33.92	50.58
$\alpha$ -Lipoic acid	32.30	23.11	16.31	12.56	21.07	58.00	47.13	36.90	26.13	42.04
Mean (A)	32.14	25.67	17.40	13.25		58.61	50.86	36.96	26.88	
LSD A	2.09					0.90				
LSD B	1.40					1.02				
LSD A*B	2.80					2.04				
Second season(2023)										
Control	28.36	24.28	14.22	9.80	19.17	53.64	45.32	29.49	18.76	36.80
Spermidine	35.93	29.47	20.13	14.84	25.09	67.78	54.29	39.43	30.15	47.91
Licorice roots extract	39.16	32.46	22.91	18.84	28.34	72.57	57.85	41.50	36.31	52.06
$\alpha$ -Lipoic acid	34.72	26.62	17.53	13.50	23.09	66.62	50.45	33.57	27.97	44.65
Mean (A)	34.54	28.21	18.70	14.25		65.15	51.98	36.00	28.30	
LSD A	2.41					2.06				
LSD B	1.28					0.94				
LSD A*B	2.57					1.88				

LSD at 5%, -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM)



**Table (7). The effect of water salinity (ppm) and bio-stimulants foliar application on the fresh and dry yield (tons/feddan) of basil plants for two seasons**

Character	Fresh yield (ton)/feddan									
	First season (2022)					Second season (2023)				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	11.75	9.25	5.46	3.18	7.41	12.43	9.93	5.85	3.41	7.90
Spermidine	13.88	10.50	7.09	5.09	9.14	14.88	11.26	7.60	5.46	9.80
Licorice roots extract	14.74	11.17	7.78	5.69	9.84	15.80	11.97	8.34	6.10	10.55
$\alpha$ -Lipoic acid	13.26	9.78	6.51	4.58	8.53	11.37	10.49	6.98	4.90	8.43
Mean (A)	13.41	10.18	6.71	4.63		13.62	10.91	7.19	4.97	
LSD A	1.68					1.17				
LSD B	0.57					0.23				
LSD A*B	1.15					0.46				
Dry yield (ton)/feddan										
	First season (2022)					Second season (2023)				
Control	1.94	1.62	1.08	0.67	1.33	1.85	1.72	1.09	0.71	1.34
Spermidine	2.36	2.02	1.42	1.11	1.73	2.59	2.02	1.48	1.12	1.80
Licorice roots extract	2.51	2.27	1.63	1.30	1.92	2.79	2.18	1.64	1.38	1.10
$\alpha$ -Lipoic acid	2.26	1.80	1.33	0.97	1.59	2.53	1.95	1.27	1.04	1.70
Mean (A)	2.27	1.93	1.36	1.01		2.44	1.97	1.40	1.06	
LSD A	0.16					0.22				
LSD B	0.11					0.13				
LSD A*B	0.22					0.26				

- LSD at 5% -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM ).

### 3.3. Na%, K %, N % and K<sup>+</sup>/Na<sup>+</sup> ratio

Results in Tables 10 and 11 shows that sodium (Na) percentages in basil plants increased significantly as water salinity rose from 1500 to 4500 ppm. Conversely, as salinity increased during both growing seasons, the contents of nitrogen (N) and potassium (K) were gradually declined. The highest Na concentrations were 3.14% and 3.17% in the first and second cuts of the second season, respectively, while the lowest K concentrations were 0.98% and 1.05% in the first and second cuts of the first season, respectively, for plants irrigated with 4500 ppm salinity (Table 10). The K<sup>+</sup>/Na<sup>+</sup> ratio also decreased significantly under these salinity conditions (Table 11).

Furthermore, foliar applications of spermidine, licorice root extract and lipoic acid significantly increased N and K percentages, as well as the K<sup>+</sup>/Na<sup>+</sup> ratio, compared to untreated plants and reduced Na level compared to control.

Of the treatments tested, licorice root extract produced the highest percentages of K and N, along with the most favorable K<sup>+</sup>/Na<sup>+</sup> ratios, followed closely by spermidine treatment under the same salinity levels. According to interaction between treatments, the maximum mean value of N, K and K<sup>+</sup>/Na<sup>+</sup> ratios were attained in plants treated with licorice root extract at 340 ppm followed by at 1500 ppm salinity level for the two seasons.

### 3.4. Proline and total amino acids (%)

Table 12 shows that proline content (mg/g) and total amino acids (%) in basil plants significantly increased with rising irrigation salinity. The highest content of proline and the highest total amino acid percentage were recorded in plants irrigated with the highest water at salinity (4500 ppm).

Our study indicated that foliar applications of spermidine, licorice root extract as well as lipoic acid elevated proline content and total amino acids in saline-irrigated basil plants compared to untreated plants in the two cuts of both seasons. Foliar application of licorice root extract produced the most significant increases in proline and total amino acids. Additionally, the highest mean values were found in plants treated with licorice extract at the 4500 ppm salinity level in the 2<sup>nd</sup> cut for the second growing season.

**Table (8). The effect of water salinity (ppm) and bio-stimulants foliar application on chlorophyll a, chlorophyll b (mg/g) of basil plants for two seasons**

Character	Chlorophyll a									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.53	0.48	0.36	0.31	0.42	0.58	0.53	0.38	0.34	0.46
Spermidine	0.64	0.58	0.49	0.38	0.52	0.71	0.64	0.54	0.41	0.58
Licorice roots extract	0.67	0.62	0.52	0.39	0.55	0.73	0.68	0.57	0.43	0.60
$\alpha$ -Lipoic acid	0.57	0.53	0.41	0.34	0.46	0.65	0.57	0.49	0.37	0.52
Mean (A)	0.60	0.55	0.45	0.35		0.67	0.61	0.49	0.39	
LSD A	0.026					0.027				
LSD B	0.021					0.023				
LSD A*B	0.046					0.044				
Second season (2023)										
Control	0.63	0.58	0.43	0.37	0.50	0.66	0.61 F	0.46	0.39	0.53
Spermidine	0.77	0.70	0.59	0.45	0.62	0.81	0.74 D	0.62	0.47	0.66
Licorice roots extract	0.80	0.74	0.62	0.47	0.66	0.84	0.78	0.65	0.50	0.69
$\alpha$ -Lipoic acid	0.71	0.63	0.53	0.40	0.57	0.75	0.65	0.56	0.43	0.60
Mean (A)	0.72	0.66	0.54	0.42		0.77	0.70	0.57	0.45	
LSD A	0.026					0.024				
LSD B	0.022					0.023				
LSD A*B	0.043					0.045				
Character	Chlorophyll b									
First season(2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.19	0.16	0.13	0.12	0.15	0.20	0.17	0.15	0.13	0.16
Spermidine	0.29	0.28	0.16	0.15	0.22	0.31	0.30	0.18	0.16	0.24
Licorice roots extract	0.33	0.31	0.19	0.16	0.25	0.36	0.33	0.21	0.17	0.27
$\alpha$ -Lipoic acid	0.27	0.24	0.15	0.13	0.20	0.29	0.26	0.16	0.15	0.21
Mean (A)	0.27	0.25	0.16	0.14		0.28	0.25	0.17	0.15	
LSD A	0.027					0.025				
LSD B	0.022					0.023				
LSD A*B	0.042					0.045				
Second season(2023)										
Control	0.20	0.16	0.14	0.12	0.16	0.21	0.17	0.15	0.13	0.17
Spermidine	0.30	0.29	0.17	0.16	0.23	0.32	0.31	0.18	0.17	0.24
Licorice roots extract	0.35	0.32	0.20	0.16	0.26	0.37	0.34	0.21	0.17	0.27
$\alpha$ -Lipoic acid	0.28	0.25	0.15	0.14	0.20	0.30	0.27	0.16	0.15	0.22
Mean (A)	0.29	0.27	0.17	0.15		0.30	0.27	0.18	0.16	
LSD A	0.026					0.027				
LSD B	0.021					0.023				
LSD A*B	0.046					0.044				

- LSD at 5% -Spermidine (2.5 mM), licorice root extract (5 g L-1) and  $\alpha$ -lipoic acid (0.5 mM).

**Table (9).** The effect of water salinity (ppm) and bio-stimulants foliar application on b-carotene (mg/g) of basil plants for two seasons

(mg/g) of bish plants for two seasons										
Character	Carotene									
First season(2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.35	0.32	0.24	0.21	0.28	0.38	0.35	0.25	0.23	0.31
Spermidine	0.43	0.39	0.33	0.25	0.35	0.47	0.43	0.36	0.27	0.38
Licorice roots extract	0.44	0.41	0.34	0.26	0.37	0.49	0.45	0.38	0.29	0.40
$\alpha$ -Lipoic acid	0.38	0.35	0.27	0.22	0.31	0.43	0.38	0.32	0.25	0.35
Mean	0.40	0.37	0.30	0.24		0.44	0.40	0.33	0.26	
LSD A	0.026					0.027				
LSD B	0.022					0.021				
LSD A*B	0.046					0.045				
Second season(2023)										
Control	0.42	0.38	0.29	0.25	0.33	0.44	0.41	0.30	0.26	0.35
Spermidine	0.51	0.46	0.39	0.30	0.42	0.54	0.49	0.41	0.32	0.44
Licorice roots extract	0.53	0.49	0.41	0.31	0.44	0.56	0.52	0.43	0.33	0.46
$\alpha$ -Lipoic acid	0.47	0.42	0.35	0.27	0.38	0.50	0.43	0.37	0.28	0.40
Mean (A)	0.48	0.44	0.36	0.28		0.51	0.46	0.38	0.30	
LSD A	0.047					0.027				
LSD B	0.040					0.023				
LSD A*B	0.079					0.046				

- LSD at 5% , -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM ).

### 3.5. Essential oil%, oil yield (ml/plant) and essential oil composition

The essential oil content and yield of basil plants were significantly affected by different water salinity levels and bio-stimulants application and their interaction. Essential oil content (%) increased gradually with increasing water salinity levels. Maximum mean values (0.83 and 0.87%) were obtained with the highest saline irrigation level (4500 ppm) in the 2<sup>nd</sup> cuts of the 1<sup>st</sup> and 2<sup>nd</sup> seasons, respectively which was non-significant with the lower level (3000 ppm) as shown in Table (13). The lowest value of essential oil content (0.65%) was obtained in the 1<sup>st</sup> cut of the 1<sup>st</sup> season in untreated bio-stimulants application. Moreover, the results showed that spermidine, licorice root extract and  $\alpha$ -lipoic acid enhanced the accumulation of sweet basil essential oil when compared to the control.

Notably, plants irrigated with water salinity at 4500 ppm and sprayed with licorice root extract showed the highest oil content (0.92%), whereas the highest oil yield (0.47 ml/plant) was obtained with the lowest saline treatment (1500 ppm) in the 2<sup>nd</sup> cut of the 2<sup>nd</sup> growing season.

The essential oil composition was largely affected by salinity stress and bio-stimulants application. Sweet basil essential oil was characterized by the identification of 22 components. The most abundant components were linalool (53.10 - 65.78%), methyl eugenol (3.66 - 16.28%), eugenol (0.44 - 12.75%), methyl (z) cinnamate (0.32 -10.33%), 1,8-cineol (2.66 - 8.74%), camphor (4.02 -12.75% and  $\alpha$ -terpineol (0.45 - 4.81%). Other components were identified in low proportions as shown in Table (14). As a result, the plant can be classified as the linalool chemo type.

Water salinity levels increased, the percentages of key compounds like linalool, 1,8-cineole, anethole, and eugenol rose compared to the control group. Salt stress also led to higher levels of methyl chavicol, geranyl acetate, and bornyl acetate.

All anti-salinity treatments enhanced linalool and eugenol percentages, especially in plants treated with licorice root extract. Conversely, control plants had the highest methyleugenol concentration (16.28%), which decreased with foliar applications of spermidine and lipoic acid, while licorice extract inhibited methyl eugenol.

**Table (10).** The effect of water salinity (ppm) and bio-stimulants foliar application on potassium%, sodium% of basil plants for two seasons

Character	Sodium%									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.49	1.14	2.22	2.81	1.66	0.52	1.79	2.57	2.99	1.97
Spermidine	0.29	0.69	1.32	1.59	0.97	0.32	0.74	1.40	1.82	1.07
Licorice roots extract	0.18	0.34	0.59	0.95	0.51	0.16	0.38	0.59	1.33	0.62
$\alpha$ -Lipoic acid	0.42	0.97	1.83	2.11	1.33	0.47	1.07	1.90	2.24	1.42
Mean (A)	0.35	0.78	1.49	1.87		0.37	1.00	1.61	2.10	
LSD A	0.43					0.19				
LSD B	0.34					0.08				
LSD A*B	0.69					0.15				
Second season (2023)										
Control	0.53	1.69	2.27	3.14	1.91	0.69	1.17	2.41	3.17	1.86
Spermidine	0.37	0.75	1.42	2.09	1.16	0.46	0.80	1.43	2.28	1.24
Licorice roots extract	0.22	0.37	0.84	1.14	0.64	0.24	0.41	1.13	1.42	0.80
$\alpha$ -Lipoic acid	0.46	1.04	1.97	2.69	1.54	0.63	1.14	2.07	3.14	1.75
Mean (A)	0.40	0.96	1.63	2.27		0.51	0.88	1.76	2.50	
LSD A	0.17					0.15				
LSD B	0.11					0.06				
LSD A*B	0.22					0.12				
Character	Potassium%									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	1.21	1.13	1.06	0.98	1.10	1.27	1.17	1.13	1.05	1.15
Spermidine	1.58	1.45	1.25	1.14	1.36	1.69	1.56	1.33	1.22	1.45
Licorice roots extract	1.69	1.51	1.37	1.20	1.44	1.80	1.63	1.46	1.28	1.54
$\alpha$ -Lipoic acid	1.35	1.27	1.14	1.04	1.20	1.44	1.36	1.21	1.11	1.28
Mean (A)	1.46	1.34	1.21	1.09		1.55	1.43	1.29	1.17	
LSD A	0.06					0.13				
LSD B	0.04					0.03				
LSD A*B	0.08					0.07				
Second season(2023)										
Control	1.37	1.28	1.24	1.15	1.26	1.46	1.39	1.30	1.23	1.34
Spermidine	1.85	1.65	1.46	1.34	1.57	1.93	1.83	1.56	1.39	1.68
Licorice roots extract	1.97	1.74	1.60	1.40	1.68	2.13	1.91	1.72	1.46	1.81
$\alpha$ -Lipoic acid	1.53	1.42	1.33	1.22	1.37	1.66	1.56	1.38	1.27	1.47
Mean (A)	1.68	1.52	1.41	1.28		1.79	1.67	1.49	1.34	
LSD A	0.12					0.14				
LSD B	0.07					0.06				
LSD A*B	0.13					0.11				

- LSD at 5% , -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM ).

**Table (11). The effect of water salinity (ppm) and bio-stimulants foliar application on K/Na ratio and Nitrogen % of basil plants for two seasons**

Character	K <sup>+</sup> /Na <sup>+</sup> ratio									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	2.49	0.99	0.48	0.35	1.08	2.46	0.65	0.44	0.34	0.97
Spermidine	5.43	2.12	0.94	0.72	2.30	5.33	2.10	0.96	0.67	2.27
Licorice roots extract	9.52	4.48	2.33	1.26	4.40	11.04	4.32	2.47	0.96	4.70
$\alpha$ -Lipoic acid	3.19	1.32	0.62	0.49	1.41	3.05	1.27	0.64	0.50	1.36
Mean (A)	5.16	2.23	1.07	0.71		5.47	2.08	1.13	0.62	
LSD A	0.21					0.29				
LSD B	0.09					0.16				
LSD A*B	0.18					0.33				
Second season (2023)										
Control	2.56	0.76	0.54	0.37	1.06	2.12	1.19	0.54	0.39	1.06
Spermidine	5.02	2.20	1.03	0.64	2.22	4.16	2.28	1.09	0.61	2.04
Licorice roots extract	8.81	4.74	1.90	1.23	4.17	8.95	4.65	1.53	1.03	4.04
$\alpha$ -Lipoic acid	3.33	1.36	0.67	0.45	1.45	2.62	1.36	0.67	0.41	1.26
Mean (A)	4.93	2.26	1.03	0.67		4.46	2.37	0.95	0.61	
LSD A	0.28					0.07				
LSD B	0.21					0.06				
LSD A*B	0.42					0.12				
Character	Nitrogen %									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	1.69	1.49	1.29	1.16 I	1.41	1.77	1.65	1.32	1.20	1.49
Spermidine	2.40	2.18	1.82	1.52	1.98	2.44	2.21	1.83	1.62	2.03
Licorice roots extract	2.65	2.47	1.95	1.67	2.18	2.71	2.53	1.99	1.74	2.24
$\alpha$ -Lipoic acid	2.22	1.87	1.40	1.28	1.69	2.24	2.04	1.46	1.33	1.77
Mean (A)	2.24	2.00	1.61	1.41		2.29	2.11	1.65	1.38	
LSD A	0.082					0.055				
LSD B	0.086					0.046				
LSD A*B	0.172					0.092				
Second season (2023)										
Control	1.78	1.57	1.36	1.23	1.48	1.86	1.74	1.39	1.27	1.57
Spermidine	2.53	2.30	1.92	1.60	2.09	2.59	2.34	1.94	1.72	2.15
Licorice roots extract	2.79	2.60	2.05	1.76	2.30	2.86	2.67	2.09	1.84	2.36
$\alpha$ -Lipoic acid	2.34	1.97	1.47	1.35	1.78	2.38	2.18	1.56	1.41	1.88
Mean (A)	2.36	2.11	1.70	1.39		2.42	2.23	1.74	1.56	
LSD A	0.077					0.313				
LSD B	0.069					0.190				
LSD A*B	0.138					0.380				

- LSD at 5% -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM ).

**Table (12). The effect of water salinity (ppm) and bio-stimulants foliar application on proline (mg/g) and total amino acids (%) of basil plants for two seasons**

Character	Proline (mg/g )									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.136	0.152	0.172	0.207	0.167	0.158	0.177	0.201	0.244	0.195
Spermidine	0.212	0.236	0.244	0.259	0.238	0.249	0.278	0.287	0.306	0.280
Licorice roots extract	0.243	0.259	0.276	0.293	0.268	0.286	0.307	0.326	0.347	0.317
$\alpha$ -Lipoic acid	0.166	0.227	0.234	0.247	0.218	0.193	0.267	0.276	0.292	0.257
Mean (A)	0.189	0.219	0.232	0.252		0.222	0.257	0.273	0.297	
LSD A	0.06					0.10				
LSD B	0.03					0.05				
LSD A*B	0.06					0.09				
Second season (2023)										
Control	0.147	0.164	0.186	0.225	0.180	0.169	0.191	0.217	0.264	0.210
Spermidine	0.229	0.257	0.265	0.281	0.258	0.269	0.302	0.312	0.332	0.304
Licorice roots extract	0.263	0.282	0.300	0.319	0.291	0.310	0.333	0.355	0.377	0.344
$\alpha$ -Lipoic acid	0.179	0.246	0.254	0.268	0.237	0.208	0.289	0.299	0.316	0.278
Mean (A)	0.205	0.237	0.251	0.273		0.240	0.279	0.296	0.322	
LSD A	0.046					0.066				
LSD B	0.036					0.041				
LSD A*B	0.073					0.082				
Character	Total amino acids (%)									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.44	0.47	0.52	0.58	0.50	0.47	0.50	0.55	0.62	0.54
Spermidine	0.60	0.64	0.67	0.70	0.65	0.65	0.69	0.72	0.75	0.70
Licorice roots extract	0.67	0.70	0.72	0.83	0.73	0.72	0.75	0.78	0.89	0.79
$\alpha$ -Lipoic acid	0.50	0.58	0.61	0.63	0.58	0.53	0.63	0.66	0.68	0.63
Mean (A)	0.55	0.60	0.63	0.68		0.59	0.64	0.68	0.74	
LSD A	0.027					0.029				
LSD B	0.023					0.024				
LSD A*B	0.046					0.048				
Second season (2023)										
Control	0.53	0.55	0.70	0.81	0.65	0.55	0.59	0.75	0.85	0.68
Spermidine	0.69	0.74	0.78	0.84	0.76	0.74	0.79	0.84	0.90	0.82
Licorice roots extract	0.80	0.88	0.96	0.98	0.90	0.86	0.94	1.02	1.06	0.97
$\alpha$ -Lipoic acid	0.62	0.70	0.75	0.81	0.72	0.66	0.75	0.80	0.88	0.77
Mean (A)	0.66	0.72	0.80	0.86		0.70	0.77	0.85	0.92	
LSD A	0.028					0.055				
LSD B	0.045					0.061				
LSD A*B	0.047					0.122				

LSD at 5% -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM)



**Table (13). The effect of water salinity (ppm) and bio-stimulants foliar application on essential oil% on dry weight basis and essential oil yield (ml/plant) of basil plants for two seasons**

Character	Essential oil%									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.58	0.63	0.69	0.71	0.65	0.60	0.65	0.71	0.74	0.68
Spermidine	0.64	0.72	0.81	0.83	0.75	0.66	0.74	0.84	0.85	0.77
Licorice roots extract	0.67	0.74	0.84	0.85	0.78	0.69	0.77	0.87	0.88	0.80
$\alpha$ -Lipoic acid	0.63	0.70	0.79	0.81	0.73	0.65	0.72	0.82	0.83	0.76
Mean (A)	0.63	0.70	0.78	0.80		0.65	0.72	0.81	0.83	
LSD A	0.03					0.03				
LSD B	0.02					0.02				
LSD A*B	0.05					0.05				
Second season (2023)										
Control	0.61	0.68	0.73	0.76	0.69	0.63	0.71	0.75	0.78	0.72
Spermidine	0.67	0.76	0.85	0.87	0.79	0.69	0.78	0.88	0.90	0.81
Licorice roots extract	0.71	0.79	0.88	0.89	0.82	0.73	0.81	0.91	0.92	0.84
$\alpha$ -Lipoic acid	0.66	0.73	0.83	0.85	0.77	0.68	0.75	0.86	0.88	0.79
Mean (A)	0.66	0.74	0.82	0.84		0.68	0.76	0.85	0.87	
LSD A	0.03					0.03				
LSD B	0.02					0.02				
LSD A*B	0.05					0.05				
Character	Essential oil yield (ml/plant)									
First season (2022)										
Cuts	First cut					Second cut				
Treatments	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)	340 ppm	1500 ppm	3000 ppm	4500 ppm	Mean (B)
Control	0.15	0.14	0.09	0.06	0.11	0.31	0.29	0.21	0.13	0.24
Spermidine	0.21	0.20	0.15	0.11	0.17	0.40	0.40	0.32	0.26	0.34
Licorice roots extract	0.24	0.23	0.18	0.15	0.20	0.44	0.47	0.38	0.30	0.40
$\alpha$ -Lipoic acid	0.20	0.17	0.13	0.10	0.15	0.38	0.34	0.30	0.22	0.31
Mean (A)	0.20	0.19	0.14	0.11		0.38	0.37	0.30	0.22	
LSD A	0.03					0.03				
LSD B	0.02					0.02				
LSD A*B	0.05					0.05				
Second season (2023)										
Control	0.17	0.16	0.10	0.07	0.13	0.34	0.32	0.22	0.15	0.26
Spermidine	0.24	0.22	0.17	0.13	0.19	0.47	0.42	0.34	0.27	0.38
Licorice roots extract	0.28	0.26	0.20	0.17	0.23	0.53	0.47	0.39	0.33	0.43
$\alpha$ -Lipoic acid	0.23	0.19	0.15	0.11	0.17	0.46	0.38	0.29	0.25	0.35
Mean (A)	0.23	0.21	0.16	0.12		0.45	0.40	0.31	0.25	
LSD A	0.03					0.03				
LSD B	0.02					0.02				
LSD A*B	0.05					0.05				

LSD at 5% -Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM)

**Table (14). The effect of water salinity (ppm), bio-stimulants foliar application and their interaction on constituents of basil essential oil of the 2<sup>nd</sup> cut in 2023 season**

Irrigation water  Spraying treatments		340 ppm				1500ppm				3000ppm				4500ppm			
		Control	Spermidine	Licorice root extract	Lipoic	Control	Spermidine	Licorice root extract	Lipoic	Control	Spermidine	Licorice root extract	Lipoic	Control	Spermidine	Licorice root extract	Lipoic
1	Linalool	53.10	55.51	56.05	54.97	57.36	60.22	60.47	59.60	61.27	62.62	63.56	61.87	63.92	65.03	65.78	64.08
2	$\alpha$ -terpineol	3.34	4.24	4.71	3.94	1.17	4.81	3.23	3.41	4.01	3.49	1.38	3.95	1.41	0.95	0.45	3.25
3	Terpinen-4-ol	1.97	1.55	-	1.21	1.95	-	-	-	-	-	2.60	-	-	-	-	1.38
4	1,8-cineol	2.66	3.60	6.60	7.57	8.72	5.71	7.70	7.25	6.70	1.23	4.90	6.11	8.74	4.25	4.35	6.17
5	Geroniol	3.98	3.23	0.32	3.36	3.32	3.25	2.13	-	2.57	3.41	2.96	2.53	2.00	2.83	2.83	2.41
6	Nerol	0.76	0.47	0.35	0.38	-	-	-	3.26	-	-	0.33	-	-	1.00	0.30	0.36
7	Methylchavicol	-	1.63	2.18	1.90	1.76	1.94	1.36	2.05	1.55	0.91	1.51	1.53	1.23	0.98	0.98	-
8	Anethole	0.97	0.95	1.12	0.96	1.43	-	1.26	-	1.00	1.59	0.95	1.00	1.13	0.84	0.84	0.92
9	Methyleugenol	16.28	3.66	-	4.86	-	-	-	-	-	-	-	-	-	-	-	-
10	Eugenol	0.44	9.17	12.75	6.79	11.52	10.71	11.18	11.03	10.16	12.06	10.05	-	9.43	12.45	12.75	10.93
11	Camphor	6.53	6.79	7.37	5.55	6.32	6.57	4.89	4.91	7.36	7.62	4.02	7.32	4.05	5.49	5.49	4.10
12	Linalyl acetate	3.87	5.20	4.13	5.33	2.80	3.36	1.85	3.73	2.91	3.57	5.28	2.95	2.14	3.89	2.98	3.39
13	Methyl(Z)cinnamate	0.68	0.32	0.48	0.34	0.85	0.37	0.42	0.61	-	0.67	-	10.33	0.42	-	-	0.33
14	Neryl acetate	1.85	0.12	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15	Bornyl acetate	-	1.08	1.89	1.63	1.63	2.09	1.68	2.05	1.72	1.67	1.12	1.66	1.27	0.91	0.91	0.95
16	Geranyl acetate	-	1.16	1.44	0.85	1.17	0.97	1.10	1.15	0.75	1.15	0.83	0.74	0.83	-	-	0.73
17	Citral	2.78	0.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18	$\alpha$ -terpinene	-	-	-	-	-	-	0.37	0.91	-	-	-	-	-	-	-	-
19	B-Caryophyllene	-	0.32	-	-	-	-	-	-	-	-	-	-	-	1.03	1.39	-
20	$\alpha$ -pinene	-	0.17	-	-	-	-	1.24	-	-	-	-	-	2.07	-	-	-
21	Myrcene	-	0.19	0.61	0.36	-	-	0.70	-	-	-	0.49	-	0.78	0.95	0.95	0.50
22	Limonene	-	-	-	-	-	-	-	-	-	-	-	-	0.38	-	-	-
TOTAL		99.21	99.6	100	100	100	100	99.58	99.92	100	99.98	99.96	99.98	99.60	99.20	100	99

- Spermidine (2.5 mM), licorice root extract (5 g L<sup>-1</sup>) and  $\alpha$ -lipoic acid (0.5 mM)

#### 4. Discussion

Our findings indicate that the morphological growth and herb production of basil plants were negatively affected by increasing salinity levels. The results of **Bashir *et al.* (2023)** and **Elhindi *et al.*, (2016 a)** are comparable to these findings. Salt stress alters metabolic processes such as meristematic activity and cell elongation reductions, these processes are linked to high respiration rates because of high energy requirements (**Ahmad *et al.*, 2022** and **Balasubramaniam *et al.*, 2023**). Additionally, salt stress resulted in smaller leaf areas because of the osmotic potential, the plant has adapted by reducing its transpiring surface or because the accelerated senescence of the leaves may result in the death (**Munns & Tester, 2008**). Licorice root extract exhibited the highest anti-salinity effects when sprayed on plant leaves producing the highest values of all characteristics, followed by spermidine then lipoic acid. **Nossier *et al.*, (2017)** highlighted the active ingredients of licorice extract, such as gibberellin and selenium, which enhance plant growth and yield under stress. Licorice root extract also contains essential mineral nutrients (such as nitrogen, zinc, calcium, potassium, sodium, magnesium and iron), bioactive components (such as amino acids, vitamins, phytohormones, and sugars), and antioxidants (such as glutathione and ascorbic acid) that promoting plant growth and production in both normal and stressful conditions (**Desoky *et al.*, 2019**). Regarding to spermidine and lipoic acid treatments, **Ekinci *et al.*, (2019)** found that exogenous spermidine mitigating the effects of salt stress on plants producing increase in plant growth, shoot length, and dry weight of leaves. In the same way, **Bashir *et al.*, (2023)** showed significant improvements in fresh and dry weights of tomato cultivars treated by lipoic acid under salt stress conditions. Lipoic acid acted to regulate the redox status of plants (**Perez Lopez *et al.* 2010**).

This study observed notable negative correlations between water salinity and the accumulation of potassium (K) and nitrogen (N), consequently affecting the K<sup>+</sup>/Na<sup>+</sup> ratio. Exogenous substances like licorice root extract, spermidine, and lipoic acid enhanced the plants' tolerance to water salinity. Salinity negatively impacts nitrogen and potassium levels due to higher concentrations of Na<sup>+</sup> and chloride ions in the soil, which disrupt the absorption of essential nutrients like nitrogen, potassium, and magnesium (**Iqbal *et al.*, 2015**). This may be due to competitive interactions which affect the ionic selectivity of cell membranes, reduce water uptake and relative water content, and inhibit plant roots' uptake of potassium and nitrate (**Howladar, 2014**).

In salt-stressed plants, the application of licorice root extract improved the K<sup>+</sup>/Na<sup>+</sup> ratio. Similarly, **Ghaloom and Faraj, (2012)** reported that licorice extract could reduce the K<sup>+</sup> outflow for good competition with Na<sup>+</sup>, hence improving the K<sup>+</sup>/Na<sup>+</sup> ratio, or may be because it increases endogenous hormones like GA<sub>3</sub> in treated plants, which increases the function of metabolic processes and their effect on tissue mineral content. Under the saline conditions, exogenous application of spermidine can decrease Na<sup>+</sup> and Cl<sup>-</sup> deposits in plants improving the K<sup>+</sup>/Na<sup>+</sup> ratios because of increased K<sup>+</sup> absorption which may have affected the competition with Na<sup>+</sup> at the binding site as well as the transport site (**Wang *et al.*, 2007a** and **Maathuis and Amtmann, 1999**). Also, lipoic acid treatment can also reduce sodium concentrations in leaves and increase potassium content under stress condition (**Bashir *et al.*, 2023**).

In the current study, the remarkable decrement in photosynthetic pigments content of basil plants as a result of increasing the salinity levels was mitigated by application of natural substances; spermidine licorice and lipoic acid. According to the previous studies, the decreased chlorophyll index and biomass production in *Ocimum basilicum* can be explained with ion toxicity caused by excessive salt levels (**Tolay, 2021**). This toxicity may result from the antagonistic effects of sodium on Mg absorption or attributed to disorganization of chloroplasts and photo-oxidation or peroxidation of chlorophyll pigments (**Hajbagheri and Enteshari, 2011** and **Paul and Roychoudhury, 2017**). Other opinions in

this respect; the decrease in carotenoids and chlorophyll content may be due to that salt stress opens porphyrin rings and harmful matters resulting from this dissolution which are transferred to vacuole and resulted in demolished green color of leaf or due to  $\beta$ -Carotene (beta-carotene) destruction and zeaxanthin formation (Parida and Das, 2005 and Sultana *et al.*, 1999).

Licorice root extract plays a key role in reducing the negative effects of salinity on leaf pigment content shown in the present study, this was emphasized by Desokya *et al.*, (2019) and Rady *et al.* (2019a). Licorice root extract (LRE) contains some nutrients and phytohormones, especially cytokinins that maintain higher leaf area and expand leaf photosynthetic pigments, and also micro-nutrients such as iron (Fe) in LRE support the main content in pretreated seedlings to promote enzymes involved in chlorophyll biosynthesis and some antioxidant enzymes that scavenge ROS and protect chlorophyll from degradation (Desokya *et al.*, 2019 and Zayed *et al.*, 2011).

Li *et al.* (2015) and Uzma *et al.* (2023) determined that the negative effect of salt stress on chlorophyll, carotenoids content was inhibited by exogenous spermidine treatment, ensuring that a sufficient supply of enzymes is available, thus promoting Chl synthesis and alleviating Chl degradation due to their acid-neutralizing and antioxidant properties, where, spermidine contains highly protonated. Also, lipoic acid application significantly raised the leaf Chl a, Chl b and carotenoid contents in both tomato cultivars under salt stress conditions (Bashir *et al.*, 2023).

In this study, basil plants accumulated more proline and amino acids in response to salt stress. In other hand, accumulation of proline and total free amino acids were a typical response to abiotic stress (Cik *et al.*, 2009). The increase in proline content could be attributed to a decrease in proline oxidase activity in saline conditions (Muthukumarasamy *et al.*, 2000). Additionally, Plants faces the osmotic pressure through accumulating osmolytes, proline in the vacuoles, to balance the osmotic potential under salt stress (Elhindi *et al.*, 2016 a). Also, significant increases in proline and total amino acids contents in basil plants sprayed with the tested biostimulants were found compared to control. Desoky *et al.* (2019) showed that LRE could overcome the salt stress in pea seedlings by increasing soluble sugars, proline, and antioxidant enzyme activities. While exogenous spermidine prevented the leakage of electrolytes as well as amino acids and recovered the damage of plasma membrane and contribute to osmotic adjustment by increasing the internal proline content thus reducing oxidative stress in plant grown under salinity stress (Duan *et al.*, 2008 and Roy *et al.*, 2005). Lastly, LA application improved antioxidant enzyme activities under both normal and stressful conditions, helping to minimize oxidative damage in LA-treated plants during salt stress (Bashir *et al.*, 2023).

Also, the study found that the percentage of essential oil in basil plants increased with rising salinity levels. These outcomes paralleled that of the study conducted by (Elhindi *et al.*, 2016b). Bernstein *et al.* (2010) stated that the increase in oil content is a concentration effect rather than a stimulation to its production. Neto *et al.* (2019) discussed the increase in essential oil content under stress conditions related to the biosynthesis stimulation and cannot be explained purely by the concentration effect. The promoting influence of studied bio-stimulants on essential oil % recorded in the present study were detected also by Abd El-Azim *et al.* (2017) and Bernstein *et al.* (2010). Higher salinity levels led to increased major constituents, mainly linalool and eugenol, whatever licorice extract as well as salinity stress providing the best essential oil quality by enhancing these compounds and inhibiting the production of methyleugenol. In a study conducted by Miele *et al.* (2001) they found that the content of eugenol and methyleugenol were correlated with plant height rather than plant age. Particularly, methyleugenol was predominant in plants up to 10 cm in height, whereas eugenol was prevalent in taller plants. They concluded that their results have to be considered, where the presence of methyleugenol could be undesirable for its carcinogenic potential.

## 5. Conclusion

The findings of this study demonstrated the licorice root extract, and spermidine respectively success of using some biostimulants in minimizing the effect of the accumulated salts and eliminate the stress conditions, and subsequently improve productivity of sweet basil.

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## تأثير بعض المحفزات الحيوية في تخفيف الإجهاد الملحي على إنتاجية الريحان الحلو

وسام محمد سراج الدين - فل الندى محمد صالح عريبي - ريم محمد الحسينى عبدالرؤوف

قسم بحوث النباتات الطبية والعطرية - معهد بحوث البساتين - مركز البحوث الزراعية - القاهرة - مصر.

### الملخص العربي

تعدّ الملوحة مشكلة رئيسية تؤثر تأثيراً سلبياً على النشاط الزراعي. ويرتبط انخفاض نمو المحاصيل الناتج عن الملوحة بالضغط الأسموزي لمحلول التربة في منطقة الجذور، مما يؤدي إلى بعض التغيرات الفينولوجية، وبالتالي انخفاض الإنتاجية. درس هذا البحث إمكانية استخدام بعض المحفزات الحيوية (مثل البولي أمين (سبيرميدين، ٢,٥ ملي مولار)، ومستخلص جذر عرق السوس (٥ جم/لتر)، وحمض ألفا ليبويك (٥,٥ ملي مولار) كرش ورقي لتقليل آثار الإجهاد الملحي الذي يتمثل في الري بالتنقيط بأربعة تركيزات من المياه المالحة (٣٤٠ (كنترول) و ١٥٠٠، و ٣٠٠٠، و ٤٥٠٠ جزء في المليون) وتأثيرهم على إنتاجية وجودة الريحان الحلو المزروع في التربة الجيرية الرملية في محطة البحوث الزراعية بعرب العوامر، محافظة اسيوط خلال موسمين زراعة (٢٠٢٢ و ٢٠٢٣). كشفت النتائج عن انخفاض تدريجي في جميع خصائص النمو والصبغات الضوئية والنيتروجين ومحتويات البوتاسيوم مع زيادة مستوى ملوحة ماء الري. بينما زاد البرولين والأحماض الأمينية الكلية والنسبة المئوية للصوديوم (Na%) بارتفاع مستويات الملوحة في كلا الموسمين. أظهر الري بالمياه المالحة عند ٤٥٠٠ و ٣٠٠٠ جزء في المليون خسائر فادحة (أكثر من ٥٠%). في المحصول الجاف. وتم الحصول على أعلى محتوى من الزيت العطري (٠,٩٢%) وأعلى إنتاج للزيت (٠,٤٧ مل/نبات) عند استخدام ملوحة (٤٥٠٠ و ١٥٠٠ جزء في المليون) على التوالي، وذلك للنباتات المرشوشة بمستخلص جذر عرق السوس. وقد وجد أن اللينالول والأوجينول هما المكونين الرئيسيين في زيت الريحان العطري. كما وجد الميثيل أوجينول في نباتات الكونترول بنسبة ١٦,٢٨% وهو مكون ضار بالصحة، ولكنه لم يظهر مع النبات المرشوشه بمستخلص عرق السوس والمرويه بالماء الملح، مما يظهر دور المستخلص في تحسين جودة الزيت العطري. ومن ثم، نلاحظ أن استخدام بعض المواد الطبيعية مثل مستخلص جذر عرق السوس والسبيرميدين كان له تأثير كبير في تخفيف الضرر التأكسدي الناتج عن الإجهاد الملحي.