

## Effect of Salinity Stress on Anise (*Pimpinella anisum* L.) Seedling Characteristics under Hydroponic Conditions

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**Abstract:** This Crop yield depends upon various soil and environmental factors. Productivity can adversely be affected due to the fluctuations in any of these factors. Anise is a vital medicinal aromatic plant; provides valuable nutrition to human body. Present study was conducted under hydroponic conditions to evaluate the effect of different salinity levels (control, 3, 6, 9, 12 dS m<sup>-1</sup>) on Anise growth and physiochemical attributes. Statistically analyzed that plant samples showed that there was significant reduction in root length (66%) shoot length (44%), shoot fresh weight (47%), root fresh (54%), shoot dry (68%), and root dry (79%) weights with increasing level of salinity. While, physiological parameters like Membrane Stability Index (28%), chlorophyll a (66%), chlorophyll b (55%) and carotenoids (42%) reduction was recorded as compare to the control treatment. While, there was significant increase in Na<sup>+</sup> concentration in shoots (60%), roots (87%) and K<sup>+</sup> concentration in shoots (44%) and root (66%) decrease with the increase in salinity. It was concluded that Anise is a sensitive crop against salinity at seedling growth stage. However, by increasing the salinity levels the growth and production of Anise significantly decreased but at lower salinity (1-3 dS m<sup>-1</sup>), crop can survive and gave a reasonable yield.

**Keywords:** Anise, salinity stress, tolerance, hydroponic.

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## 1. Introduction

Anise (*Pimpinella anisum* L.), is belongs to family Apiaceous is an annual vital spice and medicinal plant, and is considered as source of raw material for cosmetic, perfumery, pharmaceuticals and food industries (Omidbaigi et al., 2003; Rocha and Fernandes, 2016; Ross, 2001; Samojlik et al., 2012; Tepe and Tepe, 2015). In recent years, it has gained more attraction and value due to antifungal, antimicrobial, insecticidal and antioxidative effect of the Anise (Tirapelli et al., 2007). The worldwide production of Anise essential oil is about 40 to 50 tons per annum. Anise seed also contains a significant quantity of  $\gamma$ -himachalene, anisaldehyde, cis-anethole

and estragol (Ullah et al., 2014). Anise fruits (2-6%) and roots (0.05%) contains essential oil (Koul et al., 2008). Largest producers of anise oil including France, Poland, Russia, Spain and USA (Malhotra, 2012). Ground and dry Anise seed as whole and in its powder form is readily available in the spice market year around.

In aromatic and medicinal plants, growth, yield and essential oil production are subjected by numerous environmental factors, e.g. salinity stress (Aghaei and Komatsu, 2013; Akhtar et al., 2017; Penella et al., 2016). Anise is an essential medicinal spice (Lu et al., 2012; Tepe and Tepe, 2015) and its production is adversely affected by the salinity stress. The Anise production is not only depends upon the

tissue sources but also may be incorporated with the salt-induced stress factors (Sangwan et al., 2001).

Salinity is among the main drastic threats for plant in the countries of semi-arid and arid regions, including Pakistan, which adversely affect the plants yield (Schleiff, 2008). Globally over 800 million ha land is affected by sodicity (434 million ha) or salt or salinity (397 million ha) (Bakht et al., 2011). Salt affected area in Pakistan is 10.01 million hectares and due to salinity crop yield losses are feared to reach at Rs. 880 Million (Behzad et al., 2014). The ground water quality of Bahawalpur and its adjacent areas is declining rapidly. A recent survey showed that 52.78% water of Bahawalpur is unfit while 34.37% water considered as fit for irrigation and remaining 12.85% is marginal fit waters for irrigation purposes as compared to standard values (Riaz et al., 2018). Among all these factors, soil salinity is a main limiting factor for plant growth, development and yields (Yihdego, 2017).

Salinity stress is a multifaceted mechanism that affects nearly every biochemical and physiological pathways in plants (Kibria et al., 2017; Nam et al., 2017; Negrão et al., 2017; Shen et al., 2018). Exchangeable salts cause toxic effects on growth of plant in salt affected soils are produced due to (a) water stress results due to low osmotic potential of soil (b) nutritional imbalance due to higher accumulation of toxic ions (c) specific ion effect (d) and any amalgamation of above mentioned factors (Ashraf and Harris, 2004). Osmotic potential of soil decreases due to high concentration of cationic metals and salts in soil solution which results in water stress and it create difficulty for plant in absorbing the water essential for normal growth. Under these circumstances leaf water potential also decreased and ultimately crop production decline (Ali and Yun et al., 2017; Riaz et al., 2018). Under salt stress germination is the most sensitive stage, salinity hinders seed germination as well as reduces the number of

germinated seeds (Hannachi and Van Labeke, 2018; Houle et al., 2001; Mauromicale and Licandro, 2002). High degree of mortality of seeds is the principal cause of poor germination ultimately poor plant population results in low yield under high saline conditions (Pennings et al., 2005). Reduction in  $K^+$  uptake is caused by high  $Na^+$  ion concentration in soil solution and also caused chlorosis and necrosis on plant leaves (Gopal and Dube, 2003; Kere et al., 2016). Generally, reduction in leaf osmotic potential reduces the plants capability to tolerate salt stress either by accumulation of inorganic ions or by organic solutes synthesis (Hannachi and Van Labeke, 2018; Hasegawa et al., 2000).

Realizing the importance of aromatic plants and the severity of salt stress and high electrical conductivity of ground water in these areas, an attempt have been made to increase the understanding about the salinity effects on anise seedlings, physiological and ionic uptakes in the root and shoot.

## 2. Materials and Methodology

### 2.1. Experimental Site and Conditions

The experiment was conducted at rain-protected glass house of Department of Soil Science, University College of Agriculture and Environmental Sciences, The Islamia University of Bahawalpur, Pakistan. Treatments were arrangement in complete randomized design (CRD) conducted in triplicate.

The experiment was conducted during 2016. Seeds of Anise were collected from Department of Oilseed, Regional Agriculture Research Institute (RARI), Bahawalpur, Pakistan. The sterilized seeds of Anise was selected and sown in sand culture for obtaining uniform seedlings. After 25 days of sowing at two leaves stage, seedlings were transplanted into thermo pole sheet floating on half strength Hoagland solution culture in 15L iron tubs.

**Table 1: Effect of salinity stress on growth parameters of Anise**

Treatments	RL (cm)	SL (cm)	RFW (g)	SFW (g)	RDW (g)	SDW (g)
Control	42.33 a	50.24 a	5.20 a	9.32 a	0.29 a	1.10 a
T <sub>1</sub>	38.10 a	45.5 b	4.31 b	8.72 a	0.25 b	0.96 ab
T <sub>2</sub>	32.87 b	38.85 c	3.94 b	7.73 b	0.19 c	0.85 b
T <sub>3</sub>	27.82 b	31.98 d	3.51 c	6.95 c	0.15 d	0.68 c
T <sub>4</sub>	14.42 c	27.42 e	2.43 d	4.93 d	0.63 e	0.36 d
LSD	4.073	3.012	0.421	0.73	0.041	0.150

Columns sharing the same letter are non-significant at  $p < 5$ . Control:  $< 0.1 \text{ dS m}^{-1}$  EC, T<sub>1</sub>:  $3 \text{ dS m}^{-1}$  EC, T<sub>2</sub>:  $6 \text{ dS m}^{-1}$  EC, T<sub>3</sub>:  $9 \text{ dS m}^{-1}$  EC, T<sub>4</sub>:  $12 \text{ dS m}^{-1}$  EC, RL: root length, SL: shoot length, RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW: shoot dry weight.

**Table 2. Effect of salinity stress on physiological and biochemical attributes of Anise**

Treatment	MSI	Chlorophyll a ( $\mu\text{g g}^{-1}$ )	Chlorophyll b ( $\mu\text{g g}^{-1}$ )	Carotenoids ( $\mu\text{g g}^{-1}$ )
Control	44.14 a	0.52 a	2.4 a	123.3 a
T <sub>1</sub>	40.33 b	0.49 a	2.16 ab	110.0 ab
T <sub>2</sub>	37.95 bc	0.44 b	1.82 b	100.3 bc
T <sub>3</sub>	35.22 c	0.38 c	1.57 bc	87.0 cd
T <sub>4</sub>	32.22 d	0.29 d	1.11 c	72.0 d
LSD	2.98	0.046	0.60	16.488

Columns sharing the same letter are non-significant at  $p < 5$ . Control :< 0.1 dS m<sup>-1</sup> EC, T<sub>1</sub>:3 dS m<sup>-1</sup> EC, T<sub>2</sub>:6 dS m<sup>-1</sup> EC, T<sub>3</sub>:9 dS m<sup>-1</sup> EC, T<sub>4</sub>:12 dS m<sup>-1</sup> EC. MSI: membrane stability index.

Salinity was created by using different calculated doses of NaCl according to the treatments. Daily pH (7.0) of the medium was maintained. Aeration was given by bubbling air through aeration pump. The solution was changed after every seven days of period. Plants were exposed to Control = < 0.1 dS m<sup>-1</sup> EC; T<sub>1</sub> = 3 dS m<sup>-1</sup> EC; T<sub>2</sub> = 6 dS m<sup>-1</sup> EC; T<sub>3</sub> = 9 dS m<sup>-1</sup> EC; T<sub>4</sub> = 12 dS m<sup>-1</sup> EC.

## 2.2. Plant growth, physical and biochemical characteristics

The seedlings were collected after fifty days and subsequently thoroughly washed with double deionized water. To determine fresh and dry weights of plants and plant parts, 10 plants were randomly selected from each replication and well washed. These selected plants were separated into shoot and root for the determination their fresh and dry weights. After calculation of fresh weight, plant parts were oven dried at 65 °C till constant weight and dry weight was calculated. The remaining seedlings were grounded and further used for physical biochemical analysis including chlorophyll a and b, carotenoids and membrane stability index. Dried material (0.5 g) was digested in concentrated H<sub>2</sub>SO<sub>4</sub> (2 ml) subsequently Ca<sup>+</sup>, K<sup>+</sup> and Na<sup>+</sup> were determined with a flame photometer (Jenway, PFP7, Essex, UK).

## 2.3. Quality Control

All the chemicals used in this study were NIST traceable. Considering sensitivity and repeatability

standard solutions were used in sample sequence after every 8 samples. The analytical precision and accuracy were accepted only when RSD values were below 5% for the REEs, according to the results of duplicate measurements of all samples.

## 2.4. Statistical Analysis

Collected data sets were statistically analyzed using analysis of variance (ANOVA). Treatment means were compared by the least significant difference (LSD, 5%) (Steel et al., 1997). All data analyses were performed by using Statistix 8.0 (Analytical Software, 2003).

## 3. Results

### 3.1. Growth attributes

The data presented shows the impact of salinity stress on attributes of Anise (Table 1). The results showed that root and shoot length was significantly decreased as salinity increased in growth medium. Maximum lengths of both root and shoot of anise seedlings were recorded in control treatment, however gradually decreased with increasing level of salinity. Minimum shoot length (27.42 cm) and root length (14.42 cm) were observed at highest salinity level i.e. T<sub>4</sub> (12 dS m<sup>-1</sup>). Root length under medium salinity levels (T<sub>2</sub>:6 dS m<sup>-1</sup> EC, T<sub>3</sub>:9 dS m<sup>-1</sup> EC) remained statistically similar with each other, but greater than the root length produced under T<sub>4</sub>.

**Table 3. Effect of salinity stress on ionic balance in shoots of Anise**

Treatments	K <sup>+</sup> (ppm)	Ca <sup>+</sup> (ppm)	Na <sup>+</sup> (ppm)	Na <sup>+</sup> /K <sup>+</sup>	Na <sup>+</sup> /Ca <sup>2+</sup>
Control	886.5 a	232.7 a	125.8d	0.14 e	0.54 d
T <sub>1</sub>	779.9 b	197.9 b	519.4 c	0.66 d	2.59 d
T <sub>2</sub>	698.4c	156.2 c	946.1b	1.35 c	6.10 c
T <sub>3</sub>	600.76 d	104.21 d	1036.7 ab	1.72 b	9.96 b
T <sub>4</sub>	500.3e	54.5 e	1148.5 a	2.30 a	3.25 a
LSD	98	29	112	0.28	2.87

Columns sharing the same letter are non-significant at  $p < 5$ . Control :< 0.1 dS m<sup>-1</sup> EC, T<sub>1</sub>:3 dS m<sup>-1</sup> EC, T<sub>2</sub>:6 dS m<sup>-1</sup> EC, T<sub>3</sub>:9 dS m<sup>-1</sup> EC, T<sub>4</sub>:12 dS m<sup>-1</sup> EC.

**Table 4. Effect of salinity stress on ionic balance in roots of Anise**

Treatments	K <sup>+</sup> (ppm)	Ca <sup>+</sup> (ppm)	Na <sup>+</sup> (ppm)	Na <sup>+</sup> /K <sup>+</sup>	Na <sup>+</sup> /Ca <sup>2+</sup>
Control	982.1a	192.2a	132.6 e	0.13 e	0.56 d
T <sub>1</sub>	752.2 b	145.2 b	418.7 d	0.55 d	2.11 cd
T <sub>2</sub>	628.6 c	116.0c	663.4 c	1.05 c	4.26 c
T <sub>3</sub>	518.2 d	77.2 d	856.0b	1.65 b	8.22 b
T <sub>4</sub>	437.1e	32.8e	1012.4a	2.33 a	18.90 a
LSD	104	24	103	0.25	4.33

Columns sharing the same letter are non-significant at  $p < 5$ . Control :< 0.1 dS m<sup>-1</sup> EC, T<sub>1</sub>:3 dS m<sup>-1</sup> EC, T<sub>2</sub>:6 dS m<sup>-1</sup> EC, T<sub>3</sub>:9 dS m<sup>-1</sup> EC, T<sub>4</sub>:12 dS m<sup>-1</sup> EC,

Correspondingly, root fresh weights (RFW), root dry weights (RDW), results exhibited that their maximum values were produced under control treatment and showed decreasing trend with increasing level of salinity.

### 3.2. Physiological and Biochemical Parameters

The results regarding membrane stability index (MSI), carotenoid and chlorophyll (a and b) contents showed in Table 2. Our findings depicted that salinity stress significantly ( $p \leq 0.05$ ) decreased the physiological parameters with increasing levels of salinity. Maximum decline in MSI (32.22), chlorophyll-a (0.29  $\mu\text{g g}^{-1}$ ), chlorophyll-b (1.11  $\mu\text{g g}^{-1}$ ) and carotenoid contents (72  $\mu\text{g g}^{-1}$ ) was observed at the treatment T<sub>4</sub> (12 dS m<sup>-1</sup>) with respect to control and all other treatments. The declining trend was as follows: Control > T<sub>1</sub> > T<sub>2</sub> > T<sub>3</sub> > T<sub>4</sub>.

### 3.3. Ionic Balance

The behavior of K<sup>+</sup> uptake in shoot and root of Anise are presented in Tables 3, 4. The data showed that at highest level (T<sub>4</sub>:12 dS m<sup>-1</sup>) of salinity there was minimum uptake of K<sup>+</sup> in both shoot and root. It was also observed that maximum K<sup>+</sup> was accumulated in shoot when compared to root at various treatment levels.

The increasing level of salinity the potassium and calcium concentration was significantly decreased in shoot, while sodium concentration, Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> was significantly increase with salinity (Table 3). The results highlighted that increasing level of salinity the potassium and calcium concentration was significantly decreased in root, while sodium concentration, Na<sup>+</sup>/K<sup>+</sup> and Na<sup>+</sup>/Ca<sup>2+</sup> were significantly increase with salinity (Table 4).

## 4. Discussion

In present study the screening method is used to evaluate the tolerance of Anise against salinity levels at seedling and vegetative growth stage. The growth

attributes i.e. plant height, fresh and dry weights (shoot and root), significantly ( $p < 0.05$ ) decreased under salinity stress and this inhibitory effect was more on seedling and vegetative growth stage at 12 dS m<sup>-1</sup> EC.. This decreased was due to the high salts concentration which may directly affect the morphological and physiological characteristics and hence the vegetative growth declined (Talat et al. 2013; Shabani et al., 2012).

Under salinity stress excessive uptake of toxic ions like Cl<sup>-</sup> and Na<sup>+</sup>, and inhibition of cell division lead to restricted plant growth. These ions disturb the osmotic potential of vacuoles and cause cytoplasmic dehydration. In addition, high salt concentration in root zone decreases water potential leading to low availability of water and on-set of osmotic stress (Chartzoulakis et al., 2002). Due to this salt-induced osmotic stress, cell expansion and growth is inhibited resulting in poor root and shoot development. The inhibition in growth is also attributed due to the disturbance in hormonal balance (Huez-Lopez et al., 2011; Khan et al., 2003).

The results showed that plant physiological attributes, i.e. membrane stability index carotenoids, chlorophyll (a and b) contents were negatively affected by the salinity stress. The reduction in these parameters is may be due to the degradation of chloroplasts and other photosynthetic pigments (Moradi and Ismail, 2007; Kafi, 2009). Salinity effects photosynthesis by reducing efficiency of different plant mechanisms including efficient water use (Sultana et al., 1999). Chlorophyll pigments are the principal components of photosynthesis. Under salt stress, the concentration of chlorophyll (a and b) and total chlorophyll significantly decreases. Reduction in these, results in inhibition of photosynthetic activity (Xinwen et al., 2008; Redondo-Gómez et al., 2011).

The chemical analysis of leaves and root revealed that under salinity stress, plants showed higher contents of toxic ions and low level of different

essential nutrients. The results are parallel with the study of Khan et al. (2009) as they showed that under salinity stress, there was high concentration of  $\text{Cl}^-$  and  $\text{Na}^+$  ions and decrease in the concentration of  $\text{Ca}^{2+}$ ,  $\text{K}^+$  and  $\text{Mg}^{2+}$  was observed. Under salinity stress, due to higher concentration of salts plants tend to uptake excessive toxic ions which interferes the biochemistry of plant cells (Byrt et al., 2018; Munns and Tester, 2008).

The major toxic ions are  $\text{Na}^+$  and  $\text{Cl}^-$  that are taken up by plant up to toxic level (Munns, 2005; Munns and Gilliam, 2015; Bhuiyan et al., 2017; Tetsuya and Sergey, 2018). Higher concentrations of these ions in soil result in the reduced uptake of water leading to osmotic stress (Mayak et al., 2004). Zhu (2007) also reported that due to similar ionic nature of  $\text{Na}^+$  competes with  $\text{K}^+$  and plant uptake excessive  $\text{Na}^+$  instead of  $\text{K}^+$ , resulting in  $\text{K}^+$  deficiency and toxicity of  $\text{Na}^+$  in all plant parts. Decrease in  $\text{K}^+$  uptake and increase in  $\text{Na}^+$  uptake result in enhanced  $\text{Na}^+/\text{K}^+$  ratio. This  $\text{Na}^+/\text{K}^+$  ratio decrease the soluble sugars resulting in disruption of solute biochemistry in cells (Ibrahim et al., 2006). The  $\text{Na}^+$  in high concentration inhibits the activity of many cellular enzymes. This inhibition is also dependent on how much potassium is present as higher ratio of  $\text{Na}^+/\text{K}^+$  is the most damaging (Blumwald et al., 2000; Rahman et al., 2016).

## 5. Conclusion

It was concluded from present study that salinity stress adversely affected the development and growth by affecting height of plant, shoot and root dry and fresh weights, membrane stability index, chlorophyll 'a' and 'b' and carotenoid contents of Anise mainly due to higher concentration of toxic ions ( $\text{Na}^+$ ) and deficiency of essential elements especially  $\text{K}^+$  and  $\text{Ca}^{2+}$  that are mainly involved in osmoregulation. In order to reduce negative impact of salinity on agricultural productivity it is important to reduce its impact on germination and seedling growth. This study indicates that increasing in salinity level decreases the Anise production at seedling growth stage.

**List of abbreviations:** RDW, root dry weight; RL, root length; RFW, root fresh weight; SDW, shoot dry weight; SL, shoot length; SFW, shoot fresh weight.

**Author Contribution:** A.S. conducted the research study, Z.J. helped in conducting the research trial, U.R. overall prepared and formatted the manuscript. M.E. helped in result writing, M.N. Helped in graph preparing, S.A.Z. and M.A.K. helped in data statistics.

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