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Effect of Irrigation by Diluted Sea Water and Antioxidants on Growth of Cotton Plants

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IN A GREENHOUSE in the National Research Centre, a pot experiment was carried out to study the influences of irrigation by diluted sea water and antioxidants on growth of cotton plants. The treatments of salinity were irrigation by diluted sea water with 3.91 and 7.82 dS/m and by fresh water as a control. Thiamine, tryptophan and fresh water were sprayed twice at 45 and 60 days age. A split plot design was used. Nine traits were studied. Salinity decreases in growth parameters. In most cases, the higher salinity resulted in the higher depression. For antioxidants, tryptophan showed the highest values on plant height, number of leaves/ plant and fresh weight of stem, leaves/ plant and whole plant without wide differences with those of thiamine treatments. Application of thiamine and tryptophan improved the area of leaves /plant. Dry weight of stem leaves and top dry weight were improved with amino acid or vitamin. Tryptophan was more effective than thiamine. It may be recommended the use of tryptophan when irrigation by diluted sea water ranging between 3.91 and 7.82 dS/m.

Keywords: Cotton (*Gossypium barbadence* L.), Salinity, Antioxidants, Vitamin, Growth.

Cotton (*Gossypium barbadence* L.) is a leading fiber material grown, in arid and semi-arid regions of the world. Total production per unit area has reached a plateau. Sustainability and improvement of cotton yield are major challenges to meet the upcoming threats of increasing world population, deterioration of arable land, depletion of water resources and environmental stresses (UIlah *et al.*, 2008). In Egypt cotton area decreased from year to year, *i.e.*, from 1.2 million feddan in 1990 to 225 thousand feddan in 2008. This may be due to the high competition with other crops in summer cropping system, and the relative low income and higher coasts of pests control and its relatively long growth season. The extension in the new areas towards the desert is a national target where such areas suffer from the low fertility, limited water resources and salinity.

Salinity is one of the serious environmental stresses affecting plant growth and productivity. Salinity is known to induce wide range of responses in plant viz., readjustment of transport, metabolic processes and growth inhibition (Sairam & Srivastava, 2002). Salinity also induces water deficit even in well watered soils by decreasing the osmotic potential of soil solutes, making a difficult for roots to extract water from their surrounding media. Salinity stress exerts many symptoms similar to that observed under water stress deficit. In addition, to salinity is in elose with osmotic stress and ion toxicity. Salt stress is also manifested as an oxidative, all of which contribute to its deleterious effects (Root & Show, 2001 and Yazici, *et al.*, 2007).

Oxidative damage in the plant tissue is alleviated by a concerted action of both enzymatic and non-enzymatic antioxidant metabolism (Vranova & Van bernsegem, 2002). There are many reports assure the initiate relationship between enhanced constitutive antioxidant enzymes activities and increased resistance to environmental stresses (Vranova & Van brensegem, 2002 and Bor *et al.*, 2003). The exogenous application of antioxidants enhanced the oxidative defense in different plants (Sairam & Srivastava, 2002; Li *et al.*, 2008a; Abd El-Galil *et al.*, 2008 and Xie *et al.*, 2008).

Therefore, this investigation is designed to study the effect of diluted seawater and irrigation antioxidants on growth of cotton plants.

Materials and Methods

This work was designed to study the effect of spraying some antioxidants on growth and tolerance of cotton plants to salt stress. In a pot experiment in a greenhouse, National Research Centre (NRC), the study was carried out during 2007 Summar season. The treatments were as follows:

Salinity: Irrigation by diluted seawater with 3.91 and 7.82 dS/m and control (fresh water). Antioxidants: thiamine (vitamin B1), tryptophan (Amino acid) 200ppm and control (fresh water) were sprayed after planting twice (45 and 60 days).

The experiment included 9 combinations. The experimental design was split plot in 8 replicates. Metallic ten pots 35 cm in diameter and 50 cm in depth were used. Every pot contained 30 kg of air dried clay loam soil. The inner surface of the pots was coated with three layers of bitumen to prevent direct contact between the soil and metal. In this system, 2kg of gravel (Particles about 2-3 cm in diameter), so the movement of water from the base upward.

Seeds of cotton (*Gossypium barbadence* L.) CV Giza 85 was seeded on May 20^{th}. Seedlings were thinned twice; 15 and 30 days after sowing to secure two plants / pot. Calcium super phosphate (15.5 % P_2O_5) and potassium sulfate (48.5 % k_{20}) at a rate of 3.0 and 1.50 g/pot, respectively, were added before seeding. Ammonium sulfate (20.5 % N) a rate of 6.86 g / pot was added in two equal portions, the first 21 days after seeding and the second two weeks latter. Irrigation with diluted seawater in different concentrations were started 30 days after seeding (One irrigation by salt water and the next by fresh water alternatively).

The following growth characters were measuring on [illegible] days after planting:

1. Plant height (cm).
2. Number of leaves/ plant.
3. Leaves area/ plant (cm^2).
4. Fresh weight (gm) of:
   a- Stem.
   b- Leaves/plant.
   c- Whole plant.
5. Dry weight (gm) of:
   a- Stem.
   b- Leaves/plant.
   c- Whole plant.

Data obtained from each season of the study were statistically analyzed according to procedures outlined by Gomez & Gomez (1984). The differences among treatment means were compared by least significant differences test (L.S.D.) at 0.05 level of probability.

Results and Discussion

Salinity effect

It is obvious that increasing salt concentration from 0 to 7.86 dS/m significantly reduced most of growth parameters studied with the exception of fresh weight of leaves/plant, number of leaves/ plant and main stem and whole plant fresh weight (Table 1 and Fig.1). Both salt concentrations induced the same effect on the fresh weight of stem + leaves. A drastic depression in leaves area/plant was shown as a result of increasing salt concentration. These depressions were amounted by: 30.54 and 41.67% for 3.91 dS/m and 7.82 dS/m compared to the control, respectively.

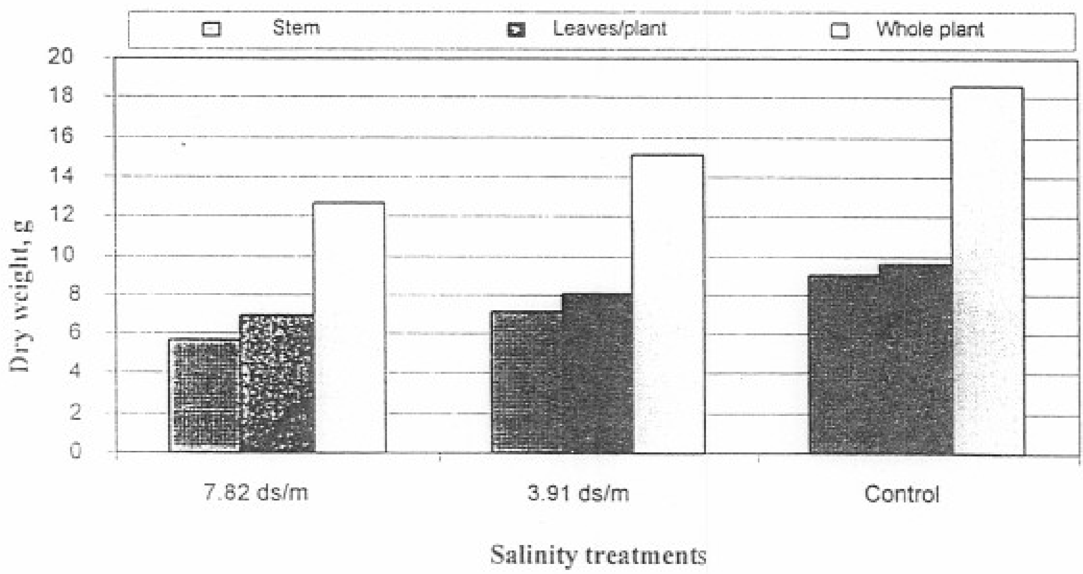

Fig. 1. Effect of salinity on dry weight of cotton.

TABLE 1. Effect of salinity on some growth parameters in cotton.

Salinity	Plant height (cm).	No. of leaves/ plant	Area of leaves/ plant (cm^2)	Fresh weight (g):			Dry weight (g):		
				Stem	Leaves/ plant	Whole plant	Stem	Leaves/ plant	Whole plant
7.82 dS/m	126.0	17.0	655	22.93	37.90	60.83	5.70	6.92	12.62
3.91 dS/m	131.6	18.5	780	24.17	35.39	59.56	7.09	8.06	15.15
Control	145.5	19.0	1123	30.94	47.15	78.09	8.97	9.60	18.57
LSD at 0.05	12.21	N.S	559	N.S	N.S	N.S	1.06	1.63	2.09

Muhling & lauchli (2002) reported an effect of salt stress on growth and metabolism of cotton plants and revealed that plant height was affected in the saline soil. A high Na^+ supply (up to 150 mmol/l NaCl) in a long-term salt treatment decline shoot yield of salt tolerant cotton. Root growth of cotton was not significantly affected under salinity. Praharaj & Rajendran (2004) observed that affected cotton leaves may be smaller and dark blue green than normal leaves. Moreover, plants exhibited appearances similar to moisture and stress condition. In excess salinity, leaves became brittle and necrotic leading to stunted growth and declining yield.

High increased salts in irrigation water or in soil firstly affected its chemical and physical properties the availability and movement of moisture as mentioned by Praharaj & Rajendran (2004). They added that reduced availability of N, P, Mn, Cu, Zn and Fe, high osmotic pressure, poor air and water movement and low microbial activity are the problems associated with salt affecting cotton soils. Sheldon *et al.* (2004) stated that salinity influenced the final content of water and osmotic strength in the soils. These effects in soil were reflected on roots and consequently water and mineral absorption too. Plant growth was reduced by osmotic stress and toxic ions increase, especially in the mature leaves (Munns, 1993; Fortmeier & Schubert, 1995; Shabaan *et al.*, 2008 and Hussein *et al.*, 2008).

The data in Table 1 showed also that negative relations were observed between the increase in salt stress and dry matter of cotton in different plant parts. An irrigation cotton plant with diluted seawater at EC 3.7 dS/m decreased dry weight of stem, leaves and stem +leaves by : 20.96, 16.04 and 18.4 %. The corresponding with EC 7.3 dS/m the depressions were 36.47, 27.92 and 31.50%. This means that the dry matter accumulation declined as salinity increased. The continuous depression in leaves area/plant (the photosynthetic surface) by the increase in salinity confirmed these results.

The effect of salt stress on dry matter of cotton were studied by Reboucas *et al.* (1989), Kramer (1990) and Leidi & Saiz (1997). Growth and yield of a crop is drastically affected by a biotic stress directly or indirectly by altering metabolism, growth and development (Garg, *et al.* 2002 and Hussein *et al.*, 2004). Salinity induced its effect through the osmotic adjustment (Meloni *et al.*, 2003) explained that limited moisture and the ion accumulation considered the main source of toxicity (Kramer, 1990; Marchener, 1990 and Muhling *et al.*, 2001). Before believed in the role of these two factors affected most metabolic processes such as water, mineral absorption and distribution, protein synthesis, photosynthesis, stomata conductance growth regulation and oxidative defense. Reduction of photosynthetic rate in cotton under limited water salt stress is documented (Ephrath *et al.*, 1993; Sultana *et al.*, 1999; Meloni *et al.*, 2003; Pettigrew, 2004 and Ullah *et al.*, 2008).

Li *et al.* (2008b) found that net photosynthetic rate (P_n) declined with increasing salinity, and the most marked reduction occurred after exposure of mangrove seedlings to a severe salinity, 400 mM NaCl. Pinheiro *et al.* (2008) on caster bean plants, mentioned that salt stress effects on chlorophyll a, chlorophyll b and chlorophyll a + b contents were evident only on experimental day 59. Dry matter accumulation of leaves, roots and stems, as well as the total dry matter, and the root to above ground ratio were lowered in salt-stressed seedlings. Meloni *et al.* (2003) reported that net photosynthesis and stomata conductance decreased in response to salt stress, but Pora showed a smaller reduction in photosynthesis than Guazuncho. The results indicated that stomata aperture limited leaf photosynthetic capacity in the NaCl-treated plants of both cultivars. However, significant reduction in the leaf chlorophyll contents due to NaCl stress was observed only on Guazuncho. In both cotton cultivars, the photochemical efficiency of PSII was not affected by salt stress.

El-Tayeb *et al.* (1999) mentioned that increasing NaCl concentration significantly reduced the radical and plumule lengths, and fresh and dry matter yields of *Cicer arietinum, Lens culinaris* and *Trigonella foenum-gracecum* especially at high salinity levels. El-Shintinawy & El-Shourbagy (2001) reported that the most abundant amino acids (cystine, arginine and metionine) constituting about 55 % of amino acid content in control wheat were reduced in 100 mM NaCl-treated plants, however, valine isoleucine, aspartic acid and proline accumulated in response to NaCl . NaCl-treated wheat seedlings showed 1.6 fold increases in total free amino acids compare to the control.

Antioxidants

Table 2 and Fig. 2 show that the differences within tested treatments reached to the level of significance for most traits studied. Leaves area/plant and fresh weight/whole plant were insignificantly responded. With all respects, a general trend tryptophan out yielded thiamine use, which did the some over control. The significant superiority of tryptophan versus thiamine was recorded on No. of leaves/plant (6.25%), dry weight of leaves/plant (27.6%) and dry weight/ whole plant (22.98%). Similarly, thiamine significantly exceeded control for plant height (11.55%), fresh weight of stem (30.68%) and dry weight of whole plant

(27.69%). This means that the use of antioxidants caused dry weight increments by about one third.

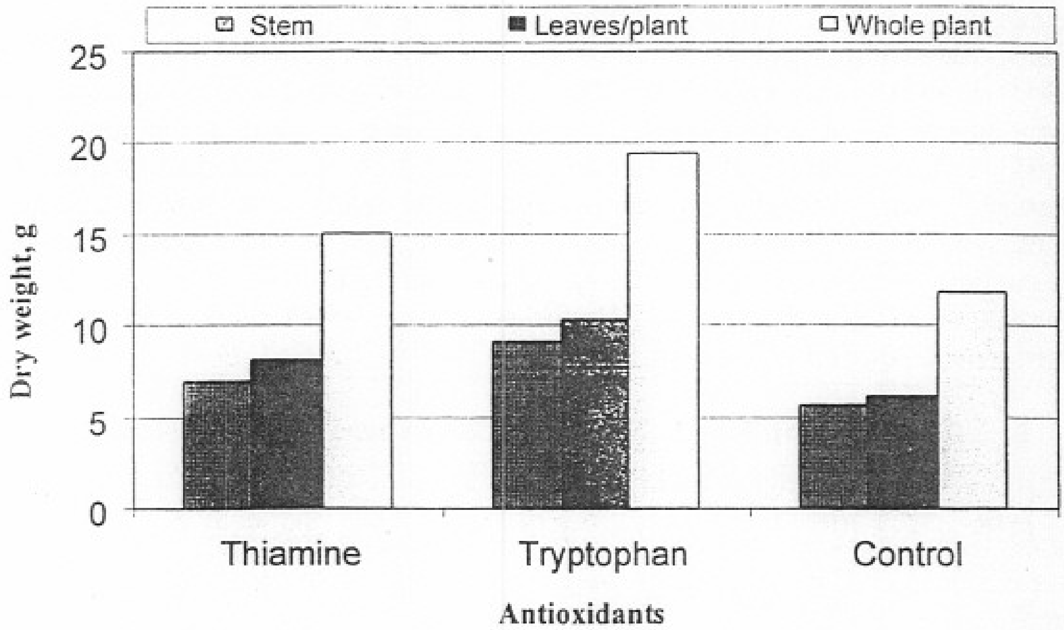

Fig. 2. Effect of thiamine and tryptophan on dry weight of cotton plant.

TABLE 2. Effect of thiamine and tryptophan on growth of cotton plants.

Antioxidants	Plant height (cm)	No. of leaves/ plant	Area of leaves/ plant	Fresh weight(g): Stem	Leaves/ plant	Whole plant	Dry weight (g): Stem	Leaves/ plant	Whole plant
Thiamine	137.1	19.2	897	27.73	42.58	70.31	6.99	8.09	15.08
Tryptophan	141.1	20.4	1080	29.09	44.52	73.61	9.10	10.33	19.43
Control	122.9	16.5	582	21.22	33.05	54.27	5.66	6.16	11.82
LSD at 0.05%	5.94	0.64	N.S	2.30	11.38	N.S	2.15	2.21	3.66

Appreciable effects of some treatments (Vit.B1 and Vit.B2) were observed on plant height, number of tillers and total fresh weight/plant (Rafaat & Balbaa, 2001). Taraaf *et al.* (1999) obtained pronounced increase in the growth parameters of lemongrass plants. Balbaa & Refaat (2002) found that most criteria of growth expressed in plant height, number of leaves and fresh weight/plant of *Tagetes minutal* l Most of thiamine in plants is represent as free vitamin, with a small proportion of diphospho-thiamine and very small amounts of triphosphothiamine, and the diposphothiamine is the biologically active form of thiamine (Balbaa & Refaat, 2002). Thiamine is known as growth regulator that influences many physiological processes such as synthesis of enzymes and nucleic acids (Hathout *et al.*, 1993). Refai *et al.* (2009) concluded that the application of AsA at 100ppm level was the superior treatment in all vegetative growth parameters of squash plants among citric acid and salicylic acid.

Numerous studies were done to investigate the response of different plants to antioxidants and its role in the oxidative defense in plants subjected to a biotic stresses (Sayed & Gaballah, 2002; Boch *et al.*, 2005 and Hussein *et al.*, 2007a & b).

Salinity x Antioxidants effect

Table 3 gives the interaction (salinity x antioxidants) means of the studied growth traits. It is clear that significant was observed only on leaves area / plant and dry weight of whole plant. Obviously, the combination (salinity, control x tryptophan) mostly yielded the highest product's. As respect to dry weight/ whole plant, the previous superior combinations insignificantly differed than all salinity control combination as well as the interactions of tryptophan with either 7.3dS/m or. 3.7 dS/m is sharply indicates that the positive value of tryptophan was when irrigation by saline water ranged between 3.7 dS/m to 7.3 dS/m. The pronounced product of whole plant dry weight, *i.e.*, 21.94 g as a result of the positive effects shown on plant height, 160.3 cm, No. of leaves/plant (22.0), area of leaves/ plant (144.4 cm^2), fresh weight of main stem (33.62g) and dry weight of main stem (10.13g).

TABLE 3. Effect of salinity and antioxidants interaction on growth of cotton plants.

Salinity	Antioxi-dants	Plant height cm.	No. of leaves/ plant	Area of leaves/ plant $(cm)^2$	Fresh weight (g): Stem	Fresh weight (g): Leaves/ plant	Fresh weight (g): Whole plant	Dry weight (g): Stem	Dry weight (g): Leaves/ plant	Dry weight (g): Whole plant
7.82 dS/m	Thiamine	132.1	19.3	814	22.77	32.77	55.54	6.15	8.22	14.37
	Tryptophan	133.0	19.3	717	28.77	47.81	76.58	7.20	7.85	15.05
	Control	113.0	17.3	458	17.25	32.22	49.47	3.76	4.70	8.46
3.91 dS/m	Thiamine	132.0	19.0	780	27.92	36.45	64.37	5.84	6.79	12.63
	Tryptophan	136.2	20.0	1079	24.88	40.76	65.64	9.97	11.33	21.30
	Control	126.7	16.0	482	19.71	28.96	48.67	5.45	6.06	11.51
Control	Thiamine	140.6	18.7	1098	32.49	58.51	91.00	8.99	9.27	18.26
	Tryptophan	160.3	22.0	1444	33.62	44.99	78.63	10.13	11.88	21.94
	Control	135.6	16.3	828	26.71	37.96	64.67	7.78	7.71	15.49
LSD at 0.05		N.S	N.S	968	N.S	N.S	N.S	N.S	N.S	6.33

Sayed & Gaballah (2002) demonstrated that the effects of salinity, thiamine and their interaction on the growth rate, water relations, photosynthetic pigments, soluble sugars and K^+/Na^+ ratio of sunflower plants were significant. Salinity was dominate for affecting the contents of $Ca^{2+}Cl$, total amino acids and membrane stability to heat and leaf water potential. The role of thiamine was dominant for Na^+, K^+ and soluble sugars contents and the contribution of interaction was dominant for growth parameters, chlorophyll and root water potential. Abbas (2004) revealed that NaCl treatments reduced fresh and dry weight of maize plants and presoaking grains in thiamine solution (200 mg/l) alleviated the inhibitory effect of NaCl. Moreover, the nutritive elements K and [illegible] and the proline contents were also increased by increasing NaCl concentration. Thiamine treatment reversed such effects.

The interaction effects of salt stress and antioxidants on dry matter of cotton plants were illustrated in Table 3 and Fig. 3. This data showed that both vitamin (thiamine) and amino acid (tryptophan) treatments enhanced the dry matter content under saline or non saline irrigation but in different degrees. The whole top plant dry weight increased when thiamine treatment was used at 7.3 dS/m , 3.7 dS/m and control by: 69.86, 9.73 and 17.88% and when tryptophan treatment was used by: 77.89, 85.06 and 41.64 % compare to plants irrigated regularly by the control. This means that tryptophan superior in ameliorate salt stress more than thiamine when plants irrigated by high level of salt concentration and the reverse was true when plants deliver moderate salt concentration and fresh water in irrigation. El-Tayeb (1997) on sorghum, found that presoaking seeds in thiamine solution (200 mg/l counteracted the adverse effects of salinity on germination and seedling growth as well as some metabolites of sorghum plants. Also, El-Tayeb *et al.* (1999) concluded that seeds soaking pre-sowing in thiamine or ascorbic acid (50 ppm) partially or completely counteracted the adverse effects of salinity on the germination, seedling growth and metabolic activities on *Cicer arietinum* and *Trigonella foenum-graecum* plants. El-Shintinawy & E-Shourbagy (2001) revealed that NaCl decreased the total content of amino acids and in contrast, increased the free amino acids in wheat seedlings. In addition, also thiamine treatment affected the protein pattern of wheat seedlings and lowered the effect of salinity on this parameter. Other investigators reported the ameliorating effect caused by antioxidants exogenous spraying among of them.

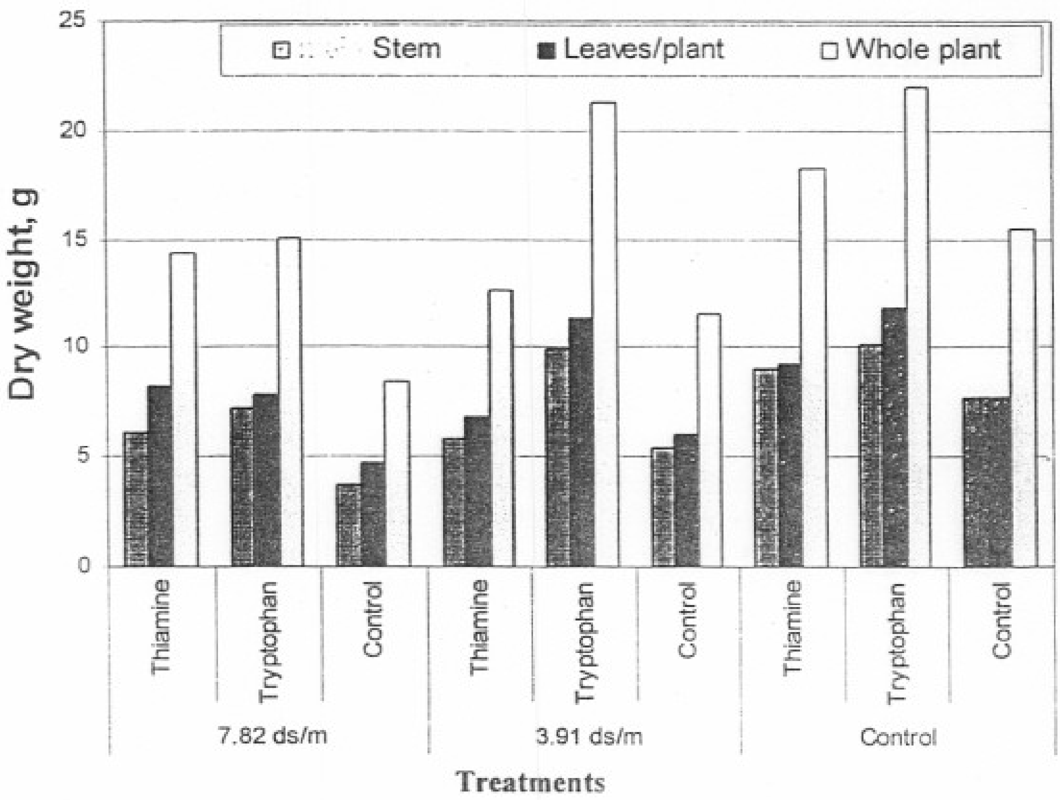

Fig.3. Effect of salinity and antioxidant interaction on growth of cotton plants.

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تأثير الرى بماء البحر المخفف ومضادات الأكسدة على نمو نبات القطن

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أجريت تجربة أصص بصوبة المركز القومى للبحوث بالدقى- الجيزة – جمهورية مصر العربية لدراسة تأثير الرى بماء البحر المخفف ٣,٩١ و ٧,٨٢ dS/m والماء العذب (كمقارنة) والرش بالثيامين و التربتوفان بمعدل ٢٠٠ جزء / المليون. والرش بالماء المقطر كمقارنة على نمو نباتات القطن صنف جيزة ٨٥ فى تجربة عامليه فى قطع منشقة .

أدت معاملات الملوحة إلى انخفاض فى نمو نباتات القطن وكان الأنخفاض أكبر مع التركيز الأعلى للأملاح بماء الرى وتفوق التربتوفان فى تأثيره الموجب على نمو نبات القطن عن الثيامين ويمكن أن نوصى بالرش بالتربتوفان عند الرى بماء البحر المخفف فيما بين تركيزات ٣,٩١ و ٧,٨٢ dS/m .

أدى الرش بالتربتوفان التأثير الموجب الأعلى على أرتفاع النبات و عدد الأوراق والوزن الغض الكلى للنبات وذلك مقارنة بالرش بالثيامين والماء المقطر. كما أدى الرش بمضادات الأكسدة (التربتوفان والثيامين) إلى تحسن مساحة الأوراق .