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# Effect of Irrigation Water Types and Spraying With Seaweeds on Vegetative Growth, Biochemical Attributes and Minerals Contents of *Ziziphus mauritiana* and *Citrus sinensis* Seedlings

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## Abstract

This experiment aimed to investigate the effect of the different irrigation water sources and spraying with seaweeds on the seedlings of two plants one is salt-sensitive (*Citrus sinensis*) and the other is salt-tolerant (*Ziziphus mauritiana*) through studying growth parameters, as well as biochemical attributes and minerals in leaves of the studied plants. Four treatments were used for irrigation water as follow: (W1)The Tap Water (control) E.c = 1.2 $dS.m^{-1}$, (W2) Shatt al-Arab river (sub river) Ec = 5.2 $dS.m^{-1}$, (W3) Shatt al-arab river (main) 6.2 $dS.m^{-1}$, (W4) The well water E.c = 8.1 $dS.m^{-1}$ with 3 levels (A1, A2, A3) of seaweed treatments prepared from (Agrosign marine) in the concentration of (0, 2, 4 ml /L), The results explained that there is a decrease in all parameters in this study with increasing the salinity of irrigation water and this is represented in well water irrigation as compared with another water types, while the interaction with-seaweeds treatments caused significant increases in the growth parameters, biochemical attributes and minerals contents. This promotion was associated with increasing the concentrations to (4 ml /L), Which gave 42.94 cm in plant height, 2.00 number of shoots, 24.61 in the number of leaves, 5.34 in stem diameter, 101.2 g in fresh weight and 19.02 g dry weight. On the other hand the interaction with seaweed extract increased significantly growth parameters, attributes biochemical and mineral content, it gave the highest value A3W1 which was 3.48 mg /g in the total chlorophyll and 73.82 mg/g in total carbohydrates, while the lowest at A1W4 which was 1.38 mg/g in the total chlorophyll and 42.92 mg / g in the total carbohydrates. The activities of the enzymes superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) have shown a marked increase when increasing the level of salinity in irrigation water as well as, the seaweeds extraction caused increasing in the content of these enzymes. The treatment (A1W4) gave the highest enzymes activities CAT, SOD, BOD which was 24.10, 21.83 and 7.93 u / g / f.w respectively.

***Keywords:*** *Irrigation water, salinity, seaweeds, minerals contents, antioxidant enzymes, Citrus sinensis, Ziziphus mauritiana*

## Introduction

Water is very necessary for growth and development of the plants. In fact, the global infrequency of water resource and increasing the salinity of soil and water which considered as one of the most important factors of irrigation water (Beltran,

1999) that appeared on the last years and it can be seen obvious in Iraq .It is known that the Downfall of Sumer civilization about 4000 years ago was caused by unsuitable agricultural techniques, that's led to soil salinization and descent in the agricultural yield in the area (Pitman and Lauchli, 2002). Currently, the decrease of water levels in Tigris, Euphrates and shatt-alarab rivers in Iraq create a big problem that's lead to use other sources like wells water. Water quality can vary from source to source. Three sources of water are commonly used in the region of this study: municipal water, rivers and well water. Well water frequently contains high levels of dissolved elements,and classified as a saline water while thewater obtained from rivers or lakes commonly has a lower level of dissolved chemicals than well water. In general, salinity is considered as a one of the major severe abiotic stress factors that effects on plant metabolism by inducing decreases in soil water potential ,which leads to adisturbance in water uptake by roots. Overall, Plants vary greatly in their ability to tolerate salt in water and classified into the sensitive plants to the salinity in spite of the difference among the species for the range of salinity tolerance degree like *citrus* (Mass,1996; Murkute *et al*., 2005) and halophytes, which are able to grow at high concentrations of salt, like *Ziziphus* (Flowers and Colmer, 2008).

*Ziziphus mauritiana* Lam (Rhamnaceae) has marked ability to tolerate salt stress and drought (Bhatt *et al*., 2008), while the citrus (Rutacea), are especially more sensitive to salinity than other plant (Boman, 1993; Maas, 1993). Current studies have focused on finding substitute natural solutions that will work to prevent and reduce the risk of salinity on plant, one of these processors are adding the seaweeds (Agrosign marine), that stimulate plant growth (Zodape, 2001; Strick *et. al*., 2003). It contains all major and minor nutrients including macro and micro elements ,amino acids and fatty acids and plant growth regulator like auxin, gibberllins and cytokinin. Seaweed extracts have been reported to stimulate the growth and yield of plants, develop tolerance to environment stress, increase nutrient uptake from soil and enhance antioxidant properties (Rathore, 2009). This work aimed to investigate the effect of various kinds of irrigation water on tolerant plant *Ziziphus mauritiana* and sensitive plant *Citrus sinensis* and increasing the tolerance to salinity by spraying the seaweeds.

**Materials and Methods**

This study was conducted in the Horticulture Department, Agriculture College, Basrah University, Basrah, Iraq from 17 November to 17 April, 1916 .The experiment was designed according to completely randomized Block design (RCBD), as a factorial experiment with three factors (Irrigation water, Seaweed Liquid extraction and plant types) four time replication, The experiment consisted of 96 plastic pots (29cm diameter), containing one year –old seedlings of same size and length taken for both experimental plants previously characterized as salt-tolerant *Ziziphus mauritiana* cv. Tuffahi (P1) and salt-sensitive *Citrus sinensis* cv. local (P2),(an experimental unit was one plant in one pot). Irrigation water salinity treatment were applied as:

1. The tap water ( control ) (W1) Ec = 1.2 dS.$m^{-1}$
2. Shatt al-arab river (sub river) (W2) Ec = 5.2 dS.$m^{-1}$
3. Shatt al-arab river (main) (W3) =6.2 dS.$m^{-1}$
4. The well water (W4) Ec = 8.1 dS.$m^{-1}$

Seaweed liquid fertilizer was prepared from (Agrosign marine, it consist of organic matter extracted from seaweeds 12%,fatty acids group 65%, natural growth regulators 25 ppm, potassium 3%, phosphor 2%,nitrogen 15%) with three levels (A1= 0, A2 = 2, A3= 4 ml /L), The solutions were sprayed once on the leaves in the early morning, every 20 days. At the end of experiment the plants were harvest and washed with distilled water then the morphological parameters were taken such us Plant height(cm), Number of shoots, number of leaves, stem diameter (mm), fresh and dry weight (gm). then dried by freeze dryer. Total chlorophyll was determined according to Goodwin (1976), total carbohydrates was determined according to Dobois *et al*., (1956), The shoot dry matter was grunden, $Zn^{+2}$ and $Fe^{+2}$ were determined by Atomic Absorption Spectrophotometer after digested the samples in $HNO_3$ and $HClO_4$ as pointed out in A.O.A.C.(1984), the N content was determined by micro Kjeldahl method (Bremner and Mulvaney, 1982), k by flame photometer, and P by spectrophotometer, after digestion in $H_2SO_4$ and $HClO_4$ (Jackson,1958). antioxidant enzymes extraction was accomplished by weighing 0.3g from leaves were collected after 3 months were directly dry frozen. The frozen tissues were pestled in a cold mortar condition. A 6.0 mL solution containing 50 mM potassium phosphate buffer (pH 7.0), 0.1 mM EDTA, 4% polyvinyll phrolidone (PVP) and 0.2 mM ascorbic acid. The extract was centrifuged at 12,000 x g at 4 °C for 20 min for purification. The enzyme assays were represented in supernatant. The CAT activity was measured as the change in absorbance of the reaction mixture at 240 nm due to hydrogen peroxide reduction (Aebi, 1984). It was calculated by the coefficient for $H_2O_2$ at 240 nm (40 mM cm). Determination of peroxidase POD activity was determined according to Kar and Choudhuri (1987) method. Activity unit was calculated using the coefficient of absorbance for tetraguaiacol at 470 nm (22.6 mM). POD activity was expressed as enzyme units per gram fresh weight (U/g fw). Superoxide dismutase (SOD) activity was determined according to Giannopotitis & Ries (1977), The absorbance readings were taken at wave length of 560nm. One unit of SOD activity was defined as the enzyme activity that reduced the photo reduction of nitroblue tetrazolium to blue formazan by 50 %. SOD activity was expressed as enzyme units per gram fresh weight (U/g fw).

**Table 1. Some physical and chemical characters for the studied soil.**

| Soil separates | % | Texture |
|---|---|---|
| **Sand** | 14.10 | Mixture |
| **Silt** | 48.40 | |
| **Clay** | 37.50 | |
| **Chemical characters** | Units | Value |
| **Electrical Conductivity(EC)** | $dS.m^{-1}$ | 2.8 |
| **pH** | — | 7.95 |
| **Organic matter** | % | 0.85 |
| **Soluble ions** | | |
| **Soluble $Ca^{++}$** | $gm.100gm^{-1}$ | 7.05 |
| **$Mg^{++}$** | *mmol.L*$^{-1}$ | 6.45 |
| **$Na^{+}$** | *mmol .L*$^{-1}$ | 4.62 |
| **$K^{+}$** | *mg. .L*$^{-1}$ | 12.08 |
| **$HCO3^{-}$** | % | 20.80 |
| **$Cl^{-}$** | *mmol .L*$^{-1}$ | 7.32 |
| **$SO4^{=}$** | *mmol .L*$^{-1}$ | 9.86 |

**Table 2. Some chemical characters for the studied irrigation water types .**

| | Units | (W1) | (W2) | (W3) | (W4) |
|---|---|---|---|---|---|
| **pH** | **-** | 7.12 | 6.85 | 6.32 | 6.16 |
| **E.Ce** | **$dS.m^{-1}$** | 1.2 | 5.2 | 6.2 | 8.1 |
| **$Cl^{-}$** | ***mmol. L***$^{-1}$ | 4.71 | 13.15 | 19.23 | 23.16 |
| **Soluble $Ca^{++}$** | ***Mmol. L***$^{-1}$ | 4.91 | 14.82 | 21.78 | 24.64 |
| **Soluble $Mg^{++}$** | ***Mmol. L***$^{-1}$ | 7.85 | 22.57 | 25.97 | 32.85 |
| **Soluble $K^{+}$** | ***Mmol. L***$^{-1}$ | 0.15 | 0.26 | 0.28 | 0.38 |
| **Soluble $Na^{+}$** | ***Mmol. L***$^{-1}$ | 3.56 | 13.30 | 20.32 | 29.81 |

## Results

**Growth parameters:** When evaluating the data of research recorded in Table (3), it is clear that different water types play an important role in plant growth with the four types of water, The results showed a significant decline in the average of growth parameters (plant height, number of shoots, number of leaves, stem diameter, fresh and dry weight). The decrease in growth parameters appeared with well water compared to tap water that having the highest value, and this effect was more obvious in the salt sensitive plant (citrus seedlings), while the *Ziziphus* seedlings were exceed in growth parameters values. On the other hand, there are many significant differences appeared when treated with seaweeds compared with untreated seedlings the highest value was represented by the treatment A3W1 which were 62.33 cm for plant height, 4.83 for number of shoots, 40.50 for number of leaves and 7.26 for stem diameter.

**Biochemical attributes**: The data of biochemical attributes are presented in Table 4 There were significant differences between treatments in total chlorophyll content and total carbohydrates, The highest value was found at treatment A3W1 for both plants which was 3.48 mg/g in the total chlorophyll and 73.82 mg /g in total carbohydrates, while the lowest at A1W4 which was 1.38 mg /g in the total chlorophyll and 42.92 mg / g in the total carbohydrates. On the other hand, it is clear from the present results that the activities of the three important anti oxidative enzymes were increased significantly with increasing the salinity of irrigation water. It was observed that the activities of CAT, BOD and SOD were increased at well water, but the lowest value in tap Water. In addition, the seaweeds raised the activities of antioxidative enzymes in *Ziziphus* seedlings as compared with *citrus* seedlings. The treatment (A1W4) gave the highest enzymes activities CAT, SOD, BOD which was 24.10, 21.83 and 7.93 u / g / f.w respectively.

**Minerals content**: A negative relationship was appeared in N, P, k, Zn, Fe content in seedling leaves for both plants with increasing water salinity, While The interaction between concentrations the seaweeds and irrigation water types gave significant differences in minerals content. The highest value of minerals were at treatment A3W1 Table (5), which were 3.03%, 1.22%, 1.66%, 77.21 µg/g, 60.39 µg/g for in N, P, k, Zn, Fe content respectively.

**Discussion**

high-quality plants can be produced only by using high-quality irrigation water. There are local differences in water characteristics with the source of the water, After analyzing the results from the experiment, the plants of this study responded significantly to the types of irrigation water, and indicated that the seedling growth was reduced at well water which has a high salinity comparing with tap water, this decrease due to the high ions concentration accumulation in the soil solution which may influenced on the enzymes activation inside the plant cells by modulating the active sites of enzymes also the $H^+$-ATP ase pumps may stop working or the membranes may be impaired ,that affect the permeability as well as the salinity affected on the Photosynthesis, respiration processes and electron transport chains (Orcutt and Nilsen,2000). There is an imbalance of nutrients due to the factor involved in the salt-induced inhibition in photosynthesis and subsequently in plant growth reduction. We found that the type of water treatments play an important role in altering the mineral nutrient distribution and decreased the absorption rates, different researchers reported that the increasing salinity of water has effects on some traits of growth and yield **(**Hamaad *et. al*., 2013; Hirich *et. al*., 2014; Algosaibi *et. al*., 2015) and reduced Zn, Fe accumulation (Bhatt *et. al*.,2008). The antioxidant enzymes were raised positively with increasing of salt concentration in irrigation water and this activation may occur due to the excessive generation of reactive oxygen species (ROS) such as superoxide and hydroxyl radicals that are concede red as a one corollary of a biotic stress exactly as high salinity and cellular homeostasis (Mittler 2002). ROSs may have the ability to interact with many cellular components, and may result in significant damage to cell structures. When ROS increases, chain reaction start, in which superoxide dismutase (SOD) catalyzes the dismutation of O2 - radical to molecular O2 and H2O2 (Meloni *et. al*., 2003).

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The H2O2 is then detoxified in the ascorbate-glutathione cycle (Mittler 2002), which involves the oxidation and re-reduction of ascorbate and glutathione through the ascorbate peroxidase (APX) and glutathion reductase (GR) enzymes (Noctor & Foyer 1998). If there is an imbalance in the cell compartment between the production of reactive oxygen species (ROS) and antioxidant defense, oxidative stress and damage occurs (Mittler 2002),many researchers reported that, (Maia *et. al*., 2010; Kahrizi *et. al*., 2012)

On the other hand,, the interaction of irrigation water type and spraying with seaweeds gave the plants the ability to tolerate the salinity stress especially the plants which were irrigated with well water , compared with the untreated plants That maybe because the sea weeds could enrich the nutrients contents of the soil and is probably due to the presence of growth promoting hormones and nutrients in more quantities. The salinity of water stimulated the decreasing of leaf chlorophyll through embarrassment of chlorophyll synthesis or accelerated degradation has been well explained by Reddy and Vora (1986),and this reverberated on total carbohydrates content. Different researchers reported that the treatmentbyseaweed caused substantial increase in growth (Thirumaran *et. al*., 2009; Taha and Salih,2012; Abdul-Jabar *et. al*., 2012 ; Hamaad *et. al*., 2013;)An attention-grabbing observation that there is a relatively salt tolerance in *Ziziphus* seedlings more than *Citrus* seedling, It is well known that most crops are salt sensitive or hypersensitive (glycophytes) on the contrary to halophytes that have the capacity to accommodate severe salinity because of the very special anatomical and morphological adaptations or avoidance mechanisms (Flowers *et al.,* 1986). These are relatively unique characteristics for which the genes are not likely to be introgressed easily into crop plant (Yokoi *et al.*,2002). From our study we can conclude that, the increasing of salinity of irrigation water over 5 dS.m$^{-1}$has a negative influence and reduction in growth parameters ,biochemical attributed and minerals contents. Also, the spraying with seaweeds act as a bio stimulator , reduce from the effects of salinity in irrigation water and encourage the plants to resist a bio stress like salinity, furthermore, type of plant play an important role in the tolerance toward salinity according to irrigation waters types ,the result shows that *Ziziphus* showed obvious tolerance compared with citrus seedling which is concedered a sensitive plant .

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# تأثير نوعية مياه الري والرش بمستخلص الأعشاب البحرية في النمو والصفات البايوكيميائية والمحتوى المعدني لشتلات السدر *Ziziphus mauritiana* والبرتقال المحلي *Citrus sinensis*

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**الملخص**

أجريت هذه الدراسة لغرض معرفة تأثير نوعية مياه الري والرش بمستخلص الاعشاب البحرية في شتلات البرتقال*Citrus sinensis* والذي يعد من النباتات الحساسة للملوحة وشتلات السدر *Ziziphus mauritiana* من خلال مقاييس النمو الخضري والصفات البيوكيميائية والمحتوى المعدني ولقد طبقت اربع معاملات من مياه الري وهي ١- مياه الحنفية معامله المقارنة ( $Ec = 1.2 \ dS.m^{-1}$ ) ٢- مياه نهر شط العرب (نهر فرعي)$Ec = 5.2 \ dS.m^{-1}$ ٣- مياه شط العرب (الرئيسي)$Ec = 6.2 \ dS.m^{-1}$ ٤- مياه البئر $Ec = 8.1 \ dS.m^{-1}$ مع ثلاث مستويات من مستخلص الأعشاب البحرية .(0, 2, 4 ml /L) وأظهرت النتائج حدوث انخفاض في جميع مقاييس النمو عند ارتفاع ملوحة مياه الري ولقد تمثلت هذه النتيجة في مياه البئر مقارنة مع باقي أنواع مياه الري والتي بلغت 42.94 سم في ارتفاع النبات و2.00 في عدد الأفرع و24.61 في عدد الأوراق و5.34 في قطر الساق و101.2 غم في الوزن الطري و19.02 غم في الوزن الجاف وبينما اظهر التداخل مع مستخلص الأعشاب البحرية زيادة معنوية ملحوظة في مقاييس النمو والصفات البيوكيميائية والمحتوى المعدني، وأعطى أعلى زيادة عند المعاملة بـ 4) ml /L) وقد سجلت أعلى معنوية عند المعاملة A3W1 وبلغت 3.48 ملغم/غم في الكلوروفيل الكلي و73.82 ملغم/غم في الكربوهيدرات الكلية و بينما كانت الأدنى عندA1W4 وبلغت 1.38ملغم/ غم في الكلوروفيل الكلي و42.92 ملغم/غم في الكربوهيدرات الكلية. وقد أظهرت فعالية الأنزيمات(superoxide dismutase (SOD و peroxidase (POD) و catalase (CAT) زيادة ملحوظة عند زيادة مستوى الملوحة في مياه الري وكذلك عمل المستخلص البحري على زيادة محتوى هذه الأنزيمات حيث أعطت المعاملة (A1W4) أعلى فعالية لأنزيم CAT, SOD, BOD وبلغت 24.10 و21.83 و7.93 وحدة/غرام/ وزن طري وعلى التوالي.