

# EFFECT OF DILUTED SEAWATER IRRIGATION AND EXOGENOUS PROLINE TREATMENTS ON GROWTH, CHEMICAL COMPOSITION AND ANATOMICAL CHARACTERISTICS OF *CONOCARPUS ERECTUS* L.

Mohammed E. El-Mahrouk<sup>1</sup>, Mohammed F. El-Nady<sup>2</sup>, Mahmoud A. Hegazi<sup>1</sup>

<sup>1</sup>Dept. of Hort, Fac. of Agric., Kafrelsheikh Univ., Egypt

<sup>2</sup>Dept. of Agric. Botany, Fac. of Agric., Kafrelsheikh Univ., Egypt

Threemelmahrouk@yahoo.com

## ABSTRACT

The objectives of this investigation were to study the effect of diluted seawater irrigation (0, 10, 15, 30 and 50%) on growth, chemical composition and anatomical structure of buttonwood plant (*Conocarpus erectus* L.). In addition, trying to counteract the adverse effect of seawater salinity by foliar application of exogenous proline (0.5 and 1%). The results showed that low levels of diluted seawater improved the growth, also proline treatments have significant effect on growth. The treatment of 30% seawater increased N, P and Fe contents in aerial parts when compared with low and high seawater levels, but 10% seawater treatment increased K level. Whereas salt stress of seawater irrigation led to direct increase in sodium content which resulted an increase in Na/K ratio in the aerial parts. The foliar application of 0.5% exogenous proline increased the accumulation of N, P, K and Zn contents in aerial parts. Diluted seawater up to 30% improved the most stem and leaf structure parameters. Foliar application of proline as osmoregulator overcame the bad effects of seawater salinity. Application of 10% seawater in combination with 1% proline achieved the highest values of stem internal growth characteristics. In contrast, 15% seawater combined with proline at 1% gave the highest values of internal leaf structure parameters

**Keywords:** *Conocarpus erectus* L., seawater, proline, osmoregulators, chemical composition, internal growth parameters.

## Introduction

Buttonwood (*Conocarpus erectus* L.), is an evergreen shrub of family *Combretaceae*, native to Florida's mangrove forest ecosystem in North America. It is found on the edges of salt flats, rock lands of the Florida Keys, borders of fresh and brackish marshes, edges of

hammocks, sometimes on spoil and other disturbed areas in South Florida. It tolerates extreme desert heat where summer temperatures may reach 47 °C and grows in soils of very low fertility (Nelson, 1996). This shrub deserves attention because it grows fairly rapidly, can endure the unrelenting, fierce tropical sun, and can tolerate the high salinity levels (halophytic plant) if it is adequately supplied with water. It provides food and cover for wildlife, protects the soil during storm surges and helps fix dunes (Popp *et al.*, 1989). It is widely planted as an ornamental evergreen in yards, parking lots, streets, and parks, and the potted plants are used to form bonsai (Gliman and Watson, 1993). The wood is durable and is used to make railroad ties, posts, boat building, fuel, and charcoal (Nelson, 1996). The bark and leaves have been used in tannery and folk medicine (Liogier, 1990).

Water scarcity is the greatest crisis facing humanity in the 21<sup>st</sup> century and possibly beyond (Singh, 2008). Water is a renewable resource, but its availability is variable and limited. Nearly every country in the world experiences water shortages during certain periods of the year (Gleick, 1993), and more than 80 countries now suffer from serious water shortages (Jin *et al.*, 2007). Agricultural production consumes more fresh water than any other human activity. To cope with the scarcity of fresh water for the sustainable development of agriculture, there is increasing awareness among agricultural scientists and planners in the utilization of seawater (at least diluted) for irrigation of crops (Fang and Chen, 1997; Jin *et al.*, 1999; Liu *et al.*, 2003).

However, the high concentration of ionic species in seawater is the main limiting factor in the utilization of seawater for irrigation (Xiao-Hua *et al.*, 2009). Salt damage of plants occurs by a combination of several causes, including mainly osmotic injury and specific ion toxicity (Munns *et al.*, 1995; Nandwal *et al.*, 2000; Di Baccio *et al.*, 2004) that affect a variety of physiological and metabolic processes in plants (Silveira *et al.*, 2001).

There is a number of variables that indicates the quality of water. Some of the basic variables are pH, electrical conductivity, salinity and hardness (Hatzikos *et al.*, 2008).

For the use of saline water effect on the soil properties and yield of many plants, Moreno *et al.*, (2001) evaluated the effects of irrigation with high and moderate saline water on soil properties as well as growth and yield of cotton and sugar beet crops. They concluded that although the soil salinity increased after saline water irrigation in comparison with freshwater irrigation, cotton yield was the same, and

sugar beet yield was higher than that with fresh water irrigation. Geng-Mao *et al.*, (2008) found that Jerusalem artichoke could be safely grown in salt-affected land with 25 and 50% seawater irrigation.

The previous studies showed that salinity may improve the antioxidant activity to stress conditions. The activation of antioxidative mechanisms in sunflower seedlings treated with 10 and 20% seawater, which although associated with different responses, confirmed the adaptation to salinity (Izzo *et al.*, 2008 and Incerti *et al.*, 2008). It has been well established that plants accumulate a variety of osmoregulator solutes including proline as an adaptive mechanism to environmental stress and salinity (Aspinall and Paleg, 1981). The use of proline as osmoregulator to overcome the bad effects of salinity, which is similar to seawater on plant growth has been reported by (Lin and Kao, 1996).

Also, Miyamoto *et al.*, (1996) and Glenn *et al.*, (1998) suggested that seawater irrigation in agriculture should be developed in the places where there is sufficiently high saline water or seawater. They suggested that using saline water to irrigate salt-tolerant crops or halophytes could be a viable strategy for developing agriculture production as well as for saving fresh water resources. In Egypt, the water used for irrigation is often mixed with seawater especially in the area near the coasts. Egypt has a long sea coast, which encourages the utilization of seawater in plant irrigation and as a mineral fertilizer. The aim of the present research was to study the effect of diluted seawater irrigation and exogenous proline treatments on growth characteristics of buttonwood plant.

### **Material and methods**

A pot experiment was conducted at the Experimental Farm of the Faculty of Agriculture, Kafr El-Sheikh University during two successive seasons of 2007 and 2008 (from April 1<sup>st</sup> to September 30<sup>th</sup> of every season). The weather during the experiment was characterized by sunny, hot dry days, and warm nights. The daily temperature during the experiment ranged from 18 to 35 °C. No rains had fallen during the experimental period.

### **Plant material and procedure:**

Four-month old buttonwood transplants of a uniform height ( $16 \pm 2$  cm in length) were obtained from a local nursery and transplanted on April 1<sup>st</sup> of each season, as one transplant /pot (30 cm diameter

plastic pots) filled with ten kg sandy clay soil (1 sand: 2 clay; v/v) and remained in open atmosphere. Initial soil samples were taken before the establishment of the study for determination of the chemical properties (Table, 1). Seawater for irrigation was taken from the sea in Alexandria town. Its salinity was approximately 42.98 and 42.10 g/l in the first and second seasons, respectively. Prior to irrigation, seawater was diluted with fresh water to the required concentration (0, 10, 15, 30 and 50 % ) in a plastic tank. The diluted seawater at different concentrations was used for irrigation at 250 ml/ pot twice weekly throughout the course of the study (6 months). The chemical properties of seawater and diluted seawater used for irrigation are shown in Tables (1 and 2). On May 1<sup>st</sup> transplants were foliar sprayed with proline at 0.0, 0.5 and 1% dissolved in a tap water (w/v).

Table (1): Chemical analysis of seawater and soil

Ions Sample	N %	P ppm	K ppm	Mg Meq/l	Ca Meq/l	Fe ppm	Zn ppm	Co ppm	Mn ppm
Seawater First season	1.4	111.38	252	176	33	0.11	0.01	0.0	0.0
Seawater second season	1.5	120.95	252	177	31	0.12	0.0	0.0	0.0
Tap water	0.3	0.2	5.3	7.8	17.6	0.0	0.5	0.0	0.01
Soil	0.5	0.56	28.8	1.6	1.6	0.01	3.26	0.0	28.0

Cont. (Table 1)

Ions Sample	Cu ppm	Ni ppm	Na ppm	Cl Meq/l	Hco <sub>3</sub> Meq/l	Co <sub>3</sub> Meq/l	pH	EC dsm <sup>-1</sup>
Seawater First season	0.0	0.0	12870	666	2.5	1.8	8.32	56.7
Seawater second season	0.0	0.0	14740	682	2.4	1.4	8.34	58.1
Tap water	0.0	0.0	311.6	1.6	2.8	0.8	7.75	3.3
Soil	4.03	0.54	1.4	0.8	3.0	0.0	7.22	0.35

Table (2): pH and EC values of the different diluted seawater treatments at the beginning of the experiment.

Seawater %	First season		Second season	
	pH	EC (dsm <sup>-1</sup> )	pH	EC (dsm <sup>-1</sup> )
0	8.06	0.49	8.32	0.47
10	7.91	5.10	8.10	7.67
15	7.98	8.47	8.11	8.70
30	7.77	17.31	8.08	18.5
50	7.97	30.4	7.71	27.8

For each season, the growth parameters (plant height, number of branches, leaf number, stem diameter, leaf area/ leaf, aerial parts fresh and dry weight, root fresh dry weights), and chemical properties were determined on plants at harvest time ( September 30<sup>th</sup>) in the second season.

### **Histological studies**

The leaf specimens including the midrib were taken from the fourth leaf from plant top. The stem specimens were taken from the fourth internode from the plant tip. Specimens were taken on day 45<sup>th</sup> of planting in the first season. Specimens were fixed in formalin alcohol acetic acid mixture (FAA, 1: 18: 1; v/v), washed and dehydrated in alcohol series. The dehydrated specimens were infiltrated and embedded in paraffin wax (52-54 °C m. p.). The embedded specimens were sectioned using a rotary microtome (Leica RM 2125) at a thickness of 8 – 10 µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranin, cleared in xylol and mounted in Canada balsam (Ruzin, 1999). Slides were examined using light microscope (Olympus optical Co., LTD, Modal: CH40RF200) each parameter was represented by an average of 10 readings taken from 3 slides.

Measurements of transverse sections of leaves of buttonwood plants i. e. thickness of leaf lamina, palisade and spongy tissues, main vascular bundles in midrib dimensions were recorded. Stem parameters i. e. stem diameter, vascular tissues, cortex thickness as well as pith diameter were calculated.

### **Measurements**

Before conducting the experiment, both seawater and soil samples were transferred to the Center Laboratory of Kafr Elsheikh University for analyzing chemical properties. Total nitrogen content was determined in digested samples by the semi-micro Kjeldahl method according to Page (1982). Total phosphorus was determined in digested samples according to Jackson (1973) by the method of Schouwenbury Van and Walinge (1967). Soluble cations of Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>++</sup>, Mg<sup>++</sup> and anions, HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, CO<sub>3</sub><sup>-</sup> were assessed in seawater and soil paste extract (Page 1982). Available micro-elements and heavy metals were extracted by diethylene triamine penta acetic acid (DTPA), (Lindsay and Norvell, 1978) and determined spectrophotometrically by atomic absorption technique using unit

PERKIN ELEMER 3300. Electrical conductivity (EC) and pH were determined according to Page (1982). At the end of experiments, the growth parameters in both seasons and chemical properties in the second season were conducted.

### **Experimental design and data analysis**

The experiments layout were set in a completely randomized design in factorial with two factors (five seawater irrigation levels x three proline concentrations). Each treatment consisted of three replicates and each replicate was represented by a plastic pot containing 8 plants. The mean and ANOVA were calculated using SPSS (version 10) software. The mean separations were carried out using Duncan's multiple range test (Duncan, 1955) and significance was determined at  $p < 0.05$ .

### **Results and discussion**

#### **1-Effect of diluted seawater irrigation, proline spraying and their interaction on growth parameters**

##### **a-Effect of diluted seawater**

The results showed significant differences among the concentrations of diluted seawater used on growth parameters on the first and second seasons, 10% seawater irrigation increased the plant height (56.7 and 58.6 cm, respectively), 15 % seawater irrigation increased the number of branches ( 14.0 and 13.7, respectively) and 30% seawater irrigation increased the leaf area ( 17.58 and 17.47 cm<sup>2</sup> respectively). However the thickest stem diameter was 0.63 and 0.62 cm via 50 and 15% seawater irrigation in the first and second seasons, respectively (Table, 3).

Highly significant effects of diluted seawater on fresh and dry weight of aerial parts and roots of *Conocarpus erectus* were observed. Table 4 indicates that 15% seawater irrigation increased the aerial parts dry weight (15.59 and 15.02 g ), roots fresh weight (8.7 and 9.55 g) and root dry weight (4.71 and 5.27 g) in the first and second seasons respectively. The heaviest aerial parts fresh weight was 41.33 and 34.73 g using 15 and 10% seawater irrigation in the first and second seasons respectively. Fresh and dry weight of the tested foliage plants increased with increasing concentration of seawater used for irrigation from 10 up to 15%. However, further increase in salinity level of the irrigation water decreased the fresh and dry weights. Similar results were obtained by Ashour *et al.*, 1997 on three grass species.

**Table (3): Effect of diluted seawater irrigation, proline spray levels and their interaction on plant height, number of branches, stem diameter and leaf area of *Conocarpus erectus* L. during two seasons**

Trait Proline % Sea water %	Plant height (cm)				Number of branches/ plant			
	0.0	0.5	1	Mean	0.0	0.5	1	Mean
First season								
0	50.0f	52.0i	40.0f	47.3C	8.2l	11.7e	12.3d	10.7C
10	54.0d	56.0c	60.0b	56.7A	10.3g	12.7c	10.7f	11.2B
15	44.0g	35.0j	67.7a	48.9B	16.8a	9.6h	15.7b	14.0A
30	33.7l	34.0k	42.0h	36.6D	10.6f	9.3i	11.7e	10.6D
50	26.3m	35.0j	26.3m	29.2E	8.3k	9.3i	9.0j	8.9E
Mean	41.6C	42.4B	47.2A		10.8B	10.5C	11.8A	
Second season								
0	63.0c	57.0e	49.3h	56.4B	7.3j	4.7k	9.7g	7.2E
10	51.7f	65.7a	58.3d	58.6A	8.3i	10.3e	12.3c	10.3B
15	44.3i	51.0g	65.3b	53.6C	12.7b	12.3c	16.0a	13.7A
30	34.0m	40.3k	40.7j	38.3D	10.7d	9.3h	10.3e	10.1C
50	22.0o	36.3l	27.3n	28.6E	10.0f	10.7d	9.3h	10.0C
Mean	43.0C	50.1A	48.2B		9.8B	9.5C	11.5A	

Cont. (Table 3)

Trait Proline % Sea water %	Stem diameter (cm)				Leaf area/ leaf (cm <sup>2</sup> )			
	0.0	0.5	1	Mean	0.0	0.5	1	Mean
First season								
0	0.55j	0.69c	0.54k	0.59E	15.02h	14.51k	14.71j	14.75C
10	0.53l	0.70b	0.64e	0.62B	15.11g	14.25m	14.73i	14.70D
15	0.66d	0.56i	0.58h	0.60D	16.18e	15.68f	16.69c	16.18B
30	0.70b	0.60g	0.55j	0.61C	17.52b	16.50d	18.71a	17.58A
50	0.75a	0.62f	0.50m	0.63A	14.47l	13.88o	14.02n	14.12E
Mean	0.64A	0.63B	0.56C		15.66B	14.96C	15.77A	
Second season								
0	0.51l	0.61e	0.71a	0.61B	14.33n	13.84o	15.15i	14.43E
10	0.60f	0.57i	0.64c	0.60C	15.40g	15.06l	15.32h	15.27C
15	0.63d	0.57i	0.67b	0.62A	16.78f	17.19c	17.01e	16.99B
30	0.55j	0.46m	0.58h	0.53E	17.12d	17.88a	17.40b	17.47A
50	0.53k	0.59g	0.55k	0.56D	14.87m	15.11j	15.08k	15.02D
Mean	0.55B	0.56C	0.63A		15.99A	15.70C	15.82B	

Different letters in each column indicate significances by Duncan (D) tests at  $P < 0.05$ .

The aforementioned results on chemical analysis of seawater indicated that seawater contained many fertilizer elements which encouraged the vegetative growth. The stimulatory effect of moderate

salinity on the growth of some halophytic plants was also reported by O'Leary (1988), and may be attributed to increased shoot osmotic status as a result of increased ion uptake (Naidoo *et al.*, 1995). Reduced growth at high salinities is probably associated with reduced turgor pressure and the high energy consumed of massive salt secretion and osmoregulation

There was a distinct effect of high concentration of seawater irrigation on the growth rates of roots and shoots of buttonwood. Excess salt decreases the leaf water potential, as in water deficit conditions, reduces water and nutrients uptake by plants, and ultimately leads to a reduced growth (Xiao-Hua *et al.*, 2009). Abiotic stresses including salt-stress induce accumulation of reactive oxygen species that are detrimental to cells at high concentrations because they cause oxidative damage to membrane lipids, proteins, and nucleic acids (Ashraf and Harris, 2004). All antioxidant enzymes were stimulated in plants exposed to seawater, and this may be a general adaptive defense response of plants to toxic saline environments during the early growth stages (Xiao-Hua *et al.*, 2009). Now, it is well established that the decrease in water potential, caused by salinity stress, leads to cell membrane damage in almost all plant species (Chen *et al.*, 1999). Cell membrane is one of the prime targets of many plant stresses and its maintenance and integrity under stress conditions is a major determinant of tolerance in plants.

#### **b-Effect of proline foliar application**

Application of exogenous proline as a foliar spray improved (increased) a biotic stress of buttonwood under seawater irrigation. Data in Table (3) showed that 1% proline increased plant height (47.2 cm), leaf area (15.77 cm<sup>2</sup>) in the first season, had thickest stem diameter (0.63 cm) in the second season and number of branches (11.8 and 11.5 in the first and second seasons, respectively). However, 0.0% proline had the thickest stem diameter (0.64 cm) in the first season and largest leaf area (15.99 cm<sup>2</sup>) in the second one while, 0.5% proline had the tallest plant (50.1 cm) in the second season.

Proline application had significant increasing effects on both fresh and dry weight of aerial parts and roots during the two seasons (Table, 4). Results indicated that 1% proline gave the heaviest fresh and dry weights of both aerial parts and roots in the first and second season when compared with 0.0 and 0.5% proline levels



**Table (4): Effect of diluted seawater irrigation, proline spray levels and their interaction on aerial parts and roots fresh and dry weight of *Conocarpus erectus* L. during two seasons**

Trait Proline % Sea water %	Aerial parts fresh weight/plant (g)				Aerial parts dry weight/plant (g)			
	0.0	0.5	1	Mean	0.0	0.5	1	Mean
First season								
0	20.33l	25.61h	25.02i	23.65D	6.20n	6.11o	6.87l	6.39E
10	30.19g	38.08b	34.23e	34.17B	13.18e	14.81c	12.65f	13.55B
15	35.38c	34.44d	54.18a	41.33A	15.12b	14.19d	17.46a	15.59A
30	20.89k	21.71j	30.98f	24.53C	8.57j	9.59i	11.07g	9.74C
50	14.00n	15.77m	13.81o	14.51E	10.77h	7.71k	6.53m	8.34D
Mean	24.16C	27.12B	31.63A		10.77B	10.48C	10.92A	
Second season								
0	32.34e	28.68h	32.83d	31.28C	13.75f	12.55h	14.07e	13.46C
10	31.18g	36.11b	36.89a	34.73A	12.71g	15.53c	15.77b	14.67B
15	35.59c	28.18j	32.33f	32.03B	15.30d	12.11j	17.66a	15.02A
30	18.96l	20.08k	28.53i	22.52D	8.21l	8.61k	12.17i	9.66D
50	14.41n	16.21m	12.08o	14.23E	6.27n	6.88m	5.41o	6.19E
Mean	26.50B	25.85C	28.53A		11.25B	11.14C	13.02A	

Cont. (Table 4)

Trait Proline % Sea water %	Roots fresh weight/plant (g)				Roots dry weight/plant (g)			
	0.0	0.5	1	Mean	0.0	0.5	1	Mean
First season								
0	4.79k	5.54h	4.48l	4.94D	2.23l	2.07m	2.46i	2.25E
10	5.18i	6.54f	6.60d	6.11B	2.40j	4.21c	4.09d	3.57B
15	9.32b	6.58e	10.22a	8.71A	5.08b	3.91e	5.15a	4.71A
30	5.17j	5.18i	7.66c	6.00C	3.10g	2.68h	3.71f	3.16C
50	3.75m	5.76g	3.09n	4.19E	2.68h	2.28k	1.85n	2.27D
Mean	5.64C	5.92B	6.41A		3.09B	3.03C	3.45A	
Second season								
0	8.41e	6.01k	9.41b	7.94B	5.01e	3.88k	5.41a	4.77B
10	6.60i	6.61h	7.16g	6.79C	4.01j	4.10h	4.33g	4.15C
15	9.15c	8.49d	11.01a	9.55A	5.33c	5.13d	5.34b	5.27A
30	7.49f	5.38l	6.35j	6.41D	4.42f	2.87l	4.05i	3.78D
50	3.25o	4.21m	3.78n	3.75E	2.08o	2.40n	2.73ia	2.40E
Mean	6.98B	6.14C	7.54A		4.17B	3.68C	4.37A	

Different letters in each column indicate significances by Duncan (D) tests at  $P < 0.05$ .

Plants are endowed with other kinds of defense mechanisms of sustaining seawater stress. It is suggested that proline is an important component of osmotic adjustment during the initial stages of salinity stress. Amino-acids such as asparagines and proline play an important

role in the osmotic adjustment of the plant under saline conditions (Gilbert *et al.*, 1998). Xiao-Hua *et al.*, 2009 showed that proline in leaves of *Hibiscus tuberosus* from different areas with different treatments of seawater irrigation increased with time as proline accumulated rapidly in plants subjected to salinity stress, where is a fall in leaf water potential (Chu *et al.*, 1974). The obtained results revealed that exogenous proline application improved growth during the two seasons under seawater irrigation stress. The exogenous application of proline increases its endogenous levels in plant tissues subjected to water stress conditions (Ali *et al.*, 2007; Ashraf and Foolad, 2007; Hoque *et al.*, 2007) which contribute to osmotic adjustment in plant tissues (Bajji *et al.*, 2000). Thus, exogenous application of proline may be an efficient mean of ameliorating the adverse effects of water stress on plants (Ali *et al.*, 2008). However, the effectiveness of proline applied as a foliar spray in triggering growth depends on the type of species, plant developmental stage, time of application and concentration (Ashraf and Foolad, 2007). The exogenous application of different concentrations of proline had an ameliorating effect on different photosynthetic parameters (Ali *et al.*, 2007). So in the present study the exogenous proline application encouraged the photosynthesis which reflected on increasing growth of the plant.

### **c- Effect of the interaction between seawater and exogenous proline**

Seawater and proline significantly affected growth parameters. Data presented in Table (3) showed different effects of the interaction between seawater irrigation and proline spray levels on tested traits in the two seasons. The tallest plants (67.7 and 65.7 cm) were obtained with 15% seawater + 1% proline and 10% seawater + 0.5% proline in the first and second season respectively, while 15% seawater + 0.0% proline and 15% seawater + 1% proline gave the highest number of branches (16.8 and 16.0 in both seasons, respectively). Also 50% seawater + 0.0% proline and 0.0% seawater + 1% proline gave the thickest stem diameter (0.75 and 0.71 cm) in both seasons respectively, whereas 30% seawater + 1% proline and 30% seawater + 0.5% proline gave the largest leaf area (18.71 and 17.81 cm<sup>2</sup> in both seasons, respectively).

Table 4 shows that 15% + 1% proline gave the heaviest fresh and dry weights of aerial parts and roots ( 54.18,1746, 10.22 and 5.15 g, respectively) in the first season, while in the second season the same

treatment gave the heaviest dry weight of aerial parts (17.66 g) as well as fresh weight of roots (11.01 g).

Under seawater salinity the osmotic potential of all plant organs significantly decreased (Geissler *et al.*, 2009) which led to reduction of plant growth parameters.

Proline is believed to protect plant tissues against stress by acting as an osmo-regulator and as a protectant for enzymes and cellular structure (Kavi Kishor *et al.*, 2005). Though proline accumulation under osmotic stresses such as salinity and drought is a much widely reported phenomenon in several biological systems, its exact link with stress tolerance remains puzzling. Synthesis of compatible solutes in plants, such as proline, in response to salinity stress is a possible strategy to engineer salt tolerance in plants and has been discussed as well as debated several times (Apse and Blumwald, 2002). Kumara *et al.*, 2009 found that *Brassica juncea* var. CS52 has an advantage of being able to accumulate the highest amounts of proline (4.5 mgg<sup>-1</sup> DW) under salinity stress and this may be directly or indirectly related to their tolerance towards salinity. From that in our work the foliar applications of proline may act to increase the tolerance of salinity stress in the plant when irrigated with high seawater concentration.

Another reason for the biomass reduction due to the salt treatments is the increased energy consumption for various salt tolerance mechanisms, e.g. for synthesizing compatible organic solutes and proteins. A higher energy use is indicated by the increased dark respiration of the plants under saline conditions (Geissler *et al.*, 2009). *Conocarpus erectus* uses proline as compatible solutes. The main function does not seem to be an osmotic, but a protective one (protection of proteins, membranes etc.). By various halophytes proline is accumulated as a reaction to NaCl salinity and to other abiotic stresses (Koca *et al.*, 2007; Ashraf and Foolad, 2007) and can enhance salt tolerance. This extremely water soluble amino acid functions as a chaperone by forming clusters with water molecules which attach to proteins and membranes and prevent their denaturation (Koca *et al.*, 2007; Ashraf and Foolad, 2007; Lee *et al.*, 2008). Due to its protective function on membranes it can also improve cell water status and ion homeostasis (Gadallah, 1999; Gleeson *et al.*, 2005), and it serves as a scavenger for hydroxyl radicals and singlet oxygen and thus reduces oxidative stress (Koca *et al.*, 2007; Ashraf and Foolad, 2007; Lee *et al.*, 2008).

## 2-Effect of diluted seawater irrigation, proline spray levels and their interactions on chemical composition

### a-Effect of diluted seawater

Data presented in Table (5) showed that 30% seawater increased the nitrogen (N), phosphors (P) and ferric (Fe) contents (3.28%, 178.08 ppm and 3.05 ppm, respectively) in aerial parts when compared with low and high seawater concentrations. Also 10% seawater increased the potassium (K) concentration (12.33 ppm) when compared with control and high seawater concentrations. However, for the other ions (such as zinc (Zn), cobalt (Co), magnesium (Mn), copper (Cu) and nickel (Ni)) that reflected on their contents in aerial parts, although the seawater is void of these ions, the results indicated that seawater decreased Cu uptake. Salt stress of seawater irrigation led to direct increase in sodium content of aerial parts, whereas 50% seawater gave the highest sodium content (39.2 ppm). The increase in sodium content resulted an increase in Na/K ratio in the aerial parts

**Table (5): Effect of diluted seawater irrigation on chemical composition of aerial parts of *Conocarpus erectus* during second season.**

Ions Seawater %	N %	P ppm	K ppm	Fe ppm	Zn ppm	Co ppm	Mn ppm	Cu ppm	Ni ppm	Na ppm	Na/ K %
0	1.78	107.19	10.14	2.82	0.15	0.0	0.0	0.35	0.0	14.6	1.44
10	2.75	99.43	12.33	2.43	0.10	0.0	0.0	0.07	0.0	19.8	1.61
15	2.75	162.05	10	2.53	0.05	0.0	0.0	0.0	0.0	26.4	2.64
30	3.28	178.08	10.79	3.05	0.15	0.0	0.0	0.12	0.0	26.73	2.48
50	1.94	129.2	7.78	2.29	0.09	0.0	0.0	0.01	0.0	36.7	4.72

Accumulation of inorganic solutes, such as cations of Na<sup>+</sup> and K<sup>+</sup> and the anion Cl<sup>-</sup>, can also play a role independently or in combination with other mechanisms in maintaining the osmotic imbalance caused by the salt stress and influence the osmotic potential adjustment of plant cells (Peng *et al.*, 2004). In the present study, the Na<sup>+</sup> content of aerial parts was increased with the increase in seawater concentration. During the experimental period, Na<sup>+</sup> and Cl<sup>-</sup> ions were accumulated in the cells. These results indicated that high concentrations of seawater can influence ions distribution, so that they can contribute to the osmotic potential, and thereby increase the protection against osmotic stress.

Under salt-stress conditions N uptake is limited by an accumulation of Cl and its competition with NO<sub>3</sub><sup>-</sup> (Alam 1999). Nitrogen content was significantly reduced by salt stress, especially in the leaves (Geissler *et al.*, 2009). In our experiments, this competition did not occur at 15 and 30% seawater irrigation, since N was accumulated when compared with the high seawater concentration (Table 5). Much of N contents under NaCl salinity were used in synthesis of specific N compounds such as amino acids (e.g. proline and aspartic acids), amides (glutamine and asparagine) and the stress-related proteins (Mansour 2000; Ashraf and Harris, 2004)

Frequently, plants exposed to NaCl inevitably absorb a large amount of Na, which subsequently causes a decrease in the contents of K (Gomez *et al.*, 1996; Hasegawa *et al.*, 2000). Generally, the most salt tolerant plants accumulate Na<sup>+</sup> in their shoots whereas sensitive plants do not. In our experiments, seawater stress did not significantly reduce K content in the plants. An accumulation of K under seawater irrigation suggests a more efficient K uptake in buttonwood plant. Jacoby (1999) proposed that K accumulation represented plant adaptation to salinity. Not only Na and K contents, but also the Na/K ratio can be used as phyto-physiological parameters for screening less sensitive plant for NaCl stress (De Lacerda *et al.*, 2005). A high Na/K ratio indicates metabolic disorders such as a reduction in protein synthesis and enzyme activities (Brady *et al.*, 1984), as well as an increase in membrane permeability (Alam, 1999). Moreover, elevated K levels act osmotically, preventing Na influx into roots and shoots (Jacoby, 1999).

The K<sup>+</sup>/Na<sup>+</sup> selectivity is an important determinant of salt tolerance and it depends on the characteristics of the transporters that mediate K<sup>+</sup> and Na<sup>+</sup> absorption. Under high salt conditions, more Na<sup>+</sup> enters the cell as the similarity in the hydrated ionic radii between Na<sup>+</sup> and K<sup>+</sup> makes it difficult for the transporter to discriminate between the two ions (Blumwald *et al.*, 2000). This ion homeostasis can actually be a reflection of several different strategies that the plant uses such as diminishing the entry of Na<sup>+</sup> ions into cells, extrusion of Na<sup>+</sup> ions out of the cell or/and vacuolar compartmentation of Na<sup>+</sup> ions.

#### **b-Effect of proline application**

The foliar application of buttonwood plant with 0.5% exogenous proline increased the accumulation of N, P, K and Zn contents, and the highest level was 3.29%, 145.22 ppm, 13.38 ppm and 0.14 ppm respectively in the aerial parts (Table, 5). However, the

accumulation of ferric, copper and sodium contents decreased with proline application.

**Table (6): Effect of exogenous proline on chemical composition of aerial parts of *Conocarpus erectus* during second season..**

Ions	N %	P ppm	K ppm	Fe ppm	Zn ppm	Co ppm	Mn ppm	Cu ppm	Ni ppm	Na ppm	Na / K %
Proline											
0.0	1.78	123.97	8.15	3.43	0.10	0.0	0.0	0.29	0.0	27.58	3.38
0.5	3.29	145.22	13.38	2.14	0.14	0.0	0.0	0.04	0.0	22	1.64
1	2.1	136.39	9.09	2.29	0.08	0.0	0.0	0.0	0.0	24.96	2.75

The increase in the shoot N content under water stress conditions was found to be due to the increased accumulation of proline leading to increased transpiration and stomatal aperture (Singh *et al.*, 1973). Foliar application of proline was effective on ameliorating the adverse effects of water stress on both maize cultivars by promoting the uptake and accumulation of essential nutrients such as N, P and K<sup>+</sup> (Ali *et al.*, 2008). Exogenously applied different concentrations of proline at different growth stages enhanced the accumulation of all these macronutrients under water stress conditions and 30 mM proline concentration was found to be the most effective as compared to the other concentrations as this concentration has already been found more effective an increasing transpiration rate in the previous studies (Ali *et al.*, 2007). All these reports support the results of the present study which suggested that 0.05% exogenous proline was effective and promoted the uptake and accumulation of essential nutrients such as N, P, K<sup>+</sup> and Zn.

### **c-Effect of the interaction between diluted seawater and exogenous proline**

The highest foliage N and K contents (5.18 % and 16.8 ppm) were obtained by the imposition of 10% seawater + 0.5% exogenous proline. Also 30% seawater + 1% proline, 15% seawater + 0.0% proline, 30% seawater + 0.5% proline, 0.0% seawater + 0.0% proline (control) and 50% seawater + 0.0% proline gave the highest contents from P (183.2 ppm), Fe (4.07 ppm), Zn (0.22ppm), Cu (1.06 ppm) and Na (39.2 ppm), respectively.

**Table (7): Effect of the interaction between diluted seawater irrigation and proline spray levels on chemical composition of aerial parts of *Conocarpus erectus* L. during the second season..**

Ions		N %	P ppm	K ppm	Fe ppm	Zn ppm	Co ppm	Mn ppm	Cu ppm	Ni ppm	Na ppm	Na / K %
Seawater %	Proline %											
0	0	1.68	67.58	8.4	3.65	0.16	0.0	0.0	1.06	0.0	16.8	2.0
	0.5	2.0	147.8	15.7	2.55	0.20	0.0	0.0	0.0	0.0	12.3	0.78
	1	1.68	106.2	6.32	2.25	0.08	0.0	0.0	0.0	0.0	14.7	2.33
10	0	1.54	97.35	8.5	3.57	0.09	0.0	0.0	0.03	0.0	22.3	2.62
	0.5	5.18	101.8	16.8	1.8	0.16	0.0	0.0	0.18	0.0	17.9	1.07
	1	1.54	99.15	11.68	1.93	0.06	0.0	0.0	0.0	0.0	19.2	1.64
15	0	1.96	156.35	7.36	4.07	0.04	0.0	0.0	0.0	0.0	29.4	3.99
	0.5	3.5	161.95	12.8	1.42	0.06	0.0	0.0	0.0	0.0	24.1	1.88
	1	2.8	167.85	9.84	2.10	0.05	0.0	0.0	0.0	0.0	25.7	2.61
30	0	1.96	177	10.4	3.03	0.09	0.0	0.0	0.36	0.0	30.2	2.90
	0.5	3.5	174.05	12.56	2.87	0.22	0.0	0.0	0.0	0.0	22.1	1.76
	1	2.66	183.2	9.4	3.25	0.13	0.0	0.0	0.0	0.0	27.9	2.96
50	0	1.73	121.55	6.1	2.85	0.13	0.0	0.0	0.0	0.0	39.2	6.43
	0.5	2.26	140.5	9.04	2.07	0.07	0.0	0.0	0.02	0.0	33.6	3.71
	1	1.82	125.55	8.2	1.95	0.07	0.0	0.0	0.0	0.0	37.3	4.55

Chemical composition of plants irrigated with seawater and treated with exogenous proline showed that the foliage of these plants contained many ions which were absorbed from both seawater and soil. Nutrient ions are dissolved in the soil solution and nutrients uptake by plants depends on water flow through the soil-root-shoot continuum (Keller, 2005). The plants treated with 0.0% proline showed a lack of elements content, but foliar application with exogenous proline at different concentrations enhanced the uptake of different nutrients. These results agree with Ali *et al.* (2008) who found that mineral nutrients (N, P, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>) of maze cultivars decreased under water stress, but exogenously applied different concentrations of proline at different growth stages enhanced the accumulation of all these macronutrients under water stress conditions

### 3- Anatomical study

Internal structures of stem and leaf lamina of buttonwood plants (*Conocarpus erectus* L.) are similar to other dicotyledons plants. The stem consists of the epidermis, ground tissue and vascular system. Ground tissue differentiated into cortex and pith. The vascular collateral bundles are arranged in complete cylinder (Fig. 1). In the first season, data illustrated in Fig. (1) and presented in Table (8) indicated that application of seawater dilutions (10, 15 and 30%)

caused an increase in stem diameter (mm) compared to control. Seawater at 10% gave the highest stem diameter value compared with the other dilutions. The stem diameter decreased gradually with increasing seawater dilutions. It is important to state that, application of proline at 0.5 and 1% slightly increased the effects in this respect compared with control (0% seawater). The combinations between seawater and proline treatments were more effective on stem diameter than seawater treatments alone. The thickest stem diameter was recorded by 10% seawater and 1% proline interaction treatment. Increasing stem diameter is due to corresponding increase in thickness of cortical cell layers, width of pith as well as the conductive tissues. For cortex thickness ( $\mu\text{m}$ ), all treatments increased the cortex expansion. No differences were found between treated plants with 0.5% proline and the control. The application of seawater at 10% in combination with proline at 1% induced the thickest cortex layers compared with the other treatments.. The increase in cortex thickness may be attributed to the increase in number of cortical cell layers and/or cell enlargement. Regarding stem vascular tissues (xylem and external phloem) thickness, application of 10 and 15% seawater in combination with proline were more effective compared with the other treatments. The highest thickness of xylem and external phloem were achieved by using 10% seawater and 1% proline interaction. The increase in differentiated vascular tissues is due to a reflection of increasing in vascular cambium activity. It is noted that, 50% seawater caused an irregular stem secondary growth. This is due to unusual behavior of typical vascular cambium (Fahn, 1990) by using high seawater salinity level.



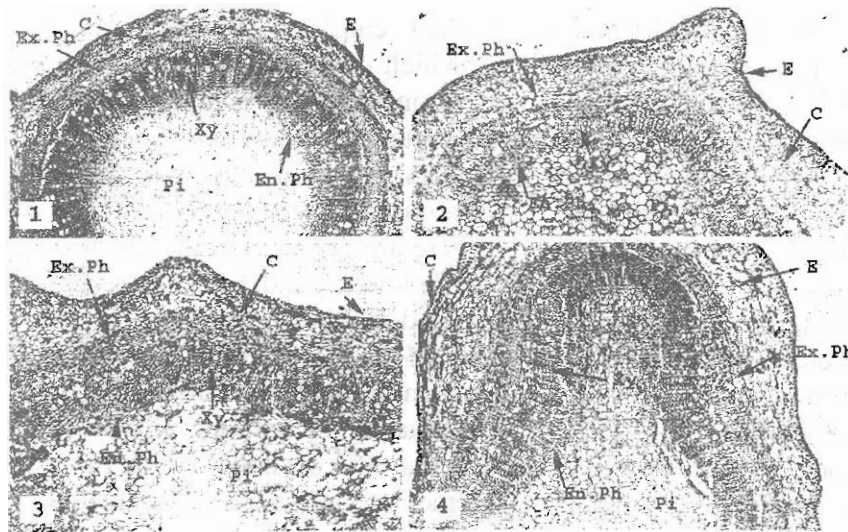


Fig (1): Cross sections through stems of buttonwood plants (*Conocarpus erectus* L.) as affected by seawater and seawater and proline interaction treatments (40x)  
 1- Control. 2- 10% seawater. 3- Combination between 10% seawater and 1% proline. 4- 50% Seawater. Epidermis (E), Cortex (C), External phloem (Ex. Ph), Internal phloem (En. Ph), Xylem (Xy), Pith (Pi).

Table (8): Effect of seawater, proline and their interaction treatments on anatomical parameters of buttonwood stems during the first season.

Treatments	Stem c. s diameters		Cortex tissue thick.		Xylem tissue thick.		Ex. phloem tissue thick	
	( $\mu$ m)	%	( $\mu$ m)	%	( $\mu$ m)	%	( $\mu$ m)	%
Control	2.76	100	232	100	216	100	133	100
Proline 0.5%	2.78	+0.72	232	0	215	-0.46	135	+1.50
Proline 1%	2.78	+0.72	255	+1.93	220	+1.85	134	+0.75
Sw 10%	3.20	+15.94	310	+33.62	244	+12.96	139	+4.51
Sw 10% + proline 0.5%	3.34	+21.01	319	+37.5	254	+17.59	143	+7.52
Sw 10% + proline 1%	3.40	+23.19	360	+55.17	256	+18.52	150	+12.78
Sw 15%	3.06	+10.87	250	+7.76	219	+1.39	135	+1.50
Sw 15% + proline 0.5%	3.14	+13.77	293	+26.29	219	+1.39	140	+5.26
Sw 15% + proline 1%	3.20	+15.94	294	+26.72	225	+4.17	140	+5.26
Sw 30%	2.81	+1.81	245	+5.60	210	-2.78	129	-3.01
Sw 30% + proline 0.5%	2.81	+1.81	245	+5.60	212	-1.85	133	0
Sw 30% + proline 1%	2.87	+3.99	251	+8.19	217	+0.46	133	0
Sw 50%	2.73	-1.09	239	+3.02	211	-2.31	120	-9.77
Sw 50% + proline 0.5%	2.88	+4.35	259	+3.02	211	-2.31	124	-6.77
Sw 50% + proline 1%	2.88	+4.35	244	+5.17	217	+0.46	127	-4.51

Seawater (Sw), External (Ex.), Cross section (c.s.)

The leaf of buttonwood plant consists of upper and lower epidermis and mesophyll tissue, which differentiate into palisade and spongy parenchyma. Epidermis is one layer of completely arranged parenchymatous cells, which are flattened parallel to the leaf surface. The palisade, is one to two layers are elongated and completely arranged. The spongy parenchymatous cells are loosely arranged with numerous intercellular spaces. There are several salt glands near vein angles on lower leaf surface. Data in Fig. (2) and Table (9) reveal that, seawater at 10, 15 and 30% increased the thickness of lamina, but the highest value was recorded at 15%. Also, proline combination treatments caused an increase in lamina thickness. Increasing in leaf thickness was a reflection of increasing in palisade and/or spongy tissues. On the other side, seawater at 50% declined lamina thickness. All seawater and proline interaction treatments augmented the thickness of leaf lamina compared with seawater dilutions alone. Regarding mesophyll tissue, proline treatments and 10 and 15% seawater and their interaction treatments increased palisade tissue thickness, while 30 and 50% seawater decreased it. At 15% seawater and 1% proline interaction treatment induced the highest thickness of palisade tissue. In contrast, the lowest palisade thickness was recorded by 50% seawater. Application of the combination between seawater (30 and 50%) and proline caused an increase in palisade thickness compared with the two seawater levels alone. For spongy tissue, all treatments induced an increase in its thickness. The thickest spongy tissue was obtained by 15% seawater and its interaction with proline. Seawater up to 30% and its interaction with proline increased midrib vascular bundle thickness. The combination between 15% seawater and 1% proline gave the highest value of midrib vascular bundle thickness. It is noted that, salinity glands volume was related with seawater level, the highest seawater level the biggest salinity glands. Internal stem and leaf growth increased parameters by using low dilutions of seawater (up to 30%) in combination with proline levels are due to seawater mineral nutrients uptake. Concern, nutrients, salinity induced a decrease in  $Cl^-$  levels in shoots and an increase in roots and the high content of  $Mg^{2+}$  in seawater restricted  $Ca^{2+}$  uptake enhancement in contrast, the accumulation of P and Mn as well as micronutrients accrued in general (D'Amico *et al.*, 2004).

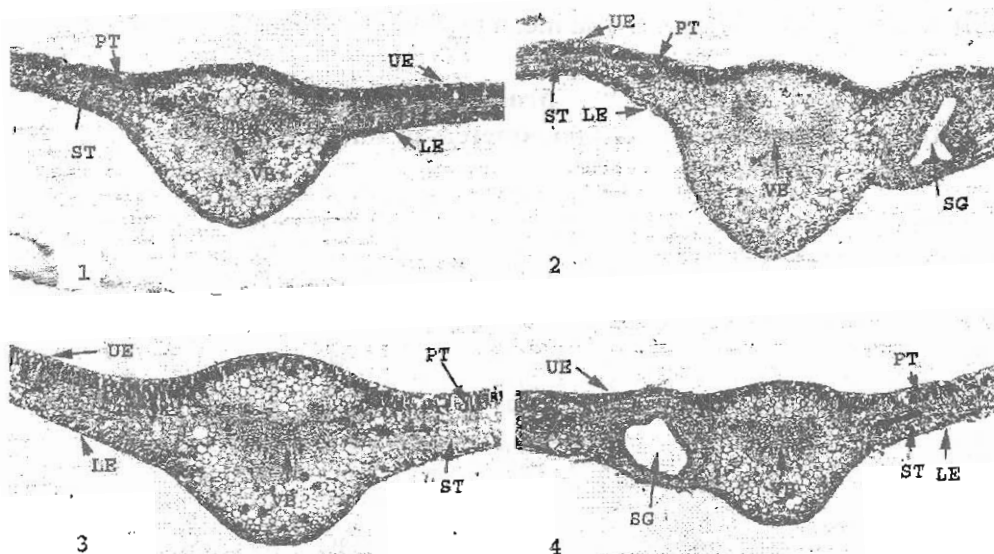


Fig (2): Cross sections through leaves of buttonwood plants (*Conocarpus erectus* L.) as affected by seawater and seawater and proline interaction treatments, (40x).  
 1- Control. 2- 15% seawater. 3- Combination between 15% seawater and 1% proline. 4- 50% Seawater. Upper epidermis (UE), Lower epidermis (LE), Palisade tissue (PT), Spongy tissue (ST), Vascular bundle (VB), Salinity gland (SG).

The reduction in internal growth parameters by using high seawater dilution (50%) are due to excessive soluble salts concentrations in the root media, due to decreasing the availability of water, by reducing the free energy of water and toxicity of one or more specific ions presented in higher relative concentrations. Application of proline as osmoregulator helps plants that limited uptake of toxic ions as  $\text{Na}^+$  and  $\text{Cl}^-$  and protected plant enzymes and cell components against deleterious effects of high salt stress (Paleg *et al.*, 1984). In addition, proline increased the succulence of leaves and causes greater increase in leaf thickness (Table, 2 and Fig. 2), which diluted seawater salinity and increased salt tolerance (Hussien, 2000). Halophytic plants similar to buttonwood play increasingly important roles for coastal ecosystem restoration, and for the development of biosaline agriculture. Biosaline agriculture will contribute to redress the pressure on limited freshwater resources, as well as the problem of increasing agricultural areas of salinized soils. It is of especial importance to focus on those halophytes that can tolerate full strength seawater salinity (Böer, 2008). There is still a lack of public

awareness, and scientific documentation regarding successfully established halophytes. What do you mean by establishment?

**Table (9): Effect of seawater, proline and their interaction treatments on anatomical parameters of buttonwood leaves during the first season.**

Treatments	Lamina thick.		Palisade tissue thick.		Spongy tissue thick.		Midrib v. b. thick.	
	( $\mu\text{m}$ )	%	( $\mu\text{m}$ )	%	( $\mu\text{m}$ )	%	( $\mu\text{m}$ )	%
Control	216	100	44	100	139	100	240	100
Proline 0.5%	220	+1.85	45	+2.27	140	+0.72	241	+0.42
Proline 1%	220	+1.85	45	+2.27	143	+2.88	240	0
Sw 10%	224	+3.70	49	+11.36	141	+1.44	256	+6.67
Sw 10% + proline 0.5%	232	+7.41	53	+20.45	141	+1.44	262	+9.17
Sw 10% + proline 1%	249	+15.28	66	+50	150	+7.91	268	+11.67
Sw 15%	257	+18.98	60	+36.36	163	+17.27	272	+13.33
Sw 15% + proline 0.5%	265	+22.69	60	+36.36	175	+26	277	+15.42
Sw 15% + proline 1%	265	+22.96	63	+43.18	174	+25.18	279	+16.25
Sw 30%	224	+3.70	42	-4.54	150	+7.91	260	+8.33
Sw 30% + proline 0.5%	224	+3.70	43	-2.27	153	+10.07	264	+10
Sw 30% + proline 1%	230	+6.48	43	-2.27	160	+15.11	266	+10.33
Sw 50%	215	-0.46	33	-25	145	+4.32	236	-1.67
Sw 50% + proline 0.5%	216	0	35	-20.45	150	+7.91	238	-0.83
Sw 50% + proline 1%	216	0	38	-13.63	148	+6.47	244	+1.67

seawater (Sw), vascular bundle (v.b.)

From the present work, it is concluded that, application of seawater at 10 and 15% improved the internal growth parameters of buttonwood plants. In addition, proline redressed plants to the adverse effects of seawater salinity.

### Conclusion

This study reports the physiological, biochemical and anatomical response of *Conocarpus erectus* to seawater stress. It was feasible to use diluted levels of seawater to irrigate *Conocarpus erectus*. It is reported that low levels of diluted seawater improved plant growth, also proline treatments have significant effects on plant growth as 30% seawater treatment increased N, P and Fe contents in aerial parts when compared with the low and high seawater levels. Therefore, 30% seawater can be used as a fertigation of *Conocarpus erectus*.

### REFERENCES

- Alam, S.M. (1999). Nutrient uptake by plants under stress conditions. In: Pessaraki, M. (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker NewYork Basel. P: 285-313.
- Ali, Q.; Ashraf, M. and Athar, H.R. (2007). Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. Pak J Bot., 39: 1133-1144.
- Ali, Q.; Ashraf, M.; Shahbaz, M. and Humera, H. (2008). Ameliorating effect of foliar applied proline on nutrients uptake in water stressed maize (*Zea mays* L.) plants. Pak J Bot. 40: 211-219.
- Apse, M.P. and Blumwald, E. (2002). Engineering salt tolerance in plants. Curr Opin Biotechnol., 13:146-50.
- Ashour, N.I.; Serag, M.S.; Abd El-Haleem, A.K. and Mekki, B.B. (1997). Forage production from three grass species under saline irrigation in Egypt. J Arid Environ., 37: 299-307.
- Ashraf, M. and Foolad, M.R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot., 59: 206-216.
- Ashraf, M. and Harris, P.J.C. (2004). Potential biochemical indicators of salinity tolerance in plants. Plant Sci., 166: 3-16.
- Aspinall, D. and Paleg, L.G. (1981). Proline accumulation: Physiological aspects. Pp 205-241. In: the physiology and biochemistry of drought resistance in plants (ed) Paleg L. G. and D. Aspinall. Academic Press.
- Bajji, M.; Lutts, S. and Kinet, J.M. (2000). Physiological changes after exposure to and recovery from polyethylene glycol-induced water deficit in callus cultures issued from durum wheat (*Triticum durum* Desf.) cultivars differing in drought resistance. J Plant Physiol., 156: 75-83.
- Blumwald, E.; Aharon, G.S. and Apse, M.P. (2000). Sodium transport in plant cells. Biochim Biophys Acta., 1465: 140-51.
- Böer, B. (2008). Halophyte research and development: what needs to be done next?. In: Kham, M. A. and D. J. Weber (eds.), Ecophysiology of high salinity tolerate plants, 397-399. Springer Science + Business Media B. V.
- Brady, C.J.; Gibson, T.S.; Barlow, E.W.R.; Speirs, J. and Wyn Jones, R.G. (1984). Salt tolerance in plants. I. Ions compatible organic solutes ant the stability of plant ribosomes. Plant Cell Environ., 7: 571-578.

- Chen, Q.; Zhang, W.H. and Liu, Y.L. (1999). Effect of NaCl, glutathione and ascorbic acid on function of tonoplast vesicles isolated from barley leaves. *J Pl Physiol.*, 155: 685–90.
- Chu, T.M.; Aspinall, D. and Paleg, L.G. (1974). Stress metabolism VI. Temperature stress and the accumulation of proline in barley and radish. *Aust J Pl Physiol.*, 1: 87-97.
- D'Amico, M.L.; Navari-Izzo, F. and Izzo, R. (2004). Alternative irrigation water: Uptake of mineral nutrients by wheat plants responding to seawater application. *J Plant Nutr.* 27: 1043-1059.
- De Lacerda, C.F. Cambraia, J. Oliva, M.A. and Ruiz, H.A. (2005). Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. *Environ Experim Bot.*, 54: 69–76.
- Di Baccio, D.; Navari-Izzo, F. and Izzo, R. (2004). Seawater irrigation: Antioxidant defence responses in leaves and roots of a sunflower (*Helianthus annuus* L.) ecotype. *J Pl Physiol.*, 161: 1359–1366.
- Duncan, D.B. (1955). Multiple range and multiple F test. *Biometrics.*, 11: 1–42.
- Fahn, A. (1990). Plant anatomy. Pp 397-407, Pergamon Press, Fourth Edition, Israel.
- Fang, S. and Chen, X. L. (1997). Using shallow saline groundwater for irrigation and regulating for soil salt-water regime. *Irrig Drain Syst.*, 11: 1–14.
- Gadallah, M.A.A. (1999). Effects of proline and glycinebetaine on *Vicia faba* responses to salt stress. *Biol Plant.*, 42: 249–257.
- Geng-Mao, Z.; Zhao-Pu, L.; Ming-Da, C. and Shi-Wei, G. (2008). Soil Properties and Yield of Jerusalem Artichoke (*Helianthus tuberosus* L.) with Seawater Irrigation in North China Plain. *Soil Sci Soc China.*, 18: 195-202.
- Geissler, N.; Hussin, S. and Koyro, H.W. (2009). Interactive effects of NaCl salinity and elevated atmospheric CO<sub>2</sub> concentration on growth, photosynthesis, water relations and chemical composition of the potential cash crop halophyte *Aster tripolium* L. *Environ Exp Bot.*, 65: 220–231.
- Gilbert, G.A.; Gadush, M.V.; Wilson, C. and Madore, M.A. (1998). Amino acid accumulation in sink and source tissues of *Coleus blumei* Benth. during salinity stress. *J Exp Bot.*, 49: 107–114.

- Gleeson, D.; Lelu-Walter, M.A. and Parkinson, M. (2005). Overproduction of proline in transgenic hybrid larch (*Larix×leptoeuropaea* (Dengler)) cultures renders them tolerant to cold, salt and frost. *Mol Breed.*, 15: 21–29.
- Gleick, P.H. (1993). *Water in Crisis*. Oxford University Press, New York
- Glenn, E.P.; Brown, J.J. and O'Leary, J.W. (1998). Irrigating crops with seawater. *Scientific American.*, 279: 56-61.
- Gliman, E.F. and Watson, D.G. (1993). *Conocarpus erectus*: Buttonwood. Fact sheet ST-179. U.S. Forest Service and Southern Group of State Foresters, Gainesville, FL., 1–3.
- Gomez, I.; Pedreno, J.N.; Moral, L. Iborra, M.R. Palacios, G. and Mataix, J. (1996). Salinity and nitrogen fertilization affecting the macronutrient content and yield of sweet pepper plants. *J Plant Nutr.*, 19: 353–359.
- Hasegawa, P.M.; Bressan, R.A.; Zhu, J.K. and Bohnert, H.J. (2000). Plant cellular and molecular responses to salinity. *Annu. Rev. Plant Physiol. Plant Mol Biol.*, 51: 463–499.
- Hatzikos, E.V.; Tsoumakas, G.; Tzanis, G.; Bassiliades, N. and Vlahavas, I. (2008). An empirical study on sea water quality prediction. *Knowledge-Based Systems.* , 21: 471–478.
- Hoque, M.A.; Okuma, E.; Banu, M.N.A.; Nakamura, Y.; Shimoishi, Y. and Murata, N. (2007). Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betaine by increasing antioxidants enzyme activity. *J Pl Physiol.*, 164: 553-561.
- Hussien, S.F.M. (2000). Structural and physiological studies and oil constituents of canola plants under salinity conditions. Ms. C Thesis Fac. Agric, Mansoura Univ. Egypt.
- Incerti, A.; Izzo, R.; Belligno, A. and Navari-Izzo, F. (2008). Seawater effects on antioxidant production in berries of three cultivars of tomato (*Lycopersico esculentum* Mill.). In: Chedly Abdelly, Münir Öztürk, Muhammad Ashraf and Claude Grignon (ed) *Biosaline Agriculture and High Salinity Tolerance*. Birkhäuser verlag/Switzerland., 44-51.
- Izzo, R.; Incerti, A. and Beertolla, C. (2008) Seawater irrigation: Effects on growth and nutrient uptake of sunflower plants. In: Chedly Abdelly, Münir Öztürk, Muhammad Ashraf and Claude Grignon (ed) *Biosaline Agriculture and High Salinity Tolerance*. Birkhäuser verlag/Switzerland., 61-69.

- Jackson, M. L. (1973). Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd, New Delhia
- Jacoby, B. (1999) Mechanisms involved in salt tolerance of plants. In: Pessaraki, M. (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker Inc., New York, Basel.p: 97–123.
- Jin, M.G.; Zhang, R.Q.; Sun, L.F. and Gao, Y.F. (1999). Temporal and spatial soil water management: A case study in the Heilonggang region, PR China. Agr Water Manage., 42: 173–187.
- Jin, Z.M.; Wang, C.H. Liu, Z.P. and Gong, W.J. (2007). Physiological and ecological characters studies on *Aloe vera* under soil salinity and seawater irrigation. Process Biochem., 42: 710–714.
- Kavi Kishor, P.B.; Sangam, S.; Amrutha, R.N.; SriLaxmi, P.; Naidu, K.R. and Rao, K.R.S.S. (2005). Regulation of proline biosynthesis, degradation, uptake and transport in higher plants :its implications in plant growth and a biotic stress tolerance. Curr Sci., 88: 424–38.
- Keller, M. (2005) Deficit irrigation and vine mineral nutrition. Am. J Enol Vitic., 56: 267-283.
- Koca, H.; Bor, M.; Qzdemir, F. and Türkan, I. (2007). The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. Environ Exp Bot., 60: 344–351.
- Kumara, G.; Purtya, R.S.; Sharmac, M.P.; Singla-Pareekb, S.L. and Pareeka, A. (2009). Physiological responses among Brassica species under salinity stress show strong correlation with transcript abundance for SOS pathway-related genes. J Pl Physiol., 166: 507—520.
- Lindsay, W.L. and Norvell, W. A. (1978). Development of a DTPA test for zinc, iron, manganese and copper. Soil Sci Soc Amer J Proc. 42: 421-428.
- Lee, G.; Carrow, R.N.; Duncan, R.R.; Eiteman, M.A. and Rieger, M.W. (2008). Synthesis of organic osmolytes and salt tolerance mechanisms in *Paspalum vaginatum*. Environ Exp Bot., 63: 19–27.
- Lin, C.C. and Kao, C.H. (1996). Proline accumulation is associated with inhibition of rice seedling root growth caused by NaCl. Plant Sci. 114: 121-128.
- Liogier, H.A. (1990). Plamas Medicinales de Puerto Rico y del Caribe. San Juan: Iberoamericana de Ediciones, Inc., 500–566.



- Liu, Z.P.; Liu, L.; Chen, M.D.; Deng, L.Q.; Zhao, G.M.; Tang, Q.Z. and Xia, T.X. (2003). Study on the irrigation systems in agriculture by seawater. J Nat Resour (in Chinese), 18: 423–429.
- Mansour, M.M.F. (2000). Nitrogen containing compounds and adaptation of plants to salinity stress. Biol Plant., 43: 491–500.
- Miyamoto, S.; Glenn, E.P. and Olsen, M.W. (1996). Growth, water use and salt uptake of four halophytes irrigated with highly saline water. J Arid Environ. 32: 141-159.
- Moreno, F.; Cabrera, F.; Fernandez-Boy, E.; Giron, I.F.; Fernandez, J.E. and Bellido, B. (2001). Irrigation with saline water in the reclaimed marsh soils of south-west Spain: Impact on soil properties and cotton and sugar beet crops. Agric Water Manag., 48: 133-150.
- Munns, R.; Schachtman, D.P. and Condon, A.G. (1995). The significance of a two-phase growth response to salinity in wheat and barley. Aust J Plant Physiol., 22: 561–569.
- Naidoo, J.; Jahnke, J. and Von Willert, D.J. (1995). Gas exchange responses of the C<sub>4</sub> grass *Sporobolus virginicus* (Poaceae) to salinity stress. In: Khan, A.M. & Ungar, I.A. (Eds), Biology of Salt Tolerant Plants, pp. 121–130. Karachi: University of Karachi.
- Nandwal, A.S.; Godara, M.; Sheokandm, S.; Kamboj, D.V.; Kundu, B.S.; Kuhad, M.S.; Kumar, B. and Sharma, S.K. ( 2000). Salinity induced changes in plant water status, nodule functioning and ionic distribution in phenotypically differing genotypes of *Vigna radiata* L. J Pl Physiol., 156: 350–359.
- Nelson, G. (1996). The Shrubs and Woody Vines of Florida. Sarasota, FL: Pineapple Press, Inc.
- O’Leary, J.W. (1988). Saline environments and halophytic crops. In: Whitehead, E.E., Hutchinson, C.F., Timmermam, B.N. & Varady, R.G. (Eds), Arid Lands: today and tomorrow, p. 773–790. Boulder, CO: Westview Press, London, and Belhaven Press. P: 1435.
- Page, A.L. (1982). Methods of Soil Analysis. Part 2: Chemical and microbiological properties.(2<sup>nd</sup> ed) Amer. Soc. Agron., In: Soil Sci. Soc. Amer. In. Madison, Wisconsin, USA.
- Paleg, L.G.; Stewart, G.R. and Bradbeer, J.W. (1984). Proline and glycine betaine influence protein salvation. Plant Physiol., 75: 974-978.

- Peng, Y.H.; Zhu, Y.F.; Mao, Y.Q.; Wang, S.W.; Su, W.A. and Tang, Z.C. (2004). Alkali grass resists salt stress through high [K<sup>+</sup>] and an endodermis barrier to Na<sup>+</sup>. J Exp Bot., 55: 939–949.
- Popp, M.; Uttge, U.L.; Cram, W.J.; Diaz, M.; Griffiths, H.; Lee, H.J.S.; Medina, E.; Schaffer, C.; Stimmel, K.H. and Thonke, B. (1989). Water relations and gas exchange of mangroves. New Physiologist., 111: 293–307.
- Ruzin, S.E. (1999). Plant microtechniques and microscopy. First Ed. Oxford University press, USA.
- Schouwenbury, J.C.V. and Walinge, I. (1967). The rapid determination of phosphorus in presence of arsenic, silicon and germanium. Anal Chem Act. 37: 271-274.
- Silveira, J.A.G.; Melo, A.R.B.; Viegas, R.A. and Oliveira, J.T.A. (2001). Salinity-induced effects on nitrogen assimilation related to growth in cowpea plants. Environ Exp Bot., 46: 171–179.
- Singh, R. (2008). Worldwide water crisis. J Membrane Sci., 313: 353–354.
- Singh, T.N.; Paleg, L.G. Aspinall, D. (1973). Stress metabolism. 1. Nitrogen metabolism and growth in barley plants during water stress. Aust J Biol Sci., 26: 45.
- Xiao-Hua, L.; Jin-He, C.; Ling, L.; Qing, L. and Zhao-Pu, L. (2009) Effect of Seawater Stress on Physiological and Biochemical Responses of Five Jerusalem Artichoke Ecotypes. Soil Sci Soc of China., 19: 208–216.

### الملخص العربي

تأثير الري بمياه البحر المخفف و المعاملة الخارجية بالبرولين علي النمو والتركيب الكيماوي والصفات *Conocarpus erectus* L. التشريحية لنبات الكونوكربس

محمد السيد المحروق<sup>١</sup>، محمد فتحي النادي<sup>٢</sup>، محمود حجازي<sup>١</sup>

<sup>١</sup> قسم البساتين، كلية الزراعة، جامعة كفر الشيخ، مصر

<sup>٢</sup> قسم النبات الزراعي، كلية الزراعة، جامعة كفر الشيخ، مصر

أجريت سلسله من التجارب في مزرعة كلية الزراعة جامعة كفر الشيخ خلال موسمي ٢٠٠٧، ٢٠٠٨ م ( في الفترة من ١ ابريل إلى ٣٠ ديسمبر من كل عام) لدراسة تأثير الري بخمسة مستويات من ماء البحر المخفف ( ٠، ١٠، ١٥، ٣٠، ٥٠%) على النمو والتركيب الكيماوي والصفات التشريحية لنبات الكونوكربس. بالإضافة إلي محاولة

تقليل التأثير السلبى لملوحة ماء البحر بواسطة معاملة الرش الخارجية بالبرولين (٠,٥% ، ١%) .

وكانت أهم النتائج المتحصل عليها كالآتي

- المستويات المنخفضة من ماء البحر المخفف حسنت النمو وكذلك كان المعاملات البرولين تأثير ايجابي على النمو
- المعاملة ٣٠% ماء بحر أدت إلي زيادة محتويات النيتروجين والفسفور والحديد في الأجزاء الهوائية عند مقارنتها بالمستويات المنخفضة والمرتفعة عنها ولكن المعاملة ١٠% ماء بحر أدت إلي زيادة محتوى البوتاسيوم
- تأثيرات الملوحة للري بمياه البحر أدت إلي زيادة مباشرة في محتوى الصوديوم والذي أدى إلي زيادة نسبة الصوديوم / البوتاسيوم في الأجزاء الأرضية.
- معاملة الرش بالبرولين الخارجية بمعدل ٠,٥% أدت إلي زيادة محتويات النيتروجين و الفسفور والبوتاسيوم الزنك في الأجزاء الهوائية
- المعاملات حتى ٣٠% ماء بحر حسنت الصفات التشريحية للأوراق والسيقان لنبات الكونوكاريس
- أيضا معاملات الرش للبرولين كمنظم اسموزي تغلبت علي التأثيرات السلبية لملوحة ماء البحر
- المعاملة ١٠% ماء بحر مصحوبة ب ١% برولين أعطت أعلى قيمة لصفات النمو الداخلي للسيقان بينما المعاملة ١٥% ماء بحر مصحوبة ب ١% برولين أعطت أعلى قيمة لصفات النمو للأوراق