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IMPROVING THE TOLERANCE OF SUNFLOWER PLANTS TO CALCAREOUS-SALINE SOIL USING SPERMIDINE, ZINC AND POTASSIUM

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ABSTRACT

Two Field experiments were carried out to study the role of foliar application of K and Zn as well as seed soaking with spermidine (Spd, 10 mg/l) alone or in combination, to improve the productivity and tolerance of sunflower (*Helianthus annuus* L.) to calcareous and salinity stress conditions. Both individual and combination treatments increased the stem diameter, shoot fresh and dry weights, yield, yield components and oil yield, as well as the concentrations of K, Ca, P and Zn and the K/Na ratio, whereas they decreased the Na concentration in the two growing seasons. The best results were obtained duc to the K + Zn + Spd treatment in both seasons. These results provide support for foliar application of K and Zn as well as seed soaking with Spd to alleviate the harmful effects of calcareous and salinity stress and enhance the ability of sunflower plants to tolerate these adverse conditions.

Keywords: Sunflower, *Helianthus annuus*, spermidine, calcareous and salinity stress, KCl, zinc.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is becoming an increasingly important source of edible vegetable oil throughout the world because of its high polyunsaturated fatty acid content and no cholesterol. In Egypt, there is a gap between the production and consumption of plant oils. Increases in the cultivated area of sunflower should be made on reclaimed lands due to the limited land available in the Nile Valley and the competition with major crops.

The presence of Ca CO_3 directly or indirectly affects the availability of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn) (Obreza *et al.,* 1993). On the other hand, salinity has deleterious effects on plant growth, development, seed germination, and seed characteristics of sunflower, especially oil content. Salinity also influences nutrient uptake.

Under these circumstances, potassium and zinc play an important role in improving plant development and the subsequent yield of oil crops. Harmati (1993) stated that K application increased the achene and oil yields. Foliar application of Zn (60 ppm applied twice,75 and 85 days after planting) increased the seed yield ha^{-1}, seed index, and seed oil content of Egyptian cotton grown on clay loam soil (Sawan *et al.,* 2006).

Decreasing soil pH improved the availability of microelements, i.e. Fe, Zn, Mn and Cu and improving chemical properties of alkaline soil as well as yield productivity and its related characteristics (Kineber *et al.*, 2004).

Polyamines (PAs) have a specific role in maintaining the cation-anion balance in plant tissues and in stabilizing membranes at high external salinity (Zhao and Qin, 2004). They have an ameliorating effect on all morphological and physiological characters and prevent the degradation of chlorophyll. Polyamines also enhanced the accumulation of organic compounds in a salinity stress study, with the exception of phenols.

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PAs may also play an important role in plant stress responses and plant development. Several fold increases in the level of free PAs in plant cells in response to a variety of abiotic stresses, including salinity, have been reported (Alcazar *et al.*, 2006).

Salt-resistant plant varieties contain higher polyamine levels under stress conditions and more recent work has shown that the application of exogenous PAs can improve the adaptation of plants to salt-stress (Lakra *et al.*, 2006).

Therefore, the present investigation aimed to improve the productivity of sunflower plants on calcareous soil with salinity stress using pre-sowing seed soaking with spermidine and the application of mineral nutrients (K and Zn) as foliar spray and/or their combinations.

MATERIALS AND METHODS

Two field experiments were conducted at experimental farm, Maryout Station, Desert Research Center, Egypt during the two growing seasons of 2007 and 2008. The mechanical and chemical analysis of the soil and irrigation water are presented in Table 1. The soil was loamy in texture and highly calcareous (31.7% $CaCO_3$), slightly saline (EC 4.7 dS m^{-1}), mildly alkaline (pH 7.9). The irrigation water was slightly saline (EC 4.6 dS m^{-1}).

Sunflower seeds were obtained from the Oil Crop Research Institute, Agriculture Research Centre, Giza, Egypt. The seeds were sown on June 1st in both seasons. The seeds were soaked in 10 mgl^{-1} spermidine (Spd) for 6 hours before sowing. Unsoaked seeds were used as the control. Two foliar applications of either potassium as KCl 2.0% and/or zinc as $ZnSO_4$ 0.01% were given, the first 5 weeks after sowing and the second after one-week interval. Distilled water was used as the control. Automatic atomizers were used for spraying of mineral nutrients after adding Tween 20 as a wetting agent at 0.05% to all spraying solutions.

The experiments were set in randomized complete block design having three replications. The experimental unit measured 9 m^2 (3 × 3 m) with row and plant spacings of 30 cm. The experimental details for treatments were as follows:

T1. Control, T2. Spermidine (Spd), T3. Potassium, KCl 2% (K), T4. Zinc, $ZnSO_4$ 0.01% (Zn), T5. K + Zn, T6. K + Spd, T7. Zn + Spd, T8. K + Zn + Spd.

The recommended fertilization for this type of soil was applied according to the Desert Research Centre as follows: Organic fertilization (47.6 m^3 per hectare) during soil preparation, $Ca(H_2PO_4)_2$ (107 kg P_2O_5/ha) before sowing, and NH_4NO_3 and K_2SO_4 (119 kg/ha each, 3 times, at 2, 4 and 6 weeks after sowing).

Growth Parameters

The first sample was taken 50 days after planting for the determination of growth parameters (plant height, stem diameter at the 10th node from the top, plant fresh and dry weights).

Biochemical Constituents

For chemical analyses (Na, K, Ca, P and Zn) from the leaves at the 7th node from the top. Crude dried leaf material was digested using the wet ashing procedure as described by Johanson and Ulrich (1959) and the contents of Na, K and Ca were determined using a Jenway PFP7 flame photometer(Brown and Lilleland, 1964). Zn and Mn were estimated using an atomic absorption spectrophotometer (Pye Unicium Spmm 1900) and phosphorus (P) according to Murphy and Riley (1962).

Yield and Yield Components

The second sample was taken at harvest (90 days after planting) to determine yield components, including head diameter, seed number/head, seed weight/head, 100-seeds weight and seed yield (T/ha).

Oil Percentage and Oil Yield

Chemical analysis was conducted on the seeds to determine the oil percentage (A.O.A.C., 2000).

The data from all the experiments were subjected to statistical analysis of variance. The differences between the means of the studied traits were judged by Duncan's multiple range test according to Gomez and Gomez (1984).

Table 1. Mechanical and chemical properties of the soil and chemical analyses of the irrigation water at Maryout Research station

Mechanical analysis

Depth (cm)	Saturation%	Coarse Sand (%)	Fine sand (%)	Silt (%)	Clay(%)	Textural Class
0-28	41.50	2.06	53.01	21.67	23.26	**Sandy Clay loam**
28-80	43.5	0.74	44.73	24.10	30.43	**Clay loam**
80-110	47.5	0.40	41.10	34.53	34.53	**Clay loam**

Chemical analyses

Depth (cm)	pH	EC (dSm^{-1})	Organic Matter (%)	$CaCO_3$ (%)	Cations (meq/L)				Anions (meq/L)			
					Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^-	HCO_3^-	Cl^-	SO_4^-
0.28	7.8	4.30	1.13	31.70	23.90	0.70	8.85	6.32	-	4.37	16.67	17.27
28-80	7.9	3.60	0.28	31.70	21.70	0.60	5.95	3.01	-	1.77	15.50	14.00
80-110	7.9	6.10	-	39.30	34.80	0.60	9.42	6.44	-	1.04	22.50	27.72

Chemical analyses of irrigation water

pH	EC (dSm^{-1})	Soluble cations (meq/L)				Soluble anions (meq/L)			
		Na^+	K^+	Ca^{++}	Mg^{++}	CO_3^-	HCO_3^-	Cl^-	SO_4^-
8.1	4.610	580	27	201	164	-	179.8	783	1050

RESULTS AND DISCUSSION

Growth Parameters

The data illustrated in Figure 1 revealed that the foliar application of K or Zn under salt soil stress increased the plant height, stem diameter, shoot fresh and dry weights of sunflower in both growing seasons. Moreover, seed soaking with Spd increased the stem diameter and shoot fresh and dry weights of sunflower plants.

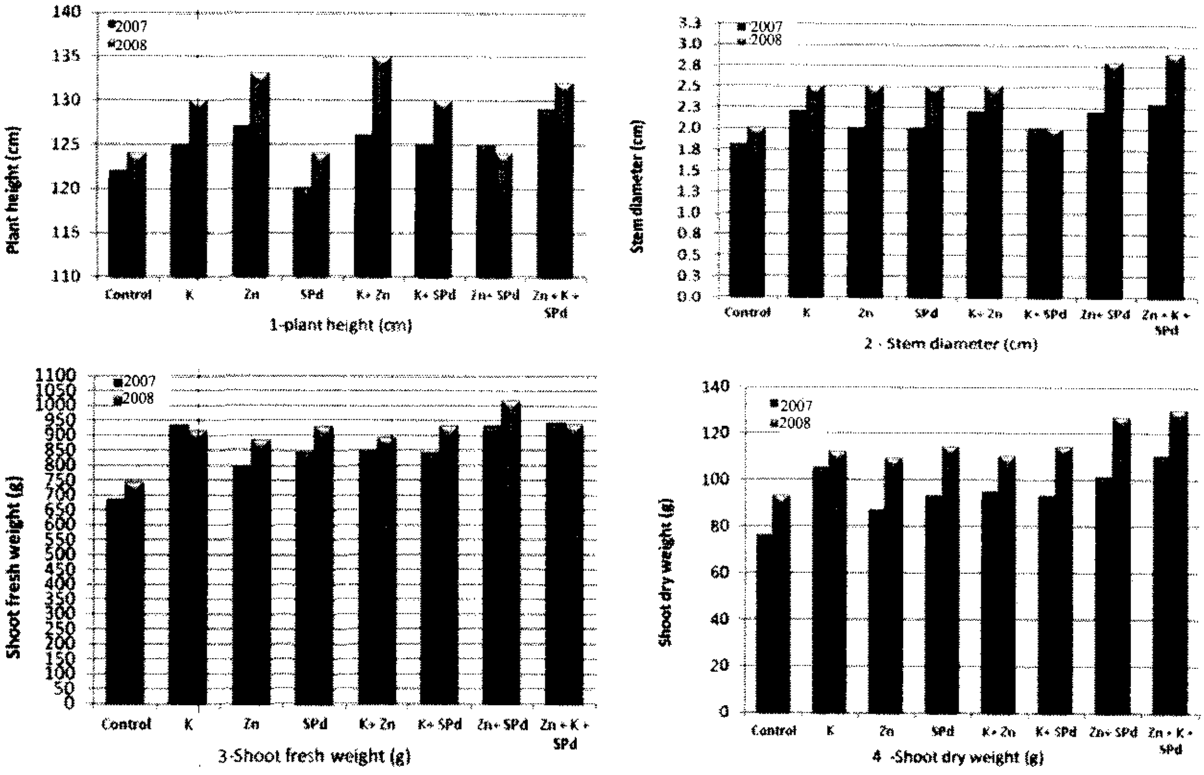
All the combined treatments increased the growth parameters of sunflower plants except for leaf number in both growing seasons. The K+ Zn+ Spd treatment was the most effective in enhancing the growth parameters and counteracting the harmful effects of calcareous saline soil, followed by Zn+ Spd.

The inhibitory effect of soil salt stress on sunflower growth may be due to a decrease in water absorption, metabolic processes, meristematic activity and/or cell enlargement (Khadr *et al.,* 1994; Sakr *et al.,* 2008) or to damage to the cells, so that they cannot perform their functions (Chen and Murata, 2002).

The reason for the promotive effect of K on sunflower growth could be that K increases the photosynthetic rate and CO_2 assimilation and has an important role in the translocation of photosynthates from source to sink(Sangakkara *et al.,* 2000).

Furthermore, K plays an important role in osmotic adjustment under saline conditions to maintain the selectivity and integrity of cell membranes (Satti and Lopez, 1994).

Zinc performs various important roles in protecting cells from the damage caused by reactive oxygen species (ROS) in plants grown under salt soil stress. Zinc is particularly needed within the environment of plasma membranes to maintain their structural and functional integrity (Cakmak, 2001).



1-plant height (cm)

2 - Stem diameter (cm)

3-Shoot fresh weight (g)

4 -Shoot dry weight (g)

Fig. 1. Sunflower plant growth parameters at 50 days after planting as affected by foliar application of zinc and Potassium as well as seed soaking with spermidine (Spd) and their combinations in two growing seasons (2007 and 2008)

Zn-deficiency- related disturbances in the cellular metabolism are responsible for oxidative damage to membrane proteins, phospholipids, chlorophyll, nucleic acids, SH- containing enzymes and IAA, thus inhibiting plant growth. Recent reports demonstrate that the shoot and root meristematic activities of plants are rapidly blocked under oxidative stress conditions as a result of DNA damage (Reicheld *et al.*, 1999). Very high concentrations of Zn in meristematic plant cells (Kitagishi and Obata, 1986; Hossain *et al.*, 1997) demonstrate the crucial roles played by Zn in highly metabolically active differentiating cells.

Many investigators have reported the effect of polyamines (PAs). It has been suggested that PAs may play a role in the antioxidative system and protect membranes from peroxidation. The alleviating effect of polyamines on plants grown under salinity stress may be due to a number of factors, including the activation of the antioxidative defence system (Chattopaydhay *et al.*, 2002), the suppression of superoxide and H_2O_2 levels (Hernandez *et al.*, 1995) thus reducing membrane damage, a reduction in ROS through the quenching of singlet oxygen and excited chlorophyll by elevating the level of carotenoids, thereby maintaining the chloroplastic membrane (Velikova *et al.*, 2000), a reduction in membrane leakage and lipid peroxidation and a decrease in the monodehydroascorbate (MDA) content, as observed in sugarcane leaves (Zhang and Kirkham, 1996), the stabilization of the membranes and an increase in ascorbic peroxidase (AXP) and glutathione reductase (GR) activity (Tiburcio *et al.*, 1994), the stimulation of chlorophyll synthesis and the prevention of chlorophyll degradation (Krishnamurthy, 1991).

It could be concluded that the mineral nutrients K and Zn alleviated the harmful effect of calcareous-saline soil on sunflower plants. The application of antioxidants (spermidine) also proved to be effective in this respect.

Yield and Yield Components

The data illustrated in Figure 2 showed that all the foliar applications of mineral nutrients increased the head diameter, number of seeds (achenes)/head, seed weight/head, 100-seeds weight and seed yield during 2007 and 2008 seasons. Zn treatment was more effective than K in this respect. Seed soaking in Spd significantly enhanced seed number/head, seed weight/head, 100-seeds weight and seed yield in both seasons.

Head diameter, number of seeds per head, (achenes)/head, seed weight/head and seed yield were significantly increased by the combined treatments during the two seasons. The highest value for each of seed number, seed weight/head and seed yield were recorded after the application of K+Zn+Spd, in comparison with control plants. It could be concluded that the K+Zn+Spd treatment was the most effective in alleviating the harmful effects of calcareous and salinity stress on yield and its components in sunflower plants.

It has been stated by other authors that the reduction in seed yield by soil salt stress is largely due to a reduction in pollen viability, which is important in pollen germination and pollen tube growth, the abscission of flowers or young fruit due to ethylene induction by salinity, decreases in pollen grain production, mean number of perfect flowers, and fruit set, and a decline in the leaf area and number per plant, resulting in a reduced supply of carbon assimilates due to lower net photosynthetic rate and biomass accumulation (Sakr *et al.*, 2008).

These results confirmed with the findings of Mekki *et al.* (1999) on sunflower plants. As K is required as a co-factor for many enzymes involved in respiration and photosynthesis, K application could result in a gain in carbon fixation and energy production, with a positive effect on oil seed yield.

Plants supplied with Zn showed a significant increase in head diameter, number of seeds, seed weight/head, 100-seeds weight and seed yield during 2007 and 2008 seasons. These results are in agreement with the findings of Sawan *et al.* (2006) on the seed yield of Egyptian cotton.

In wheat plants, decreases in grain yield due to salinity stress were more marked in Zn-deficient plants (Ekiz *et al.*, 1998). By affecting the synthesis and activity of antioxidative enzymes, Zn is an important factor against destructive O2 species in plant defence systems. Thus, an improvement in the Zn nutritional status of plants may be of great importance for their survival under oxidative stress conditions such as (drought, chilling, high light levels, ozone and salinity (Cakmak, 2001).

The efficiency of Zn in improving the productivity of sunflower may be due to its ability to increase the auxin level and to promote the hormonal balance within the plant tissues and the condensation of amino acids into protein (Jefferey, 1987).

As for the role of exogenous antioxidants on alleviating salinity stress effects, PAs such as spermine (Spm, a tetramine), spermidine (Spd, a triamine) and their obligate precursor putrescine (a diamine) are implicated in the induction of plant adaptation to stresses (Mishra *et al.*, 2003). It has been suggested that PAs may play a role in the antioxidant system and protect membranes from peroxidation.

The antioxidants applied in the present work were able to alleviate or minimize the harmful effect of NaCl salinity on sunflower growth.

Biochemical Constituents

The data presented in Table 2 show that the foliar application of K and Zn or seed presoaking in spermidine, significantly decreased Na content and increased K, K/Na, Ca, P and Zn during the two seasons. The combined treatments reduced Na content, for which higher values were recorded in control plants grown on calcareous saline soil in the two seasons. The application of K+ Zn+ Spd gave the best results in terms of the lowest values of Na and the highest values of K, K/Na, Zn and P in the two growing seasons.

In a saline environment, plants take up excessive amounts of Na+ and Cl–, resulting in high Na+/Ca2+ and Na+/K+ ratios, which may impair the selectivity of the root membrane (Khan *et al.*, 1997). The greater accumulation of Na+ in plant roots may be due to a regulatory mechanism located within the roots that prevents

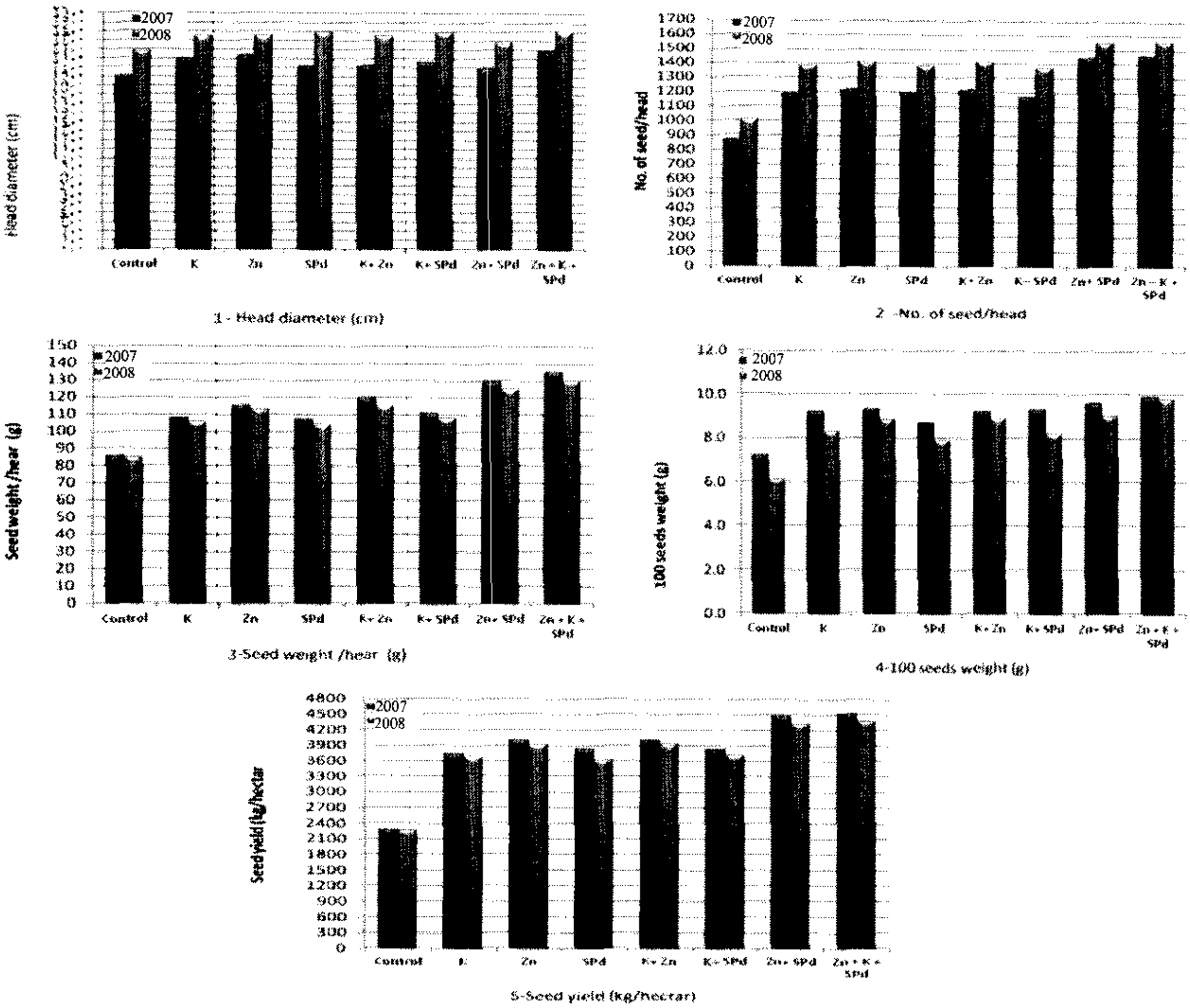


Fig. 2. **Sunflower Yield and yield components at 90 days after planting as affected by foliar application of zinc and Potassium as well as seed soaking with spermidine (Spd) and their combinations in two growing seasons (2007 and 2008)**

Table 2. Sunflower mineral element concentrations at 50 days after planting as affected by foliar application of zinc and Potassium as well as seed soaking with spermidine (Spd) and their combinations in two growing seasons (2007 and 2008)

Treatments	Na (mg/g dwt)		K (mg/g dwt)		Ca (mg/g dwt)		K/Na ratio		P (mg/g dwt)		Zn mg/100 gm d.wt	
	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008	2007	2008
Control	18.4c	15.8d	44.4a	46.5a	79.0a	66.6a	2.41a	2.94a	0.28a	0.22a	1.16a	0.91a
K	16.1b	13.9bc	47.1b	54.9b	82.9b	69.0c	2.93b	3.94b	0.40c	0.40c	1.55b	1.10b
Zn	16.4b	14.4c	46.9b	52.2b	81.3b	67.5b	2.86b	3.62b	0.36b	0.33b	1.54b	1.10b
SPd	15.9ab	13.9b	47.3b	53.2b	89.3c	71.0cd	2.97b	3.76b	0.40c	0.40c	1.65b	1.30c
K+Zn	16.5b	14.5c	48.0c	55.0b	83.0b	70.0c	2.90b	3.80b	0.40c	0.40c	1.60b	1.10b
K+SPd	16.0b	13.7bc	47.0b	53.0b	90.0c	70.0c	2.94b	3.89b	0.40c	0.30b	1.65b	0.90a
Zn+SPd	14.0a	12.8a	49.0cd	60.0c	86.0d	70.0c	3.50c	4.69c	0.40c	0.52d	1.80c	0.95a
Zn+K+SPd	13.5a	12.0a	50.0de	63.0c	92.0e	72.0e	3.70c	5.25d	0.45d	0.55d	1.85c	1.35c

Values within the same column having the same letter are not significantly different at the 5% level of probability (Duncan's Multiple Range Test) the translocation of excessive cations, such as Na+, from the root to aerial parts, resulting in Na+ retention.

Chloride is a more sensitive indicator of salt damage than sodium, since it is stored by the plant. The accumulation of Cl- may cause leaf injury, thereby decreasing photosynthesis and productivity. Ahmed *et al.* (2002) proposed that the relatively greater uptake of Cl- than Na+ in salt-stressed plants could be responsible for growth reduction by depressing the uptake of other anions.

The reduction of internal potassium concentration could be related to an increase in potassium efflux into the growth medium (Cramer *et al.,* 1989), the disruption of membrane integrity by Na+ and the inhibited transport of this ion into the roots and up to the shoots (Wu *et al.,* 1999), the antagonism between K+ and Na+ cations, which increased considerably as salinity increased (Sairam and Srivastava, 2002), or the passive accumulation of Na+ in the roots and shoots leading to a high Na+/K+ ratio and reduced plant growth.

It was found that the application of K and trace elements increased the P concentration in soybean, which could be attributed to the role of K in osmotic adjustment and in maintaining the selectivity and integrity of cell membranes (Satti and Lopez, 1994).

It appears that trace elements play a role in the regulation of ion uptake in sunflower grown under salinity. Trace elements may also modify the movement of nutrients within the plant, causing changes in nutritional requirements under salinity (Hatung, 2004).

The decrease in P concentration associated with salinity conditions may be ascribed to high pH values, which might hinder P availability to the plants (Greenway and Munns, 1980) or to a decrease in the translocation of P upward through the stem because of an increase in the osmotic pressure of the root medium (Sakr *et al.,* 2008).

The decrease in calcium concentration under salinity may be due to the reduced activity of calcium in the solution at high sodium levels, a decrease in the amount of calcium available for uptake by the plant, the antagonism between sodium and calcium at the site of uptake in the roots, or to inhibition of uptake processes.

In the present investigation, better growth and yield were associated with lower Na+ content, higher K content and a higher K/Na ratio as a result of Spm treatment. In this context, many investigators reported that Spd has a diminishing effect on Na concentration and has a promotive effect on K, P and Zn (Tiburcio *et al.,* 1994; Velikova *et al.,* 2000; Chattopadhyay *et al.,* 2002).

It could be concluded that the application of exogenous plant antioxidants could alleviate the harmful effects of salinity stress on the biochemical constituents of wheat plants.

Oil Percentage and Oil Yield

The data presented in Table 3 clearly demonstrate that the foliar application of K and Zn or seed presoaking in spermidine significantly increased the oil percentage and oil yield (kg/hectare) of sunflower seeds in both seasons. The application of Zn resulted in the highest mean values of oil % and oil yield compared with control plants in both seasons.

The combined treatments K+Zn, K+SPd, Zn+SPd and K+Zn+SPd increased the values of oil % and oil yield (kg/hectare) compared with untreated plants in both seasons. Zn acted synergistically with Spd, showing higher values compared with the joint application of Zn and K. The K+Zn+Spd treatment gave the best results in the two growing seasons.

The enhancing effect of Zn, K and Spd on growth parameters, the accumulation of dry mass and the biochemical constituents of sunflower plants may be reflected in the increasing oil yield.

Table 3. Sunflower oil% and oil yield (kg/ha) at 50 days after planting as affected by foliar application of zinc and Potassium as well as seed soaking with spermidine (Spd) and their combinations in two growing seasons (2007 and 2008)

Treatments	Oil %		Oil yield Kg/hectare	
	2007	2008	2007	2008
Control	34.3a	34.7b	971a	975a
K	35.1a	35.8b	1306bc	1318b
Zn	37.7bc	37.9c	1549d	1561c
SP	36.4b	36.2c	1368c	1347b
K+Zn	36.0bc	35.3bc	1380c	1368b
K+ SP	36.6c	36.0c	1287b	1299b
Zn+ SP	38.0d	32.0a	1594e	1606d
Zn+K+ SP	38.2d	38.0d	1606e	1611d

Values within the same column having the same letter are not significantly different at the 5% level of probability (Duncan's Multiple Range Test)

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تحسين تحمل نباتات عباد الشمس للأراضي الملحية و الجيرية باستخدام السبرميدين والزنك والبوتاسيوم

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في دراسة حقلية تم الرش الورقي لنبات عباد الشمس بالزنك والبوتاسيوم وكذلك نقع البذور في مادة سبرميدين بتركيز (10 mg/L) كل منفرد أو متداخلة بهدف تحسين المقاومة للإجهاد الملحي والجيري وزيادة الإنتاج. أوضحت النتائج أن المعاملات منفردة أو متداخلة أدت الي زيادة معنوية في قطر الساق والوزن الغض والجاف للمجموع الخضري والمحصول ومكونات المحصول ومحصول الزيت وكذلك تركيز البوتاسيوم والكالسيوم والفوسفور والزنك ونسبة الصوديوم إلى البوتاسيوم. بينما أدت المعاملات السابقة إلى نقص تركيز الصوديوم خلال موسمي النمو. وكانت أفضل النتائج فى المعاملة (K+ Zn+ Spd) في كلا الموسمين. وتشير هذه النتائج أن الرش الورقي بالبوتاسيوم والزنك و نقع البذور فى السبرميدين ضروري لتقليل الأثر الضار للإجهاد الناتج عن استخدام الأراضي الملحية الجيرية وزيادة مقدرة نبات عباد الشمس علي مقاومة الأثر الضار لهذه الظروف الغير ملائمة.