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# Effects of Salinity and Calcium on the Growth, Ion Concentration and Yield of Olive (*Olea europaea* L.) Trees

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# ABSTRACT

In this study, we investigated the interaction effects of salinity (NaCl) and CaCl<sub>2</sub> on olive trees. The experiment was conducted with three levels of NaCl (0, 40 and 80 mg  $L^{-1}$ ) and four levels of  $Ca^{2+}$  (0, 50, 100 and 150 mg  $L^{-1}$ ). The effects of NaCl and CaCl<sub>2</sub> on the growth and ion concentrations in olive (Olea europaea cv. Manzanillo) were investigated. The results showed that the annual and accumulated yield, fruit size and vegetative growth ratio were affected by salts. Shoot length was higher in plants treated with CaCl<sub>2</sub>, although shoot growth was reduced at 50 mg  $L^{-1}$  NaCl. The NaCl concentrations in plants were also affected by the  $Ca^{2+}$ ,  $K^+$  and N concentration.  $Ca^{2+}$  supply linearly increased leaf  $Ca^{2+}$  concentration and decreased leaf  $Na^+$  concentration. Leaf  $Ca^{2+}$ ,  $K^+$ , and N decreased under salt stress. The results obtained from this experiment showed that salt stress caused a significant reduction in plant growth and leaf number and weight. Therefore, the high-Ca tolerance index for yield was effective in screening for high fruit number and high yield of olive, and the high-salinity tolerance caused to decrease for olive yield. The effect of CaCl<sub>2</sub> and salinity was significant the yield and growth of olive plants.

Key words: Olea europaea, salinity, toxicity, yield and growth of olive

# INTRODUCTION

Salinity stress represents a worldwide increasing environmental problem for crop production [6]. Olive (*Olea europaea*) locally known as zaitoon, is a small-growing evergreen tree, native to parts of southern Europe and Asia Minor [20]. Cracked green "seasoned" Manzanilla is a table olive specialty that is progressively gaining the favor of consumers and increasing its production, which reached 7,000,000 kg in 2005/2006 season [1]. Olive trees are mainly grown in semiarid regions with Mediterranean climate, where scarce and irregular rainfall causes low yields. Around the Mediterranean Basin, olive trees have been traditionally cultivated in dry lands. However, the water demand for irrigation is increasing in olive orchards, because of enhanced yields and profits [21], leading to the use of low-quality water resources. Olive trees are considered moderately tolerant to salinity [25]. Olive trees cultivation in saline soils depends on the cultivar [16]. Olive grows successfully in saline soils, where other fruit tree crops cannot be grown [5]. The reduction of photosynthesis in the plants treated with salinity was reported by many researchers [28]. Study on the mechanism of salt tolerance in olive is low. The existence of intraspecific variability

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for salt tolerance in olive has been reported [29].  $Ca^{2+}$  supply to the saline soil solution regulates  $Na^+$  uptake by plants and can prevents the accumulation of toxic levels of  $Na^+$  [15].  $Ca^{2+}$  supply in the irrigation water probably had a positive effect on protection of the cell wall and the plasmatic membrane and regulates the selectivity of ionic uptake [18, 30]. An apparent increase in salt tolerance has been noted when  $Ca^{2+}$  levels supplied under saline conditions [30]. High salt concentration in soils inhibits crop growth and yield and is one of the major constraints in agricultural production in arid regions [17]. The role of  $Ca^{2+}$  has not been sufficiently studied in some perennial trees such as the olive tree. The leaf injury symptoms associated with Na. Effect of  $Ca^{2+}$  on alleviating the toxic effect of  $Na^+$  depends on the  $Ca^{2+}$  concentration [8]. Current study was aimed to evaluate the effect of different concentrations of NaCl and  $CaCl_2$  on the growth, ion concentration and yield of olive (*Olea europaea* cv. Manzanillo). In addition, we studied the role of  $Ca^{2+}$  nutrition on the incidence of salinity in olive.

# MATERIALS AND METHODS

#### **Plant Materials and Treatments**

Plants were grown in the different treatments for 3 months before being examined for symptoms of toxicity. This study was carried out on the effects of salts in mature olive trees in a long term field experiment (during 2010-2011). Six-year-old olive trees (*Olea europaea* cv. Manzanillo) were cultivated under drip irrigation with saline water composed of a mixture of NaCl and CaCl<sub>2</sub>. Plants were initially watered daily for 21 days. After 21 days of acclimation, plants were irrigated two times per week with different concentrations of NaCl (0, 40 and 80 mg L<sup>-1</sup>) and CaCl<sub>2</sub> (0, 50, 100 and 150 mg L<sup>-1</sup>) for each treatment. The chemical analysis of the growing medium is presented in Table 1.

#### **Plant Growth Measurement**

At the end of the experiment and after application of treatments, plant growth parameters (leaf area and leaf fresh weight) were measured. The areas of primary leaves were determined by an area meter (Crump Scientific Products, UK. [10]. Leaf fresh weight was obtained by a digital balance.

#### Leaf Nutrient Analysis

At the end of the experiment, eight leaves were randomly sampled per plant for module of Ca, K, N and Na concentration. Leaf samples from the middle of the shoot were collected, then were washed once with tap and twice with distilled water, after that they were dried at 75°C for 24 h and were ground to a fine powder to pass a 30 mesh screen. A portion of 0.5 g of the fine powder of each sample was dried (as ash) in a muffle furnace at 515°C for 5 h. Then, the ash was dissolved in 3 mL of 6-N-hydrochloric acid and diluted with double distilled water up to 50 mL and the concentrations of Ca, N, K, and Na elements were determined by atomic absorption spectroscopy. The concentration of microelements and macronutrients was expressed as dry weight percentage.

#### Yield Measurement

At the end of the experiment, the numbers and weight of fruits per plant computation were measured in each of the plants per treatment. Fruit weight was calculated by a digital balance.

#### **Statistical Analysis**

Experiment was designed on the basis of completely randomized block design with 3 replications per treatment and 36 olive trees. Analysis of variance (ANOVA) was done using SPSS statistical software and means were compared using Duncan's test (DMRT).

#### **RESULTS AND DISCUSSION**

The results of the present study indicated that young olive plants were subjected to high saline conditions. The application NaCl in olive plants may be due to the reduced uptake and transport of  $Ca^{2+}$ . These results are in disagreement with those observed by Sotiropoulos and Dimassi [26] in kiwifruit; where, K<sup>+</sup> and Ca<sup>2+</sup> decreased in the presence of NaCl. Ca<sup>2+</sup> supply to the saline solution and consequently increasing in the Ca<sup>2+</sup>: Na<sup>+</sup> ratio enhanced plant growth (Table 2). Salinity treatments resulted in a slower plant growth and a smaller final shoot weight and shoot length. The two main saline ions also had a different influence on growth parameters. The 12 weeks after the beginning of the proliferation experiment, the following growth parameters were evaluated: number of shoots longer than 7 mm per explants, their length and productivity (number of shoots the average shoot length) and the fresh and dry weights. Shoot length was measured at 3 month intervals on each experimental plant. Relative growth was

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determined as the length of the shoot of salt-treated plants expressed as a percentage of that of the control plants. Addition of 90 mg L<sup>-1</sup> NaCl to growth medium, significantly reduced leaf nitrogen and potassium concentrations (Table 3). Little growth was obtained when  $CaCl_2$  was not supplied to the olive trees. Shoot length, significantly increased with CaCl<sub>2</sub> concentration, showing a quadratic response that indicates a reduction in shoot growth at the highest CaCl<sub>2</sub> concentrations. Result showed significant difference ( $p \le 0.05$ ) in the yield of treatments. Sodium Chloride treatment only produced leaf toxicity symptoms. Growth reduction following salt treatment in olive is generally attributed to excessive salt accumulation in growing tissues [14]. It is may be due to the decreased transport of an essential nutrient from the roots to the shoot and feedback control by the shoots [31]. Among the different concentrations of NaCl, 30 mg L<sup>-1</sup> did not significantly reduce the productivity of the ex-plants and produced significant increases of the fresh and dry weights with respect to  $0 \text{ mg } L^{-1}$  NaCl. Similar results have been observed in the other woody species [26]. Shiyab et al. [27] also observed a significant decrease in the growth parameters at and above 150 mM NaCl in explants from seeds of sour orange (Citrus aurantium) in proliferation. Ca<sup>2+</sup> in the irrigation water is thought to decrease Na<sup>+</sup> uptake and transport to the shoot [29]. Salinity reduces shoot growth [13, 29]. Growth and salt-induced defoliation of a navel orange scion on Cleopatra mandarin rootstock was increased by addition of Ca<sup>2+</sup> to the root medium but decreased for navel orange scion on cv. Citrange [2].

Our studies showed the impact of different NaCl rates on total yield (Table 2). Results showed significant difference (p≤0.05) in the yield. Salinity decreased fruit number compared to the control, so that increasing was seen after application of 150 mg  $L^{-1}$  CaCl<sub>2</sub> In our study, the highest fruit weight was obtained using 150 mg  $L^{-1}$  CaCl<sub>2</sub> (Table 2). It is difficult to assess the relative contributions of osmotic and ion specific toxicity effects on growth and fruit yield reduction. It has been generally reported that a significant yield reduction occurs in olives cultivated under high saline conditions [32]. Salinity has an important role in pollen viability and germination, number of flowers and fruits [4]. Salinity effects on yield depend on the concentration [12, 32]. The increase of Ca<sup>2+</sup> concentration in the plant medium under saline conditions increased the number of fruits per plant and total yield fruit was affected because the fruit weight was increased [24]. Results obtained in this study showed no differences was observed in annual or accumulated yield among treatments, as it was also reported by Bouaziz [3] and Weissbein et al. [33]. In stone-fruit trees, salinity has been attributed to reduce the plant total yield [9].

Table 1: The main chemical	properties of the growing medium
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Type of soil	Potassium (ppm)	Phosphorus (ppm)	Nitrogen (ppm)	EC (×10)	pН	Sand (%)	Clay (%)	Lay (%)
Silt-loam	251	22	0.17	3.0	7.4	22	59	19

Treatments (mg L <sup>-1</sup> )	Leaf area (cm <sup>2</sup> )	Leaf fresh weight (g/plant)	Fruit No.	Fruit weight (g)	Yield (g)
$CaCl_20 + NaCl 0$	530.32e	32.30g	8. 4ef	4.7de	39.90h
$CaCl_20 + NaCl 40$	418.451	28.63h	7. 7fg	4.3f	33.111
$CaCl_20 + NaCl 80$	341.33m	20.46k	7.0g	4.0g	28.00m
$CaCl_2 50 + NaCl 0$	563.43c	35.42d	9.4cd	5.1c	47.94f
$CaCl_2 50 + NaCl 40$	492.54k	32.37g	8.9de	4.8d	42.72g
$CaCl_2 50 + NaCl 80$	523.45f	28.66h	8.2ef	4.5ef	36.90k
$CaCl_2 100 + NaCl 0$	615.67b	37.62b	10.1bc	5.6b	56.56d
$CaCl_2 100 + NaCl 40$	520.65g	35.86c	9.5cd	5.2c	50.35e
$CaCl_2 100 + NaCl 80$	503.57h	32.75f	9. 3cd	5.1c	47.43f
$CaCl_2 150 + NaCl 0$	674.53a	39.84a	11.2a	6.1a	68.32a
$CaCl_2 150 + NaCl 40$	574.24c	37.51b	10.9ab	5.8b	63.22b
$CaCl_2 150 + NaCl 80$	537.51d	34.75e	10.5ab	5.6b	58.80c

Tables 2: The effect of different concentrations of NaCl and C	CaCl <sub>2</sub> on the vegetative characteristics in olive tree
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In each column, mea ns with the similar letters are not significantly different at 5% level of probability using F test

The data obtained from leaf ion concentration of the plants related to salinity and Ca levels are presented in Tables 3. Results showed significant difference (p≤0.05) in the yield of plants treated with NaCl and CaCl<sub>2</sub>. Results also show that leaf  $Ca^{2+}$  concentration was increased when  $Ca^{2+}$  rises in the saline solution, and this increase seemed to be related with a notable decrease in leaf Na<sup>+</sup> concentration that, consequently, may also be regulated by leaf Ca<sup>2+</sup> concentration. CaCl<sub>2</sub> affected on the concentration of leaf Na<sup>+</sup>, Ca<sup>2+</sup> and K<sup>+</sup> in olive plants irrigated with NaCl. Leaves were sampled and analyzed 84 days after the beginning of the treatments. However, the concentration of leaf Na<sup>+</sup> rapidly decreased as CaCl<sub>2</sub> increased from 0 to 90 mg L<sup>-1</sup>. The decrease in leaf potassium was 0.96, as compared to the control (1.16) in highest salinity (Table 3). One of the primary plant responses to salinity is the decrease in  $Ca^{2+}$  and  $K^+$  concentration in leaves [7, 11]. A reduction in  $K^+$  concentration and  $K^+/Na^+$  ratio in saline conditions was reported by Rush and Epstein [23]. The decrease of N is accompanied by a high NaCl uptake [22].  $Ca^{2+}$  supply in the irrigation water reduced uptake and transport of Na<sup>+</sup> to the shoot and leaf [19]. The direct factor might be salinity (such as osmotic effect, Cl or Na toxicity) as was reported by Xu et al. [34].

Tables 3: The effect of different concentrations of NaCl and CaCl2 on Na, N, Ca and K levels of olive tree leaf.

	NT (0())	NT (Q()	G (0()	TT (0/)
Treatments (mg L <sup>-1</sup> )	Na (%)	N (%)	Ca (%)	K (%)
$CaCl_2 0 + NaCl 0$	0.11g	1.61de	2.14de	1.16 a
$CaCl_2 0 + NaCl 40$	0.24c	1.50f	2.06e	1.04b
CaCl <sub>2</sub> 0 + NaCl 80	0.38a	1.35g	1.95f	0.97c
$CaCl_2 50 + NaCl 0$	0.12g	1.68c	2.23cd	1.10b
$CaCl_2 50 + NaCl 40$	0.22cd	1.58e	2.12e	0.93c
$CaCl_2 50 + NaCl 80$	0.30b	1.46f	2.04ef	0.85d
$CaCl_2 100 + NaCl 0$	0.08h	1.83a	2.40b	0. 93c
$CaCl_2 100 + NaCl 40$	0.15f	1.76b	2.32bc	0.88d
$CaCl_2 100 + NaCl 80$	0.21d	1.66d	2.24c	0.81d
$CaCl_2 150 + NaCl 0$	0.05k	1.88a	2.52a	0.93c
$CaCl_2 150 + NaCl 40$	0.12g	1.77b	2.41b	0.90c
$CaCl_2 150 + NaCl 80$	0.18e	1.68c	2.35b	0.83d

In each column, means with the similar letters are not significantly different at 5% level of probability using F test

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