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Salt Effects on Growth and Leaf Chemical Constituents of *Simmondsia chinensis* (Link) Schneider

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This study was conducted at Biology Department, Faculty of Science,Taif University, Saudi Arabia to investigate the effect of different salinity concentrations 8.6, 12.9, 17.2, 25.9, 34.5, 51.7, 86.2, 103.4 and 120.7 mM NaCl, beside control treatment (tap water) on growth parameters, leaf measurements and leaf chemical constituents of jojoba (*Simmondsiachinensis* (Link) Schneider). The results indicated that salinity treatments, especially the highest level significantly decreased plant height, number of both leaves and branches on the stem, as well as, the number of nodes on the stem compared to other salinity treatments, in most cases. Similar trend has been observed for the leaf measurements (length, width, area and stomatal density). The root parameters followed the same trend of shoot growth. Leaf chlorophyll content, protein, N, P, K and Ca were decreased with increasing salinity concentrations. Meanwhile, sodium, chloride and carbohydrates were gradually increased with increasing the concentration of salinity especially, with higher levels.

Keyword:Salinity,Jojoba, Chlorophyll, Stomata, Carbohydrate

1. Introduction

Jojoba (*Simmondsiachinensis* (Link) Schneider) is a relatively new crop that is adapted to hot, dry climates. It is a new industrial crop being grown commercially in hot arid and semiarid regions. *Simmondsia chinensis* (Link) Schneider is a perennial evergreen shrub. It is dioeciously, with male and female flowers present in separate plants. Jojoba has become an attractive alternative cropbecause of the promising commercial applicationsfor its seed oil in cosmetics. Many countries arelooking toward developing jojoba culture to solveoverproduction and low price for their food andother traditional crops **(Ayerza, 1996)**. **Botti *et al.* (1998)** indicated that jojoba plantations now have a better chancefor economic and agricultural success. In recent years, renewed interest in commercial cultivation of jojoba has begun as the useful properties of the liquid wax obtained from the seed have been confirmed **(Wisniak,1987 and Brown et al., 1996)**.

Salinization threatens the productivity of agricultural land (**Mckee et al., 2004**) and agricultural sustainability **(Waisel, 2001)**.Salinity is a major problem that negatively affects agricultural activities in many regions in the world, especially the Near East and North Africa region. Generally, salinity problems increase with increasing salt concentration in irrigation water. Crop growth reduction due to salinity is generally related to theosmotic potential of the rootzone soil solution (**Abou-Hadid, 2003**).Water used for irrigation can vary greatly in quality depending on type and quantity of dissolved salts. Almost 50% of the irrigated land is affected by high salinity (**Zhu, 2001**), often resulting in secondary salinization due to inappropriate use of saline irrigation water. Despite the essentiality of chloride as a micronutrient for all higher plants and of sodium as mineral nutrient for many halophytes and some species, salt accumulation may convert agricultural areas in unfavorable environments, reduce local biodiversity, limit growth and reproduction of plants, and may lead to toxicity in nonsalt-tolerant plants, known as glycophytes **(Ashraf and Harris, 2004, and Parida and Das, 2005)**. The effects of salinity are generally summarized as water stress, salt stress and stress due to ionic imbalance **(Greenway and Munns, 1980)**. Therefore at least one part of salt stress is associated with water stress, which is a general condition, and it can be expected that plant adaptation to salinity may show features similar to those characteristic of adaptation to water stress.

It believed to be future arid plant, in general, jojoba developed reasonably well under salinities of 8 dSm^{-1}**(Benzioni et al., 1990)**. **Thomson (1982)** investigated that jojoba tolerated 4 dSm^{-1} salts in the irrigation water. Salinity and drought are considered to be the most serious growth-limiting factors for crop plants **(Boyer, 1982, and Vinocur and Altman, 2005).** Increasing salinity of irrigation water has contributed to progressive salinization of agricultural soils inhibiting agricultural productivity in many semi-arid and arid regions of the world **(Qadir et al., 2000)**. Sodium chloride (NaCl) is the most commonly encountered source of salinity **(Li et al., 2006)**. Much of the strain in salinity stress is related to water stress arising from excessive uptake of salts by the plants and the resulting reduction in water potential.

For this crop to be an economically profitable alternative for arid and semi-arid zones, it is necessary to first select plants of high productivity that also possess sufficient resistance to abiotic stresses. Salinity is considered to be the most serious growth-limiting factors for crop plants **(Boyer, 1982 and Vinocur and Altman, 2005).** In spite of information showing that jojoba tolerates fairly high levels of salinity (**Benzioni et al.,1996**) and water stress (**Foster and Wright, 1980**), the selections to date have not been intended for use in regions with extremely high levels of salinity and water stress **(Botti et al., 1998b)**. Therefore, the aim of this study was to evaluate the effect of salinity concentrations on shoot and root growth, leaf measurements and chemical constituents of jojoba leaves.

2. Materials and Methods

This study was carried out at Biology Department - Faculty of Science -Taif University - Saudi Arabia at the seasons of 1433 and 1434. Salinity treatments were. These salinity concentrations equal 8.6, 12.9, 17.2, 25.9, 34.5, 51.7, 86.2, 103.4 and 120.7 mM NaCl, besides control (tap water). Each plot comprised four pots. To balance the evaporative losses, and also to maintain plants at the required levels of salts, they were watered every alternative day **(Rawat and Banerjee, 1998).** The measurements of shoot and roots were taken.

The study was conducted in order to study the effect of different salinity treatments on vegetative growth (plant height, number of branches, number of leaves, number of nodes on the stem of each plant, stem diameter, fresh and dry weight of the branches, fresh and dry weight of roots, root length, leaf measurements; its length, with, area and stomatal density) as well as the leaf chemical constituents (leaf chlorophyll, total carbohydrates, protein and elemental content i.e. nitrogen, phosphorus, potassium, sodium, chloride and calcium) of jojoba leaves. Seeds of jojoba were sown directly into plastic pots (30 x 20 cm). Each pot included four seeds. The physical and chemical characteristics of the soil used in this study were shown in **Table (A).**

Table (A) Physical and chemical properties of used soil

Particle size distribution (%)				pH	E.C. dSm^{-1}
Sand	Silt	Clay	Texture grade		
82.40	7.10	10.50	Sandy	8.37	3.68

Total $CaCO_3$ (%)	Organic matter (%)	Soluble ions (meq/L) [soil paste]						Total N (%)	Total P (%)	Total K (%)
		Anions				Cations				
		Cl^-	$CO^=_3$	HCO^-_3	$SO^=_4$	Ca^{++}	Na^+			
0.98	0.11	0.67	-	2.33	47.55	44.75	3.67	0.17	0.036	0.043

The shoot characters taken in this experiment were plant height (cm), number of main branches/plant, stem diameter (cm), shoot weight (fresh & dry) and number of nodes/stem. The root measurements were root weight (fresh & dry) and root diameter (cm). Data recorded concerning leaf were number of leaves/plant, leaf width, length (cm) and its area (cm^2) and stomatal density (number/mm^2).

2.1 Leaf area

Blade area was measured using digital image analysis according to the methodof **Matthew *et al.* (2002)**. Digital image of the leaf blade was created in digital format using a Hewlett- Packard scanner (Hewlett Packard, Cupertino, ca), image was scanned at dot per inch (100 dpi), the blade area was measured using public domain software (scion image version 4.02).

2.2 Stomatal density measurement

Stomatal density (number/mm^2) was measured according to the method as described by (**Botti *et al.*, 1998 a**).

2.3 Chemical constituents

2.3.1 Chlorophyll content

Randomly samples of fresh leaves were taken by the end of October from the middle part of stem for chlorophyll determination. Chlorophyll content was determined according to **Sadasivam and Manickam (1992)** by using spectrophotometer (Pharmacia, LKB-Novaspec II) at wave length of 663 nm for chlorophyll (a), 644 nm for chlorophyll (b). Total chlorophyll fractions calculated as mg/gm fresh weight of leaves.

2.4 Total carbohydrates

Total carbohydrates percentages were determined in leaf samples taken by the same way and in the meantime of chlorophyll samples. The leaf samples were dried in an electric oven at 70 °C for 24 hours according to **A.O.A.C. (1995)**. Then, the fine powder used to determine total carbohydrates percentage in leaves. Total carbohydrates including polysaccharides in leaves of jojoba were calorimetrically determined with the anthrone sulphuric acid method according to **El-Enany (1986)**.

2.5 Leaf Mineral Content

Nitrogen, phosphorus, potassium, calcium, sodium and chloride were determined in dried leaf samples, digested using sulphuric and perchloric acids method as mentioned by **Piper (1967)**,according to the methods described by **Black *et al.* (1965)**, (**Chapman and Pratt, 1961**), **Jackson (1973) and Johnson and Ulrich (1959).** Protein percentage in leaves calculated by using the conversion factor of 6.25 based on the assumption that the protein contains 16 % nitrogen according to **Ranganna (1978)**.Data were subjected to statistical analysis using "F" test according to **Snedecor and Cochran (1973)** and least significant difference (L.S.D.) values at 0.05 was used for comparison between means according to **Gomez and Gomez (1984)**.

3. Results

3.1. Shoot Growth Characteristics

The presented clearly show that jojoba shoot growth was affected by different salinity treatments (Fig. 1, 2 and 5). The plant height, branch number/plant, shoot weight (fresh & dry) and nodes number/plant were gradually decreased with increasing salinity concentration. Salinity levels led to decrease the previously mentioned characters since the lowest values in this respect were obtained by the maximum dose of salinity. However, the control plants recorded the highest values of shoot growth in comparison with any salinity level. The statistical analysis of results indicated that the differences between the treatments were significant, in most cases.

3.2. Root Growth Parameters

It is clear from the obtained data that applying salinity levels resulted in a significant reduction in fresh and dry weight of roots as well as root diameter compared to the control. Increasing salinity concentration caused a significant decreased in shoot/root ratio (fresh & dry) and this reduction was gradually with increasing the salt levels (Fig. 3 and 4).

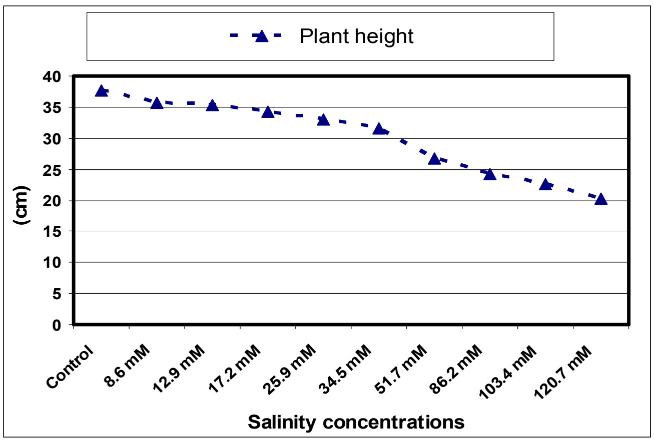

Fig 1: Plant Height of jojoba as Affected by Different Salinity Treatments

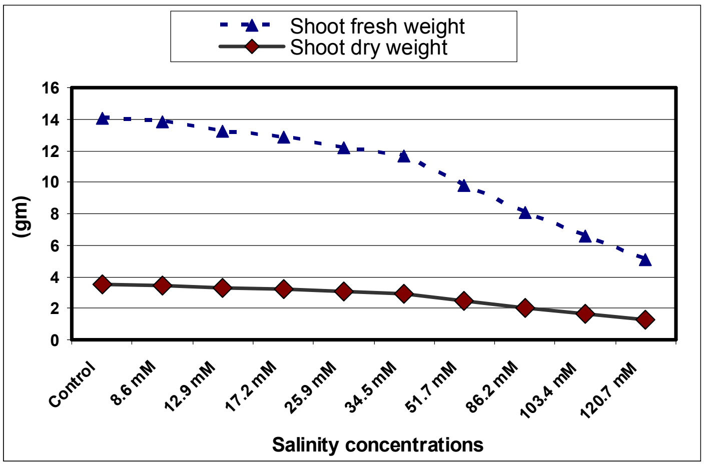

Fig 2: Shoot weight (fresh & dry) of jojoba as Affected by Different Salinity Treatments

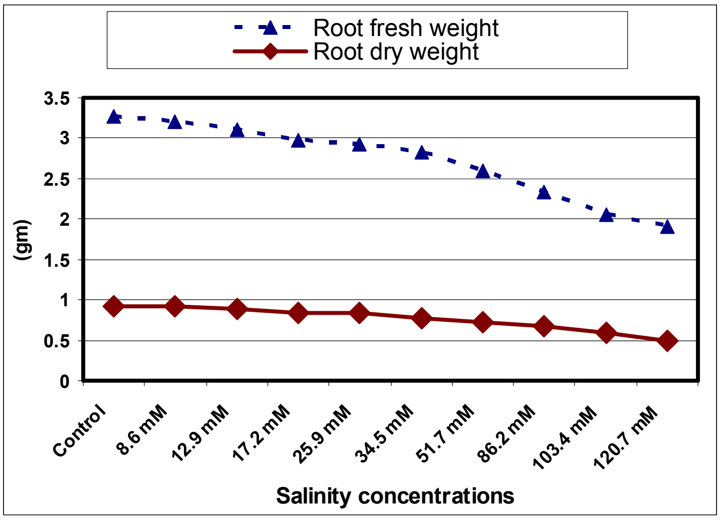

Fig 3: Root Weight (fresh & dry) of jojoba Plants as Affected By Salinity Treatments.

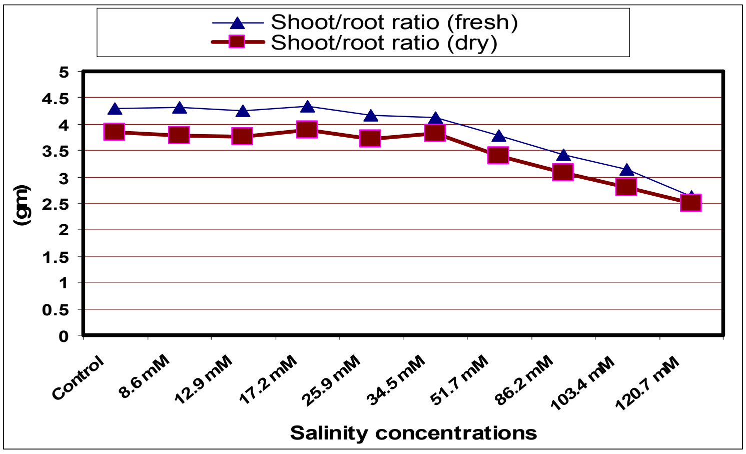

Fig 4: Shoot/root Ratio (fresh & dry) of jojoba as Affected by Different Salinity Treatments

3.3. Leaf Measurement

3.3.1. Leaf Length, Width and Area

The leaf length, width and its area were significantly decreased by using different salinity concentrations (Fig. 6). The increase of the salinity concentration, the decrease of leaf measurements was occurred. The shortest leaf length was recorded by the highest salinity level, also the previous treatment recorded the narrowest leaves and the smallest leaf area compared to the untreated control.

3.3.3. Leaf Number/Plant

The obtained results clearly indicate that the leaf number was significantly decreased with increasing the salinity concentrations since the control plants resulted in the highest leaf number compared highest salinity concentration (Fig. 5).

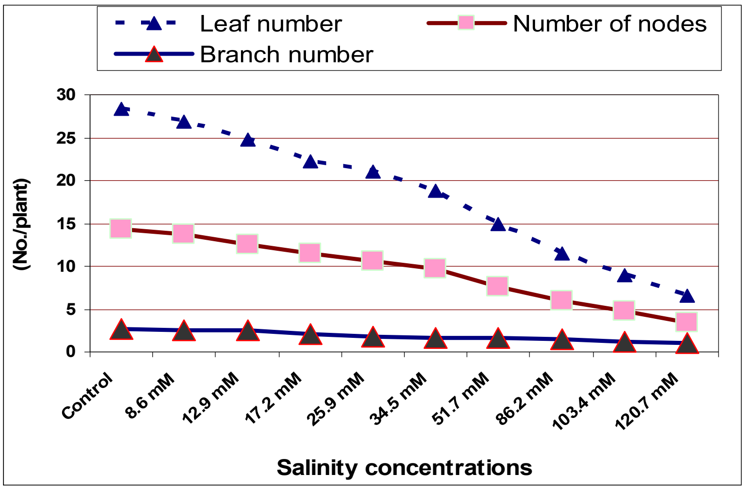

Fig 5: Branch number, leaf number and number of nodes/plant of jojoba as affected by different salinity treatments

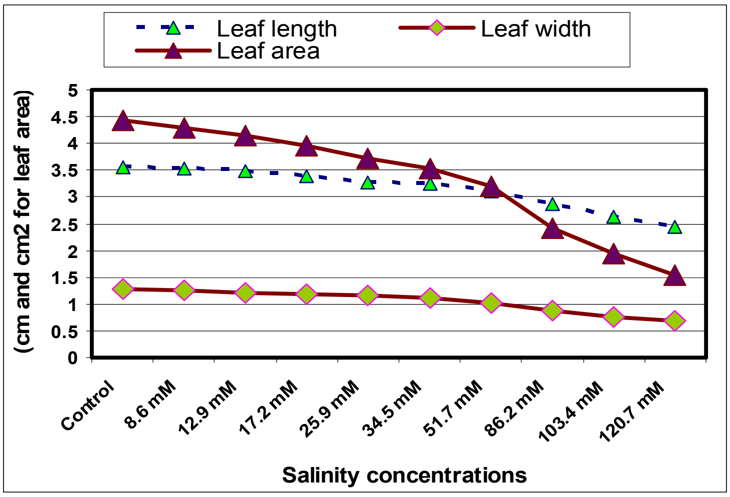

Fig 6: Leaf length, width and its area of jojoba as affected by different salinity treatments

3.3.2. Stomata Density (no/mm^2)

It was observed that, there was significant effect of salinity levels on stomatal density of jojoba leaves (Fig. 7). Mostly, salinity levels significantly decreased stomatal density of jojoba leaves compared with control. Stomatal density decreased gradually with increasing salinity concentrations, the lowest stomatal density were obtained by the highest salinity treatments.

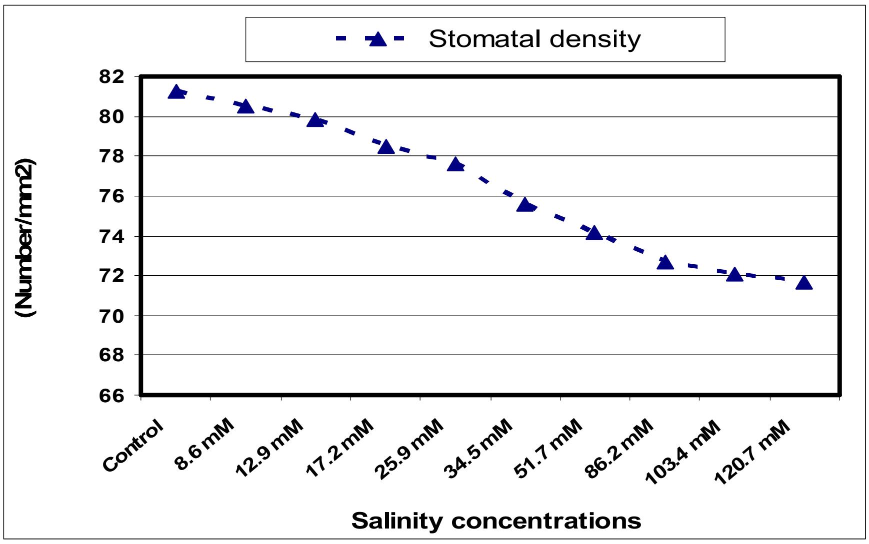

Fig 7: Stomatal density of jojoba leaves as affected by different salinity treatments

3.4. Chemical Constituents

3.4.1. Elements Content

The obtained results indicated that N, P and K^{+1} percentages were influenced by using different salinity concentrations (Fig. 8). The obtained data revealed that, insignificant differences were observed among control, 8.6, 12.9 or 17.2 mM, in most cases.

Increasing the concentrations over 17.2 mM significantly decreased these elements compared to lower ones. In the same trend of the previous three cations, Ca^{+2} content was higher in untreated leaves and salinity treatments led to a gradual decrease in its concentrations.

Increasing salinity concentration gradually increased Na^{+1} and Cl^{-1} content (Fig. 8). The differences among salinity treatments were significant for both anions.

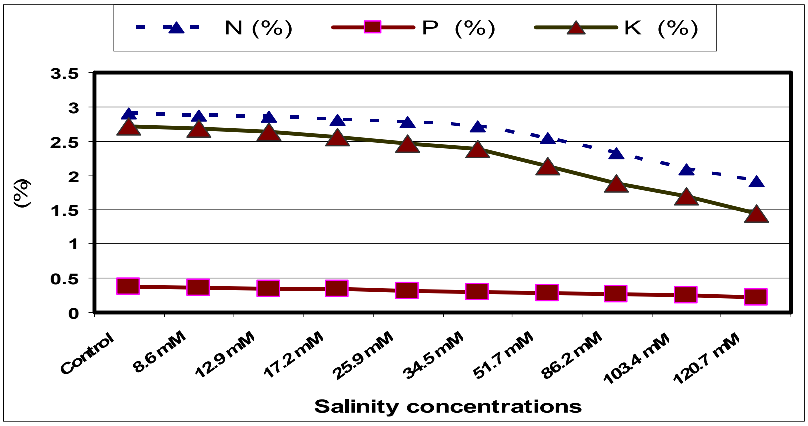

Fig 8: Leaf mineral content (N, P and K) in jojoba leaves as affected by Different Salinity Treatments

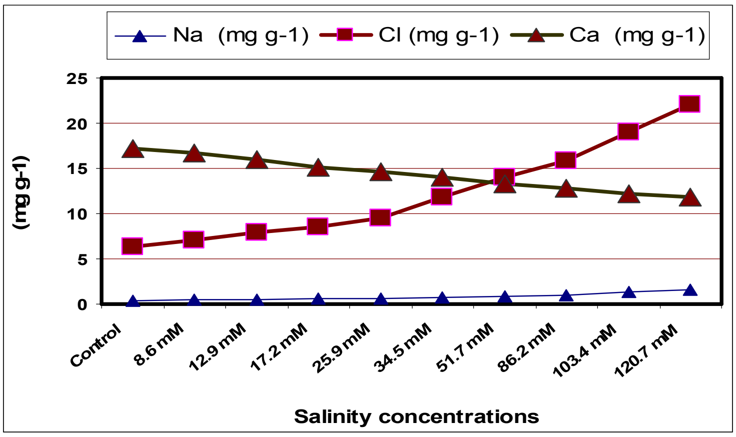

Fig 9: Leaf mineral content (Na, Cl and Ca) in jojoba leaves as affected by different salinity treatments

3.4.2. Leaf Chlorophyll Content

Data concerning the effect of salinity concentrations on the chlorophyll content of jojoba leaves revealed that increasing salinity concentrations significantly decreased chlorophyll "a", "b" and total chlorophyll. The highest salinity treatment resulted in the lowest total chlorophyll content however, the highest value in this respect was obtained when the control treatment (without salinity) was applied (Fig. 10).

3.4.3. Protein Percentages

The obtained data indicated that, salinity treatments showed significant differences protein percentage among control, the other salinity treatments. Also, the treatment of 34.5 mM significantly increased protein percentage in comparison with 86.2, 103.4 or 120.7 mM treatments (Fig. 11).

3.4.4. Total Carbohydrates Percentage

The present results postulated that the highest salinity concentrations improved carbohydrate percentage of jojoba leaves compared to the other ones (Fig. 11). Total carbohydrate percentages were increased with increasing salinity concentration from 8.6 to 120.7 mM, the maximum carbohydrate percentages were observed with salinity treatment of 103.4 and 120.7 mM.

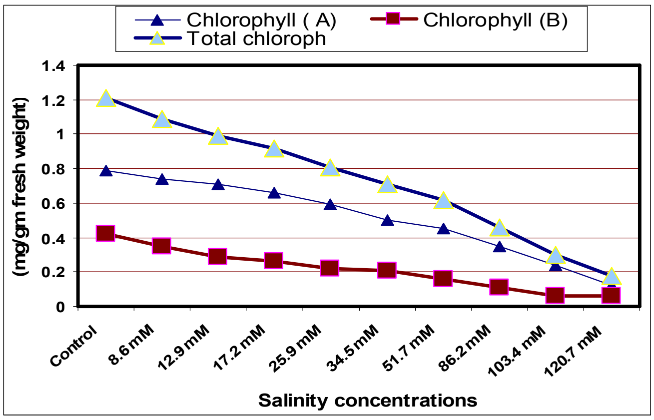

Fig10: Leaf content of chlorophyll of jojoba leaves as Affected by Different Salinity Treatments

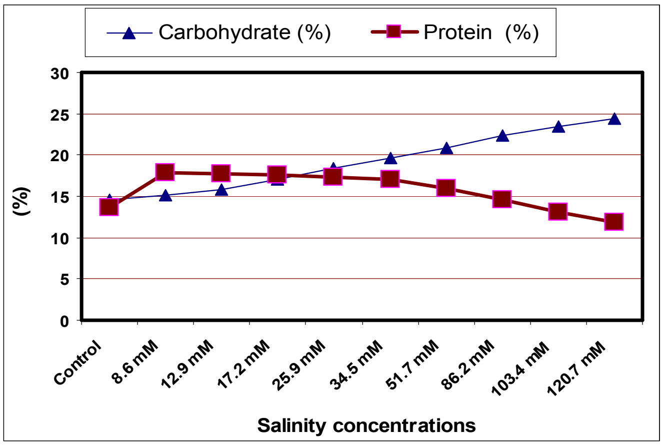

Fig 11: Leaf content of carbohydrates of jojoba as affected by different salinity treatments

4. Discussion

The effective regulation of water is central to resistance of many stresses, including salt stress. A common indicator of salt stress is the reduction of growth due to inadequate water uptake **(Munns, 2002 and Borsani *et al.*, 2003).** In our results, the plant growth was negatively correlated with increasing salinity concentrations. The values of plant height, branch number, number of leaves and nodes, shoot weight (fresh & dry), shoot/root ratio and leaf measurements were decreased with salinity levels increased, especially with higher salinity concentrations **(Figures, 1,2,4,5 and 6)**. Inhibition of shoot growth has been considered a whole plant adaptation to salt stress or water stress **(Meloni *et al.*, 2001; Akhtar *et al.*, 2003; Mulholland *et al.*, 2003 and Qaderi *et al.*, 2006).** The suppression of shoot and root growth under salt-stress may either be due to osmotic reduction in water availability or to excessive accumulation of ions, known as specific ion effect **(Marschner, 1995).** These results confirm the others obtained by (**Katerji *et al.*, 2004andMansour *et al.*, 2005**).

It is very important here to refer to salt stress on leaf measurements and stomatal density as our data revealed that there is a reduction in these characters were recorded by salt stress **(Fig. 7)**. Some researchers indicated that the most salt-resistant plants had the lowest stomata, stomatal density and leaf area tended to lower with higher salinity (**Botti et al,. 1998a**). This reduction may be occurred to make an adaptation to salt and inhabitation of its uptake. Further studies are needed to supply information concerning the previous relationship. Regarding nutrients contents of jojoba leaves as affected by salinity levels, it was, mostly, noticed that N, P, K and Ca contents were higher with control or lower salinity levels **(Fig. 8 and 9).** On the contrary of the above results Na^{+1} and Cl^{-1} showed opposite trend, whereas, they increased gradually with increasing salinity concentrations **(Fig. 9).** When salinity results from the excess of NaCl, which is by far the most common type of salt stress, the increased intercellular concentration of Na^{+1} and Cl^{-1} is deleterious to cellular systems **(Serrano *et al.,* 1999).** In addition, the homeostasis of not only Na^{+1} and Cl^{-1} but also of essential cations such as K^{+1} and Ca^{2+} is disturbed **(Serrano *et al.*,1999;Tattini *et al.*, 2002; Roussos et al., 2007).** Plant survival and growth under salt stress depend on adaptations that re-establish ionic homeostasis, thereby reducing the cellular exposure to ionic imbalances. High concentrations of salt impose a hyperosmotic

shock by decreasing the chemical activity of water, thereby causing loss of turgor.
Our data showed that increasing levels of NaCl induced a progressive absorption of Na^{+2} and Cl^{-1} in jojoba leaves, agreeing with **Chavan and Karadge (1986)**, **Taban et al. (1999) and Turan et al. (2007)**. Accumulation of Cl^{-1} in the root tissue is disruptive to membrane uptake mechanisms, and hence may increase translocation of Cl^{-1} to the shoots**.** When NaCl was applied to the soil, the levels of K in plant were reduced in accordance with the antagonism between Na and K **(Alberico and Cramer, 1993 and Azevedo and Tabosa, 2000).** Salinity levels considerably decreased leaf content of chlorophyll "a", chlorophyll "b" and total chlorophyll compared to the control **(Fig. 10).** Apparently, the lowest leaf content of chlorophyll was obtained from 120.7 mM. These results 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 and Allen, 1995).** Our results are in agreement with many authors who revealed that, the total chlorophyll content of leaves was reduced by increasing NaCl level (**Cha-Um and Kirdmanee, 2009**). The salinity could seriously change the photosynthetic carbon metabolize, leaf chlorophyll content as well as photosynthetic efficiency. It was observed that the high levels of salinization induced a significant decrease in the contents of pigment fractions (chlorophyll a and b) and consequently of the total chlorophyll content as compared with control plants **(Seeman and Critchley, 1985** and **Sharkey et al. 1985)**. The decreased levels in chlorophyll content under saline stress is commonly reported phenomenon and established that it may be due to different reasons; one of them is related to membrane deterioration (**Ashraf and Bhatti, 2000**).

Concerning carbohydrates percentage, our results concluded that carbohydrate content of jojoba leaves significantly increased with increasing salinity levels **(Fig. 11)**. This increment may be occurred in order to regulate the osmotic potential under salt stress **(Sasiakala and Prasad, 1993 and Teixeira and Pereira, 2007).** These results are in agreement with **Dhanapackiam andMuhammad (2010)**. Many plants, which are stressed by NaCl salinity, accumulated starch and soluble carbohydrates (**Greenway and Munns, 1980 and Rathert, 1984**). This accumulation has been attributed to impaired carbohydrate utilization (**Munns and Jermaat, 1986**). It has been generally recorded that salinity adverselyaffects seedlings growth and some relevant metabolic processes of glycophytic plants (**Hampson and Simpson, 1990 and Zidan and Al-Zahran 1994).**Interestingly, our data indicated that the protein content of jojoba leaves was decreased with increasing salinity concentrations in the same direction of nitrogen content in leaves. These data were in the opposite trend of **Pruvot et al. (1996)** who mentioned that salt stress increased protein content since they found the salt stress increased the proline amino acids. The negative effect of water and salt stresses were clearly appeared when the combination between them was occurred, consequently, shoot and root growth and chemical constituents of jojoba plant were inhibited. Even thought, the irrigation intervals was decreased the limitation of water absorption and biochemical processes were happened. These results were in accordance with the findings of **Cusido et al. (1987) and Parida and Das (2005).**

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