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# EFFECT OF PROLINE FOLIAR SPRAY ON GROWTH AND PRODUCTIVITY OF SWEET BASIL (*Ocimum basilicum*, L.) PLANT UNDER SALINITY STRESS CONDITIONS

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**ABSTRACT:** Two pot experiments were carried out at Ornamental Nursery, Faculty of Agriculture, Zagazig University, Egypt, during the two consecutive seasons of 2016/2017 and 2017/2018 to study the effect of different saline irrigation water levels (0.0, 1000, 2000 and 3000 ppm), proline concentrations (0.0, 100 and 200 ppm) and their combinations on plant growth, salt resistance index, volatile oil production, photosynthetic pigments and proline content of sweet basil (*Ocimum basilicum*, L) plant. Plastic pots in 40 cm diameter filled with 6 kg clay soil were used in this experiment. This experiment was set up in a split-plot design with three replicates. The main plots were occupied by saline water levels and the sub plots were entitled to proline concentrations. The results showed a significant decrease in most studied plant growth parameters (plant height, number of branches/plant and herb and root dry weight/plant), salt resistance index, volatile oil production (volatile oil percentage and volatile oil yield/plant) and photosynthetic pigments (Chlorophyll a and chlorophyll b) with increasing the levels of salinity, but an opposite impact occurred with increasing the proline concentrations. Also, proline content was decreased as increasing the levels of salinity to reach the maximum values with 3000 ppm salinity level. However, for the combination among the levels of saline water and proline, the combination treatment (1000 ppm salinity level + 100 ppm proline) showed the highest increment in the growth and productivity of sweet basil plant under Sharkia Governorate conditions.

**Key words:** *Ocimum basilicum*, Saline irrigation water, proline acid, growth, chlorophyll.

## INTRODUCTION

Basil or sweet basil (*Ocimum basilicum* L.) is an perenial plant belongs to family Lamiacea, native to the tropical regions of Southeastern Asia and Africa, that includes around 30 species, which are much differentiated in respect to morphological and chemical features (**Vina and Murillo, 2003; Telci *et al*., 2006**). Sweet basil is the smell of the plant aromatic good savoring, added to food to impart delicious taste and fine odor and basil oil uses are diet benefactor and enters in scented soaps and aromatherapy industry. Sweet basil contains some antibiotics for some types of cancer, antioxidant, vitamins, beta-carotene and metal salts; such as Mn, Cu, Mg and K (**Marotti *et al*., 1996**). Sweet basil medical antiseptic properties are including the gut, repelling gas and anthelmintic, assistant digestion, lowers cholesterol, blood sugar and tonic for the immune system. It also inserts in the treatment of some diseases; such as intestinal colic, dysentery, boiled chronic diarrhea, vomiting in children, dental pain, kidney stones, headaches, colds, coughs and asthma (**Ozcan and Chalchat, 2002; Sajjadi, 2006**).

Salinity is one of the main abiotic factors negatively affecting plant production all over the world (**Koca *et al*., 2007**). Increasing salt concentrations in soil decreases the plant ability to absorb water, adversely affects metabolic processes and affects osmotic balance, nutrient absorbance, hydraulic conductivity, stomatal conductance, net photosynthetic rate, and

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intercellular $CO_2$ concentrations, all of this results in negatively affecting the plant ability to grow and develop (**Al-Karaki *et al*., 2001**). Furthermore, salinity stress affects the plant ability to uptake water in the root zone through decreasing the water potential of the soil (**Sabir *et al*., 2009**). This deficiency in available water under saline condition raises the potential of cells to be dehydrated which is a result of the osmotic stress caused by salinity. The higher ratios of toxic ions like $Na^+$ and $Cl^-$ damage the balance between ions through reducing the plant ability to absorb other ions like $K^+$, $Ca^{2+}$, and $Mn^{2+}$ (**Hasegawa *et al*., 2000**).

Accumulation of compatible solutes in high levels is needed to adjust the osmotic imbalance when plants confront several abiotic stresses including drought, salinity and chilling. Others include amino acid derivatives such as proline (**Parvaiz and Satyawati, 2008**). Application of proline for improvement of environmental stress tolerance was reported in several plants. **Hossain and Fujita (2010)** demonstrated that exogenous proline provided a protective action against salt-induced oxidative damage by reducing $H_2O_2$ and lipid peroxidation level and by enhancing antioxidant defense and methylglyoxal detoxification systems.

The present study was intended to evaluate whether the adverse effects of irrigation by saline water on sweet basil growth, salt resistance index, volatile oil production, photosynthetic pigments and proline content could be mitigated by proline foliar spray.

## MATERIALS AND METHODS

Two pot experiments were carried out at Ornamental Nursery, Faculty of Agriculture, Zagazig University, Egypt, during the two winter consecutive seasons of 2016/2017 and 2017/2018. This work was conducted to investigate the effect of different levels of saline irrigation water, proline concentrations and their combinations on plant growth, salt resistance index, volatile oil production, photosynthetic pigments and proline content of sweet basil plant (*Ocimum basilicum*, L).

The sweet basil seedlings were obtained from privt Nursery in Belbas District, Sharkia Governorate, Egypt. Seedlings were planted in the experimental plots on 15th October during the two seasons. The experimental unit was containing 12 pots. Pots in 40 cm diameter were filled with 6 kg clay soil (contained 48.24% clay, 28.45% silt and 23.31% sand) and irrigated with standard nutrient solution with different levels of saline water. the plants were irrigated immediately after plating with tap water for 2 weeks prior to application of the tredeatments Electrical conductivity (EC), pH, and the concentration of cations and anions in the salt and in the soil used in the pot experiment are shown in Table 1 according to **Chapman and Pratt (1978)**.

Seedlings were planted to be one plant/pot. The plants were irrigated with four salinity levels. The four levels of the artificial sea water were used by dissolving known weights of the natural salt crust of sea water(obtained from AL – max shipping company) in tap water. The four salinity levels were (0.0, 1000, 2000 and 3000 ppm). The plants were irrigated when needed to maintain soil moisture at 65-70 % of field capacity moisture percentage was maintained by adding required amount of water to overcome the loss through evaporation and transpiration (measured with a weighing balance) every day.

The source of proline acid [Pyrrolidine-2-carboxylic acid ($C_5H_9NO_2$)] was TECHNO GENE Company, Dokky, Giza, Egypt. Proline concentration treatments 0.0, 100 and 200 ppm were applied as foliar application weekly after two weeks of transplanting date as well as after two weeks of first cut. Each experimental unit received 2 L solution using spreading agent (Super Film at a rate of 1ml /l). The untreated control plants were sprayed with tap water with spreading agent.

This experiment was set up in a split-plot design with three replicates. The main plots were occupied by four saline water levels. The sub plots were entitled to three proline concentrations. The combination treatments between saline water levels and proline concentrations were 12 treatments.

### Data Recorded

#### Plant growth

In both seasons, the plants were harvested twice annually by cutting the aerial parts of each plant (5 cm) above the soil surface. The two cuts

**Table 1. Electrical conductivity (EC), pH, and the concentration of cations and anions of the salt extract and in the soil used in the pot experiment (average of two seasons)**

| Parameter | EC (mmhos/cm) | pH | Cations (meq/l) | | | | Anions (meq/l) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $Ca^{++}$ | $Mg^{++}$ | $Na^{+}$ | $K^{+}$ | $HCO_3^{-}$ | $SO_4^{--}$ | $Cl^{-}$ |
| **Soil clay** | 0.96 | 7.78 | 12.22 | 6.45 | 3.47 | 18.65 | 2.01 | 2.68 | 4.88 |
| **Salt extract at (5:1)** | 161.3 | 7.59 | 9.45 | 8.78 | 1542.54 | 1.98 | 5.68 | 78.62 | 1622.35 |

were taken on 15th January and 15th April in both seasons. In addition, plant height (cm), number of branches/plant and total herb dry weight/ plant (it is dried in oven at 45ºC) were determined. Also, sweet basil dry roots weight/ plant (g) was recorded, besides, the salt resistance index (SRI %), as a real indicator for salinity tolerance was calculated from the equation mentioned before by **Wu and Huff (1983)**: SRI (%) = Mean root length of the salt treated plants/mean root length of control one × 100.

### Volatile oil production

After the two cuts the volatile oil from dried herb of sweet basil plants was isolated by hydro distillation for 3 hr., in order to extract the volatile oil according to **Guenther (1961)** and the volatile oil yield per plant (ml) was calculated.

### Chemical constituents

In fresh leaf samples taken from the second cut during both seasons, photosynthetic pigments (chlorophyll a and b mg/g as fresh weight) were measured according to the methods of **Saric *et al*. (1967)**. Furthermore, In dry leaves of the two cuts during both seasons, the free amino acid proline (mg/100 g as dry weight) by the method explained by **Bates *et al*.(1973)** were determined.

## Statistical Analysis

The statistical layout of this experiment was split-plot experiment in completely randomized block design. Data were analyzed according to **Gomez and Gomez (1984)**. The means were compared using computer program of Statistix version 9 (**Analytical software, 2008**).

# RESULTS AND DISCUSSION

## Plant Growth

As shown in Tables 2, 3, 4 and 5, using salinity treatments, generally, decreased plant height, number of branches/plant as well as herb and root dry weights/plant of *Ocimum basilicum*, except 1000 ppm, compared to control in cuts during both seasons. Such decrease was significant with the levels of 2000 and 3000 ppm. A real indicator for salt tolerance (the salt resistance index), was as 100% for general control plants grown in soil without saline water irrigation and without foliar spray with proline acid (Table 6). However, the percentage of this index was increased to more than 100% for plants irrigated with either fresh water or saline water at low level (1000 ppm) in the two cuts during both seasons.

The decrease in dry weight of herb and root/plant might be due to that salinity increased osmotic pressure which caused a drop in plant water content as found by **Sanchesconde and Azura (1979)**. Moreover, **Pessarakli and Touchane (2006)** found that mechanism of salt may result in cell division inhibitory and hence, reduces the rate of plant development.

**Elhindi *et al*. (2017)** observed that negative effects were more apparent in the fresh and dry weight parameters clearly proves that salinity has a significant decreasing growth of sweet basil plants. **Shehata and Nosir (2019)** stated that under high levels of salinity (2000 and 4000 ppm NaCl), the plants present lowest values of plant height, shoot length, branch numbers/plant compared to control.

Tables 2, 3, 4, 5 and 6 shows that proline treatments at 100 ppm followed by 200 ppm concentration, significantly increase *Ocimum basilicum* growth parameters and salt resistance index percentage in the two cuts during both seasons compared to control. Moreover, **El-Sherbeny and Da Silva (2013)** found that a foliar application of 100 mg·l proline increased plant height, number of branches, fresh and dry weights of leaves of (*Beta vulgaris* L.) plant. **Dawood *et al*. (2014)** indicated that the foliar application of 25 mM proline caused significant

**Table 2. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* plant height (cm) in the two cuts during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) | 0.0 | 100 | 200 | Mean (S) |
| | 2016/2017 season | | | | 2017/2018 season | | | |
| | First cut | | | | First cut | | | |
| **0.0** | 37.67 | 45.00 | 41.67 | **41.44** | 38.67 | 42.00 | 38.67 | **39.78** |
| **1000** | 38.67 | 46.00 | 41.67 | **42.11** | 36.67 | 40.33 | 39.33 | **38.78** |
| **2000** | 30.33 | 34.67 | 33.00 | **32.67** | 28.33 | 33.33 | 31.67 | **31.11** |
| **3000** | 27.33 | 29.00 | 28.67 | **28.33** | 19.67 | 22.33 | 22.67 | **21.56** |
| **Mean (P)** | **33.50** | **38.67** | **36.25** | | **30.83** | **34.50** | **33.08** | |
| **LSD at 5%** | **(S)= 1.59** | **(P)= 1.12** | **(S×P)= 2.42** | | **(S)= 1.16** | **(P)= 1.11** | **(S×P)= 2.16** | |
| | Second cut | | | | Second cut | | | |
| **0.0** | 37.33 | 42.67 | 40.67 | **40.22** | 36.00 | 38.67 | 36.33 | **37.00** |
| **1000** | 39.00 | 44.33 | 41.33 | **41.56** | 37.33 | 39.33 | 38.33 | **38.33** |
| **2000** | 31.67 | 35.67 | 33.67 | **33.67** | 29.67 | 32.00 | 30.67 | **30.78** |
| **3000** | 23.00 | 28.33 | 25.33 | **25.56** | 18.00 | 23.67 | 22.67 | **21.44** |
| **Mean (P)** | **32.75** | **37.75** | **35.25** | | **30.25** | **33.42** | **32.00** | |
| **LSD at 5%** | **(S)= 1.20** | **(P)= 1.01** | **(S×P)= 2.04** | | **(S)= 1.61** | **(P)= 0.64** | **(S×P)= 1.92** | |

**Table 3. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* number of branches /plant in the two cuts during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) | 0.0 | 100 | 200 | Mean (S) |
| | 2016/2017 season | | | | 2017/2018 season | | | |
| | First cut | | | | First cut | | | |
| **0.0** | 16.00 | 21.33 | 18.33 | **18.56** | 14.67 | 19.67 | 16.33 | **16.89** |
| **1000** | 22.67 | 26.67 | 24.67 | **24.67** | 19.67 | 24.33 | 21.67 | **21.89** |
| **2000** | 14.33 | 18.33 | 17.00 | **16.56** | 14.00 | 16.33 | 15.00 | **15.11** |
| **3000** | 12.67 | 16.67 | 13.67 | **14.33** | 11.00 | 15.33 | 13.33 | **13.22** |
| **Mean (P)** | **16.42** | **20.75** | **18.42** | | **14.83** | **18.92** | **16.58** | |
| **LSD at 5%** | **(S)= 0.70** | **(P)= 0.54** | **(S×P)= 1.12** | | **(S)= 0.77** | **(P)= 0.74** | **(S×P)= 1.43** | |
| | Second cut | | | | Second cut | | | |
| **0.0** | 16.67 | 20.67 | 18.00 | **18.44** | 14.33 | 17.33 | 15.67 | **15.78** |
| **1000** | 21.67 | 25.67 | 23.67 | **23.67** | 19.00 | 23.67 | 20.67 | **21.11** |
| **2000** | 15.33 | 17.33 | 15.33 | **16.00** | 12.67 | 14.67 | 13.67 | **13.67** |
| **3000** | 13.00 | 16.00 | 13.33 | **14.11** | 11.33 | 15.00 | 12.33 | **12.89** |
| **Mean (P)** | **16.67** | **19.92** | **17.58** | | **14.33** | **17.67** | **15.58** | |
| **LSD at 5%** | **(S)= 1.09** | **(P)= 0.69** | **(S×P)= 1.57** | | **(S)= 0.81** | **(P)= 0.56** | **(S×P)= 1.21** | |

**Table 4. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* herb dry weight/plant (g) in the two cuts during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) | 0.0 | 100 | 200 | Mean (S) |
| | 2016/2017 season | | | | 2017/2018 season | | | |
| | First cut | | | | First cut | | | |
| **0.0** | 15.32 | 15.92 | 15.78 | **15.67** | 15.80 | 16.48 | 16.05 | **16.11** |
| **1000** | 15.52 | 16.35 | 15.55 | **15.81** | 15.59 | 16.29 | 15.81 | **15.90** |
| **2000** | 11.80 | 11.97 | 11.47 | **11.74** | 11.38 | 11.65 | 11.22 | **11.42** |
| **3000** | 10.97 | 11.33 | 10.93 | **11.08** | 10.62 | 10.78 | 10.78 | **10.73** |
| **Mean (P)** | **13.40** | **13.89** | **13.43** | | **13.35** | **13.80** | **13.47** | |
| **LSD at 5%** | **(S)= 0.15** | **(P)= 0.14** | **(S×P)= 0.28** | | **(S)= 0.21** | **(P)= 0.23** | **(S×P)= 0.43** | |
| | Second cut | | | | Second cut | | | |
| **0.0** | 14.66 | 15.06 | 14.82 | **14.85** | 14.18 | 14.68 | 14.51 | **14.46** |
| **1000** | 14.62 | 15.46 | 14.89 | **14.99** | 14.05 | 15.01 | 14.71 | **14.59** |
| **2000** | 10.47 | 10.84 | 10.44 | **10.58** | 10.05 | 10.45 | 9.98 | **10.16** |
| **3000** | 9.84 | 10.20 | 10.02 | **10.02** | 9.45 | 9.72 | 9.45 | **9.54** |
| **Mean (P)** | **12.40** | **12.89** | **12.54** | | **11.93** | **12.46** | **12.16** | |
| **LSD at 5%** | **(S)= 0.16** | **(P)= 0.08** | **(S×P)= 0.21** | | **(S)= 0.11** | **(P)= 0.14** | **(S×P)= 0.25** | |

**Table 5. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* root dry weight/plant (g) in the second cut during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | |
|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) |
| | 2016/2017 season | | | |
| **0.0** | 7.96 | 11.78 | 8.69 | **9.48** |
| **1000** | 8.21 | 12.72 | 10.43 | **10.45** |
| **2000** | 6.04 | 7.65 | 5.93 | **6.54** |
| **3000** | 4.35 | 5.79 | 4.57 | **4.90** |
| **Mean (P)** | **6.64** | **9.48** | **7.40** | |
| **LSD at 5%** | **(S)= 0.67** | **(P)= 0.57** | **(S×P)= 1.14** | |
| | 2017/2018 season | | | |
| **0.0** | 6.29 | 10.50 | 7.44 | **8.08** |
| **1000** | 8.57 | 11.21 | 8.85 | **9.54** |
| **2000** | 5.78 | 7.43 | 6.16 | **6.46** |
| **3000** | 3.25 | 5.11 | 4.13 | **4.16** |
| **Mean (P)** | **5.97** | **8.56** | **6.64** | |
| **LSD at 5%** | **(S)= 0.95** | **(P)= 0.65** | **(S×P)= 1.42** | |

**Table 6. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* salt resistance index (%) in the second cut during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | |
|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) |
| | | 2016/2017 season | | |
| **0.0** | 100.00 | 109.97 | 107.57 | **105.85** |
| **1000** | 110.64 | 119.80 | 114.44 | **114.96** |
| **2000** | 79.74 | 93.29 | 82.67 | **85.23** |
| **3000** | 57.15 | 81.22 | 61.66 | **66.68** |
| **Mean (P)** | **86.88** | **101.07** | **91.58** | |
| **LSD at 5%** | **(S)= 4.83** | **(P)= 2.00** | | **(S×P)= 5.82** |
| | | 2017/2018 season | | |
| **0.0** | 100.00 | 120.66 | 111.13 | **110.60** |
| **1000** | 107.92 | 127.81 | 119.89 | **118.54** |
| **2000** | 72.27 | 92.10 | 83.42 | **82.60** |
| **3000** | 61.93 | 77.05 | 64.24 | **67.74** |
| **Mean (P)** | **85.53** | **104.40** | **94.67** | |
| **LSD at 5%** | **(S)= 3.42** | **(P)= 2.58** | | **(S×P)= 5.42** |

increases in growth parameters of *Vicia faba* compared with the control. Also, **Rady *et al.* (2016)** demonstrated that all proline levels (3, 6 or 9 mM) caused a significant increase in growth characteristics and physiological attributes (shoot length, number of leaves and branches per plant, and shoot fresh and dry weights per plant)of both lupine varieties compared to the control.

Increasing the concentration of proline significantly influenced the plant height, branch number/plant, herb dry weight/plant, root dry weight/plant and salt resistance index under salinity levels (Tables 2, 3, 4, 5 and 6). It is clear from the obtained results that treating basil plants with proline at 100 ppm significantly was followed by stimulating the vegetative growth and root parameters under salinity levels in the two cuts during both experimental seasons. In most cases, there are no significant deference between the combination treatments of control (un-salinized plants) and 1000 ppm combined with 100 ppm proline concentration in both cuts during both seasons. Also, amino acids are a well known biostimulant which has positive effects on plant growth and significantly mitigates the injuries caused by a biotic stresses (**Kowalczyk and Zielony, 2008**).

Foliar application of proline is a shotgun approach in minimizing deleterious effects of salinity. Moreover, crop plants show resistance to oxidative damage by inducing high levels of antioxidants, accumulation of certain organic osmolytes and reducing the toxic ions ($Na^+$ and $Cl^-$). Furthermore, **Siddique *et al.* (2015)** demonstrated that foliar application of proline resulted in a significant increase in plant growth parameters of rice. The plant height, root growth and effective tillers /plant decreased with increased salinity levels. When the salt treated plants were supplied with exogenous proline, they produced significant amount of growth parameters. **Butt *et al.* (2016)** found that foliar application of praline under salt stress conditions stimulated chilli shoot and root length, plant fresh and dry mass and photosynthetic rate.

## Volatile Oil Production

Results presented in Tables 7 and 8 show that, volatile oil percentage and volatile oil yield/ plant were significantly decreased by saline water irrigation levels in the two cuts during both seasons compared to the lowest level (1000 ppm) and cotrol. However, the decrease in this connection were about 4.99 and 6.06 as well as 10.68 and 8.80 % for the salinity level at 3000 ppm in the first and second cuts during the first and second seasons, respectively. However, this result may be due to salt-induced water stress reduction of chloroplast stoma volume and regeneration of reactive oxygen species in playing an important role in the inhibition of photosynthesis seen in salt stressed plants (**Price and Hendry, 1991; Allen, 1995**). In this connection, it was found that increasing of salinity stress decreased almost essential oil amount in Chamomile (**Razmjoo *et al*., 2008**). There are report of an increase in essential oil percentage due to lower levels of salinity was also found in thyme (**Ezz El-Din *et al*., 2009**). Also, **Keramati *et al*. (2016)** showed that *O. basilicum* essential oil content and essential oil yield were decreased significantly with salinity increase. However, there was a slight increase (3% compared to the control) in essential oil content when basil plants were subjected to moderate salinity stress (3 dS $m^{-1}$ NaCl). **Polanski *et al*. (2018)** indicated that drought stress motivated a significant reduction in all of the growth parameters and essential oil yield and percent of peppermint plant.

Results of both seasons in Tables 7 and 8 indicate that, using proline at 100 ppm under irrigated with saline water at 1000 ppm resulted in significant increase in volatile oil percentage and volatile oil yield/plant compared to the highest rate of proline (200 ppm) combined with the same level of salinity. Concerning volatile oil production of sweet basil, it was found that the highest levels of salinity (2000 and 3000 ppm) combined without proline application gave the minimum values in this regard compared to the other combination treatments in the two cuts during both seasons. However, **Rady and Mohamed (2018)** reported that foliar spraying of 10 mM proline to wheat plants was effective in reducing the effects of irrigation with saline water on plant growth and salt tolerance which reflected on chemical constituents of *Triticum aestivum* plant.

## Photosynthetic Pigments and Proline Content

Results listed in Tables 9 and 10 suggest that, using salinity treatment at higher levels of 2000 and 3000 ppm decreased chlorophyll a or b content in leaves as fresh weight compared to control and the lowest water salinity level (1000 ppm) in the second cut during the two seasons. There was a decrease in chlorophyll content as salinity levels increased from 1000 to 2000 and then 3000 ppm. Such result confirmed the findings reported earlier by **Nouman *et al.* (2012**) on *Moringa oleifera* and **Ali *et al.* (2013**) on *Simmondsia chinensis*. In contrast, proline content in leaves as dry weight was increased as saline water irrigation levels increased to reach its maximum values with 3000 ppm in the two cut during the two seasons (Table 11). The increase in proline content could be attributed to a decrease in proline oxidase activity in saline conditions (**Muthukumarasamy *et al*., 2000**). Proline occurs widely in higher plants and accumulates in larger amounts than other amino acids. Plants under salt stress may present increased levels of certain compounds.

Chlorophyll a and b content was increased by using proline as foliar spray compared to control in both seasons (Tables 9 and 10). However, the highest values in this parameter were achieved with 100 ppm in the first and second seasons compared with control and the other one under study. While, the highest values in proline content were achieved with control when compared with 100 ppm in the first and second seasons (Table 11). Exogenous application of proline to culture medium resulted in an increase in the free proline content in callus cells of alfalfa (**Hayat *et al*., 2012**). Also, proline at the lower concentration (10 mg.l) improved various tomato plant chlorophyll a fluorescence parameters compared with control (**Kahlaoui *et al*., 2014**).

Moreover, Chlorophyll content (a or b) in sweet basil leaves was increased as a result of the treatment of proline at 100 ppm combined to those of salinity at 1000 ppm followed by control in comparison to those of salinity alone

**Table 7. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* volatile oil percentage in the two cuts during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) | 0.0 | 100 | 200 | Mean (S) |
| | 2016/2017 season | | | | 2017/2018 season | | | |
| | First cut | | | | First cut | | | |
| **0.0** | 0.730 | 0.850 | 0.767 | **0.782** | 0.757 | 0.847 | 0.783 | **0.796** |
| **1000** | 0.753 | 0.863 | 0.813 | **0.810** | 0.773 | 0.857 | 0.817 | **0.815** |
| **2000** | 0.703 | 0.803 | 0.727 | **0.744** | 0.693 | 0.763 | 0.703 | **0.720** |
| **3000** | 0.707 | 0.780 | 0.743 | **0.743** | 0.677 | 0.760 | 0.697 | **0.711** |
| **Mean (P)** | **0.723** | **0.824** | **0.762** | | **0.725** | **0.807** | **0.750** | |
| **LSD at 5%** | **(S)= 0.017** | **(P)=0.016** | **(S×P)=0.031** | | **(S)=0.020** | **(P)=0.013** | **(S×P)=0.029** | |
| | Second cut | | | | Second cut | | | |
| **0.0** | 0.707 | 0.777 | 0.743 | **0.742** | 0.710 | 0.760 | 0.707 | **0.726** |
| **1000** | 0.737 | 0.793 | 0.773 | **0.768** | 0.720 | 0.777 | 0.740 | **0.746** |
| **2000** | 0.673 | 0.750 | 0.697 | **0.707** | 0.637 | 0.720 | 0.640 | **0.666** |
| **3000** | 0.680 | 0.723 | 0.687 | **0.697** | 0.640 | 0.683 | 0.663 | **0.662** |
| **Mean (P)** | **0.699** | **0.761** | **0.725** | | **0.677** | **0.735** | **0.687** | |
| **LSD at 5%** | **(S)=0.019** | **(P)=0.011** | **(S×P)=0.026** | | **(S)=0.008** | **(P)=0.011** | **(S×P)=0.021** | |

**Table 8. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* volatile oil yield /plant (ml) in the two cuts during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) | 0.0 | 100 | 200 | Mean (S) |
| | 2016/2017 season | | | | 2017/2018 season | | | |
| | First cut | | | | First cut | | | |
| **0.0** | 0.547 | 0.677 | 0.580 | **0.601** | 0.577 | 0.667 | 0.603 | **0.616** |
| **1000** | 0.587 | 0.707 | 0.633 | **0.642** | 0.597 | 0.693 | 0.630 | **0.640** |
| **2000** | 0.400 | 0.473 | 0.397 | **0.423** | 0.377 | 0.447 | 0.380 | **0.401** |
| **3000** | 0.347 | 0.403 | 0.370 | **0.373** | 0.323 | 0.387 | 0.347 | **0.352** |
| **Mean (P)** | **0.470** | **0.565** | **0.495** | | **0.468** | **0.548** | **0.490** | |
| **LSD at 5%** | **(S)=0.016** | **(P)=0.015** | **(S×P)=0.030** | | **(S)=0.011** | **(P)=0.007** | **(S×P)=0.017** | |
| | Second cut | | | | Second cut | | | |
| **0.0** | 0.517 | 0.587 | 0.550 | **0.551** | 0.513 | 0.583 | 0.527 | **0.541** |
| **1000** | 0.547 | 0.617 | 0.570 | **0.578** | 0.503 | 0.593 | 0.557 | **0.551** |
| **2000** | 0.353 | 0.410 | 0.370 | **0.378** | 0.320 | 0.383 | 0.330 | **0.344** |
| **3000** | 0.313 | 0.350 | 0.320 | **0.328** | 0.290 | 0.327 | 0.307 | **0.308** |
| **Mean (P)** | **0.432** | **0.491** | **0.452** | | **0.407** | **0.472** | **0.430** | |
| **LSD at 5%** | **(S)=0.019** | **(P)=0.009** | **(S×P)=0.024** | | **(S)=0.007** | **(P)=0.009** | **(S×P)=0.016** | |

**Table 9. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* chlorophyll a content (mg/100g as fresh weight) in second cut during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | |
|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) |
| | | 2016/2017 season | | |
| 0.0 | 2.69 | 2.83 | 2.73 | **2.75** |
| 1000 | 2.75 | 2.90 | 2.79 | **2.81** |
| 2000 | 2.57 | 2.66 | 2.55 | **2.59** |
| 3000 | 2.42 | 2.50 | 2.45 | **2.45** |
| Mean (P) | **2.60** | **2.72** | **2.63** | |
| LSD at 5% | (S)= 0.03 | (P)= 0.04 | | (S×P)= 0.07 |
| | | 2017/2018 season | | |
| 0.0 | 2.74 | 2.87 | 2.82 | **2.81** |
| 1000 | 2.78 | 2.87 | 2.85 | **2.83** |
| 2000 | 2.50 | 2.60 | 2.52 | **2.54** |
| 3000 | 2.42 | 2.51 | 2.48 | **2.47** |
| Mean (P) | **2.61** | **2.71** | **2.67** | |
| LSD at 5% | (S)= 0.02 | (P)= 0.02 | | (S×P)= 0.03 |

**Table 10. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* chlorophyll b content (mg/100g as fresh weight) in second cut during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | |
|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) |
| | | 2016/2017 season | | |
| 0.0 | 0.79 | 0.87 | 0.85 | **0.83** |
| 1000 | 0.83 | 0.89 | 0.86 | **0.86** |
| 2000 | 0.72 | 0.77 | 0.72 | **0.74** |
| 3000 | 0.70 | 0.76 | 0.72 | **0.73** |
| Mean (P) | **0.76** | **0.82** | **0.79** | |
| LSD at 5% | (S)= 0.02 | (P)= 0.02 | | (S×P)= 0.04 |
| | | 2017/2018 season | | |
| 0.0 | 0.85 | 0.89 | 0.84 | **0.86** |
| 1000 | 0.86 | 0.91 | 0.86 | **0.88** |
| 2000 | 0.68 | 0.78 | 0.70 | **0.72** |
| 3000 | 0.69 | 0.75 | 0.69 | **0.71** |
| Mean (P) | **0.77** | **0.83** | **0.77** | |
| LSD at 5% | (S)= 0.01 | (P)= 0.01 | | (S×P)= 0.02 |

**Table 11. Influence of saline water irrigation level (S), proline concentration (P) and their combination (S×P) treatments on *Ocimum basilicum* proline content (mg/g as dry weight) in the two cuts during 2016/2017 and 2017/2018 seasons**

| Saline water level (ppm) | Proline concentration (ppm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.0 | 100 | 200 | Mean (S) | 0.0 | 100 | 200 | Mean (S) |
| | 2016/2017 season | | | | 2017/2018 season | | | |
| | First cut | | | | First cut | | | |
| **0.0** | 3.42 | 2.97 | 3.23 | **3.20** | 3.63 | 3.30 | 3.53 | **3.49** |
| **1000** | 4.20 | 3.57 | 4.00 | **3.92** | 4.53 | 3.73 | 4.27 | **4.18** |
| **2000** | 4.83 | 4.13 | 4.43 | **4.47** | 5.20 | 4.30 | 4.80 | **4.77** |
| **3000** | 6.87 | 5.67 | 6.43 | **6.32** | 7.07 | 5.53 | 6.70 | **6.43** |
| **Mean (P)** | **4.83** | **4.08** | **4.52** | | **5.11** | **4.22** | **4.82** | |
| **LSD at 5%** | **(S)= 0.13** | **(P)= 0.13** | **(S×P)= 0.25** | | **(S)= 0.06** | **(P)= 0.10** | **(S×P)= 0.18** | |
| | Second cut | | | | Second cut | | | |
| **0.0** | 3.07 | 2.80 | 2.93 | **2.93** | 3.13 | 3.03 | 3.00 | **3.05** |
| **1000** | 3.83 | 3.03 | 3.67 | **3.51** | 3.23 | 3.50 | 4.13 | **3.62** |
| **2000** | 4.07 | 3.67 | 4.07 | **3.93** | 3.57 | 3.83 | 4.23 | **3.88** |
| **3000** | 6.47 | 5.40 | 6.47 | **6.11** | 6.57 | 5.30 | 6.33 | **6.07** |
| **Mean (P)** | **4.36** | **3.72** | **4.28** | | **4.12** | **3.92** | **4.42** | |
| **LSD at 5%** | **(S)= 0.14** | **(P)= 0.17** | **(S×P)= 0.31** | | **(S)= 0.15** | **(P)= 0.07** | **(S×P)= 0.19** | |

(2000 and 3000 ppm) or those of the other ones of combination between proline and salinity in the two seasons. On the other hand, the best values in proline content were achieved without the treatment of proline combined to those of salinity at 2000 and 3000 ppm in comparison to those of salinity alone (1500 ppm) or those of the other ones of combination between proline and salinity in the two seasons. This improved efficiency of photosynthetic pigments may be attributed to stimulating chlorophyll biosynthesis and/or inhibiting its degradation, as well as the more efficient scavenging of reactive oxygen species (ROS) and stabilizing photosynthetic reactions by proline and other antioxidant compounds (**Abdelhamid *et al*., 2013**). For the combination among the levels of NaCl and proline, the interaction treatment (12 ds. $m^{-1}$ NaCl + 150 mg.l proline) showed the highest increment in total chlorophyll and true proline **(Al-Mayahi and Fayadh, 2015)**.

## Conclusion

From above mentioned results, it is preferable to spray *Ocimum basilicum* plants with proline at 100 ppm under moderate salt stress (1000 ppm) to enhance the plant growth, salt resistance index, volatile oil production, photosynthetic pigments and proline content of sweet basil plant under Sharkia Governorate conditions.

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# تأثير الرش الورقي بالبرولين على نمو وإنتاجية نبات الريحان تحت ظروف الإجهاد الملحي

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أجريت تجربتان أصص في مشتل الزينة، كلية الزراعة، جامعة الزقازيق، مصر خلال الموسمين المتتاليين ٢٠١٦ / ٢٠١٧ و ٢٠١٧ / ٢٠١٨ لدراسة تأثير مستويات الري بالماء المالح (صفر، ١٠٠٠ ، ٢٠٠٠ و ٣٠٠٠ جزء/مليون)، وتركيزات البرولين (صفر ، ١٠٠ و ٢٠٠ جزء / مليون) والتداخلات بينهما على نمو النبات ودليل تحمل الملوحة و إنتاج الزيت العطري والصبغات النباتية والمحتوى من البرولين لنبات الريحان . استخدمت أصص بلاستيكية بأقطار ٤٠ سم تم تعبئتها بـ ٦ كجم تربة طينية لهذه التجربة، كان تصميم هذه التجربة قطع منشقة مرة واحدة في ثلاث مكررات، وزعت مستويات الماء المالح في القطع الرئيسية و تركيزات البرولين في القطع تحت الرئيسية، أظهرت النتائج نقص معنوي في معظم قياسات النمو (ارتفاع النبات وعدد الأفرع/ نبات والأوزان الجافة للعشب والجذور) ودليل تحمل الملوحة وإنتاج الزيت العطري (النسبة المئوية للزيت العطري ومحصوله / نبات) والصبغات النباتية (كلوروفيل أ و ب) مع زيادة مستويات الملوحة، لكن علي النقيض من ذلك أدى إضافة البرولين إلى زيادة كل من النمو الخضري ودليل تحمل الملوحه وإنتاج الزيت العطري وكذلك الصبغات النباتية، مع ذلك، لدراسة التداخل بين مستويات الماء المالح والبرولين، كانت أعلى القيم لنمو وإنتاجية الريحان تحت ظروف محافظة الشرقية مع معاملة التداخل بين تركيز الملوحة ١٠٠٠ جزء/مليون + ١٠٠ جزء/مليون برولين.

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