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A Novel Strategy for Controlling Damping-Off and Charcoal Rot Diseases of Sunflower Plants Grown Under Calcareous-Saline Soil using Spermine, Potassium and Zinc

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Abstract: Two field experiments were carried out to investigate the role of Spermine (SP) as seed soaking and/or foliar application of K and/or Zn in reducing the harmful effects of charcoal rot caused by *Macrophomina phaseolina* and damping-off caused by *Rhizoctonia solani* as well as improving the productivity of sunflower plant in calcareous-saline soil. From five tested locations of Maryout region, Egypt, *M. phaseolina* and *R. solani* were isolated from infected plants. Both fungi were pathogenic and cause typical symptoms of damping-off. *Macrophomina phaseolina* showed to be highly virulent fungus. It gave the highest values of pre- and post-emergence damping-off compared with *R. solani*. Application of SP+Zn+K increased germination percentage and reduced damping-off by both fungi under greenhouse conditions. Under greenhouse and field conditions, all treatments of SP and/or K and/or Zn significantly reduced the incidence of damping-off disease compared with control treatment. Presoaking of seeds in SP and foliar spraying of K and/or Zn increased most of growth parameters, yield and its components and oil content of seeds, as well as the contents of K, Ca, P and Zn and K/Na ratio, whereas decreased Na concentration. All treatments significantly increased the activity of total peroxidase, ascorbic peroxidase, superoxide dismutase and catalase enzymes in plants, in addition to the improvements in the content of chlorophyll A, chlorophyll B, caroteinoids and total phenols compared with control plants in both seasons. Therefore, application of SP+Zn+K could be recommended to alleviate the harmful effects of damping-off and charcoal rot diseases in calcareous-saline soil by enhancing the tolerance of sunflower plant to these adverse conditions.

Key words: *Helianthus annuus*, spermine, calcareous-saline soil, damping-off, charcoal rot, *Macrophomina phaseolina*, *Rhizoctonia solani*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) represents one of the main oil crops in the world. In Egypt, there is a gap between production and consumption of plant oils. Increasing the cultivated areas of sunflower should be done in the reclaimed lands due to the limited areas of the Nile valley and the competition with the main crops. In calcareous sandy soils, the presence of $CaCO_3$ directly or indirectly affects the availability of nitrogen, phosphorous, potassium, magnesium, iron, manganese and zinc, moreover, salinity has deleterious effect on plant growth, development, nutrient uptake and even seed germination and in particular, seed characteristics of sunflower, mainly oil content. Under these circumstances, potassium and zinc play an important role in improving the plant development and subsequent yield of oil (Harmati, 1993; Obreza *et al*., 1993).

Fungal infection by *Macrophomina phaseolina* and *Rhizoctonia solani* limits sunflower planting, growth and productivity because of the partial interruption in the number of harvested heads as a result of broken plants (Conte *et al*., 1989). Charcoal rot of sunflower caused by *Sclerotium bataticola* (Taub.) [*M. phaseolina* (Tassi) Goid.] affects plants and shows grey to black discoloration at the base of the stem, which may extend upward thus hollowing the interior portion of the stem. Later the bith becomes shriveled and discolored (Ilyas *et al*., 1982; Baumer and Hajdu, 1984). The disease also causes root and basal stem rots, premature ripening and drying of stalks (Mirza *et al*., 1984).

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Using microelements either as soil application or as foliar spraying improve growth and yield of sunflower plants (Hegazy, 2004). For instance, foliar application of Zn increases seed and oil yields of Egyptian cotton grown under clay loam soil, this effect was extended to improve oil quality (Sawan *et al.*, 2006). The resistance of plant could be induced by manganese and zinc (Marschner, 1986; Abd El-Hai *et al.*, 2007; El-Baz, 2007). The specific role of polyamines is to maintain a cation-anion balance in plant tissues by stabilizing membranes at high external salinity, polyamines has ameliorating effect on all morphological and physiological characters and prevents degradation of chlorophyll, however, polyamines enhances accumulation of organic compounds except phenols under salinity stress conditions (Zhao and Qin, 2004).

The present study aimed to improve growth, yield and oil production as well as reducing damping-off and charcoal rot diseases of sunflower plants grown under calcareous saline soil using spermine (as pre-sowing seed soaking) and mineral nutrients i.e., K and Zn (as foliar spraying).

MATERIALS AND METHODS

Sunflower seeds and chemicals: Sunflower seeds (Sakha 53) were obtained from Oil Crop Research Department, Agricultural Research Center, Giza, Egypt and chemicals from Al-Gomhuria Co., El-Mansoura, Egypt.

Isolation, purification and identification of the causal pathogens: The causal pathogens were isolated from sunflower plants (Sakha 53) showing typical symptoms of damping-off and charcoal rot diseases. Samples were collected from five different locations of Maryout region, Egypt. The infected plant parts were thoroughly washed in sterilized tap water then placed on PDA plates supplemented with streptomycin sulfate (0.035 g L^{-1}). The growing fungi were purified and identified as *M. phaseolina* (Ellis, 1976) and *R. solani* (Sneh *et al.*, 1992).

Preparation of the fungal inocula: The inocula of *M. phaseolina* and *R. solani* were prepared using sorghum: coarse sand: water (2: 1: 2) medium. The ingredients were mixed, bottled and autoclaved for 30 min at 121°C. The sterilized medium was inoculated using agar discs, obtained from the periphery of 6 days old colony of each of the isolated fungus. The inoculated media were incubated at 28±1°C for 15 days. The resulted cultures were used for soil infestation in a greenhouse experiment for studying the pathogenicity test.

Greenhouse experiments

Pathogenicity test: Pots experiment was carried out at Faculty of Agriculture, Mansoura University, Mansoura, Egypt. The previously prepared fungal inocula were used to infest soil at a rate of 4% (w/w) in plastic pots (30 cm diameter) filled with 5 kg autoclaved calcareous-saline soil taken from experimental farm of Maryout Station, Desert Research Center, Egypt. The pots were inoculated with *M. phaseolina* or *R. solani* watered and left for 3 days to ensure the distribution of pathogenic fungi. Three pots were used for each treatment as replicates. Seeds of sunflower were surface sterilized in 1% Sodium hypochlorite solution for 5 min followed by 3 successive rinses in sterilized tap water. The excess water was removed by placing the treated seeds between sterilized tissue paper until dryness. Seeds were sown at the rate of five seeds pot^{-1}. The percentages of pre- and post-emergence damping off were calculated (on the basis of total number of planted seeds) after 20 and 50 days from planting, respectively.

Germination and damping-off disease of sunflower as affected by tested treatments: To study the effect of seed soaking in spermine and/or foliar spraying of the developed plants with K and/or Zn on germination percentage and damping-off disease of sunflower plant under greenhouse. The previously prepared fungal inocula were used to infest soil of the pots at a rate of 4% (w/w). The pots were inoculated with *M. phaseolina* or *R. solani* watered and left for 3 days to ensure the distribution of inoculated fungi. During that period, soil was moistened when necessary. Seeds were soaked in SP (10 mg L^{-1}) for 6 h before sowing. Foliar applications of potassium as KCl (2.0%) and/or Zinc as $ZnSO_4$ (0.01%) were started at the 5th week after sowing. Tween 20 was used as wetting agent at 0.05%. A daily observation for germination and damping off were recorded.

Field evaluation of spermine, K and Mn: Two field experiments were conducted in the experimental farm of Maryout Station, Desert Research Center, Egypt during the two growing seasons of 2007 and 2008. The mechanical and chemical analysis of the farm soil are prepared in Table 1. This farm soil is characterized as highly calcareous (31.7% $CaCO_3$), slightly saline (EC 4.7 dS m^{-1}), mild alkaline (pH 7.9) and loamy in texture. Irrigation water is slightly saline (EC 4.6 dS m^{-1}).

Table 1: Mechanical and chemical properties of the experimental farm soil and chemical analysis of 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 analysis

					Cations (meq L^{-1})				Anions (meq L^{-1})			
Depth (cm)	pH	EC (dS m^{-1})	Organic matter (%)	$CaCO_3$ (%)	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 analysis of irrigation water

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

Sunflower seeds were soaked in Spermine at 10 mg L^{-1} for 6 h before sowing. Seeds were sown on June 1st in both seasons. Foliar applications, one week interval of Kcl (2.0%) and/or $ZnSO_4$ 0.01% were started at the 5th week after sowing. Distilled water served as control. Tween 20 was used as wetting agent at 0.05%. Automatic atomizer was used. Treatments were arranged in complete block design with three replicates. The experimental unit area was 9 m^2 (3×3 m) with 30 cm apart rows and plants spacing 30 cm. Recommended fertilization for this type of soil was applied, according to Desert Research Center, Egypt. Treatments were as follows:

- Control (without any treatment)
- Spermine (SP) (10 mg L^{-1})
- Potassium, KCl (2%)
- Zinc, $ZnSO_4$ (0.01%)
- K+Zn
- SP+K
- SP+Zn
- SP+Zn+K

Germination and damping-off (*R. solani*) as well as disease incidence of charcoal rot (*M. phaseolina*) were recorded after 30 and 90 days after sowing. Two samples were taken. The first was taken at the 50th day after sowing for determination of (1) growth parameters (plant height, stem diameter at the 10th node from the top, leaves number of fresh and dry weights of plant, (2) crude dried materials of the leaves which were digested using the wet ashing procedure as described by Johnson and Ulrich (1959), (3) the contents of Na, K and Ca which were determined using Jenway PFP7 flame photometer model (Brown and Lilleland, 1964), (4) Zn that was estimated using atomic absorption spectrophotometer (Pye Unicium Sp 1900) and (5) phosphorus (P) which was determined according to Murphy and Riley (1962). All chemical analysis were determined in leaves taken from the 7th node from the top. The second sample was taken at harvesting time (90 days after sowing) for the determination of yield components including, head diameter, seeds number/head, seeds weight/head, 100 seeds weight (seed index) and the seeds yield (t ha^{-1}). Oil content of seeds was determined according to AOAC (1970).

Determination of total phenolic compounds: Total phenolics were determined at 60 days after sowing. Samples of fresh shoot (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10000 rpm for 15 min under cooling and the supernatants were saved. The residues were extracted in 80% ethanol. The supernatants were evaporated to dryness at room temperature. Residues were dissolved in 5 mL distilled water. One-hundred microliter of each extract was water diluted to 3 mL, 0.5 mL of Folin-Ciocalteau reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was mixed thoroughly to the extract. The developed color was spectrophotometrically measured at 650 nm after 60 min. Catechol was used as a standard. The results were expressed as mg catechol 100 g^{-1} fresh weight (Singleton and Rossi, 1965).

Determination of enzymatic activities: Ascorbate Peroxidase (APX) activity was assayed spectrophotochemically according to Fielding (1978). The assay was carried out at 25°C in 1.0 cm light path cuvette

and the reaction mixture consisted of 1500 μL phosphate buffer (pH 7.5), 20 μL EDTA, 1000 μL sodium ascorbate and enzyme extract (20 μL). After mixing, the reaction was initiated by adding the 480 μL H_2O_2 and decreasing in optical density at 290 nm against blank (without extract) was continuously recorded every minute (for 2 min).

Total Peroxidases (TPX) activity was assayed spectrophotochemically according to Amako *et al*. (1994). The assay was carried out at 25°C in 1.0 cm light path cuvette and the reaction mixture consisted of 1500 μL phosphate buffer (pH 7.5), 1000 μL pyrogallol and 480 μL H_2O_2 solution. After mixing the reaction was initiated by adding the enzyme extract (20 μL) and the increase in optical density at 430 nm against blank (without extract) was continuously recorded every minute (for 3 min).

Super Oxide Dismutase (SOD) activity was determined in leaves collected in an ice bucket and brought to the laboratory. Leaves were then washed with distilled water and surface moisture was wiped out. Leaf samples (0.5 g) were homogenized in ice cold 0.1 M phosphate buffer (pH 7.5) containing 0.5 Mm EDTA with pre-chilled pestle and mortar. The homogenate was transferred to centrifuge tubes and was centrifuged at 4°C in Beckman refrigerated centrifuge for 15 min at 15000x g. The supernatant was transferred to 30 mL tubes and referred to enzyme extract. SOD activity was estimated in 3 mL of reaction mixture containing 0.1 mL of 1.5 M sodium carbonate, 0.2 mL of 200 mM methionine, 0.1 mL of 2.25 Mm nitro-blue tetrazolium, 0.1 mL of 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer (pH, 7.5), 1 mL distilled water and 0.05 mL of enzyme were taken in test tubes. Tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL riboflavin (60 mM) and placing the tubes below a light source of two 15 w florescent lamps for 15 min, reaction was stopped by switching off the light and covering the tube with black cloth. Tubes without enzyme developed maximal color. A non-irradiated complete reaction mixture which did not develop color served as blank. Absorbance was recorded at 560 nm and one unit of enzyme activity was taken as the quantity of enzyme which reduced the absorbance reading of samples 50% in comparison with tubes lacking enzymes (Dhindsa *et al*., 1981).

Catalase (CAT) activity was determined by measuring the rate of H_2O_2 conversion to O_2 at room temperature using a lipid-phase O_2 electrode (Hansatech, Norfolk, UK). Approximately 0.5 g of plant tissue, consisting of the apical region of the shoot including the cotyledons, was extracted in 1.5 mL of 0.1 mM Hepes/KOH buffer (pH 7.4) and then centrifuged at 10000 g for 5 min. The rate of O_2 production was measured by adding 50 μL of the supernatant to 0.1 M Hepes (pH 7.4) containing 530 mM H_2O_2. Catalase activity was calculated on a fresh weight basis to keep the data uniform with the H_2O_2 measurements and to reduce the chances of distribution as a result of protein synthesis alteration due to heat shock (Vierling, 1991; Bettany, 1995).

Determination of photosynthetic pigments: Photosynthetic pigments (chlorophyll A, B and carotenoids) were determined in the 2nd leaf from plant tip by extracting in 90% methanol for 24 h at room temperature after adding traces of sodium carbonate (Robinson and Britz, 2000) then photosynthetic pigments were determined spectrophotometrically according to Mackinney (1941).

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

RESULTS

Isolation of the pathogenic fungi: As shown in Fig. 1, *M. phaseolina* and *R. solani* were isolated in high frequency from the five tested locations of Maryout region. Both of them were isolated from plants showed typical symptoms of damping-off and charcoal rot diseases. *Macrophomina phaseolina* was isolated at high frequency compared with *R. solani* in the five locations.

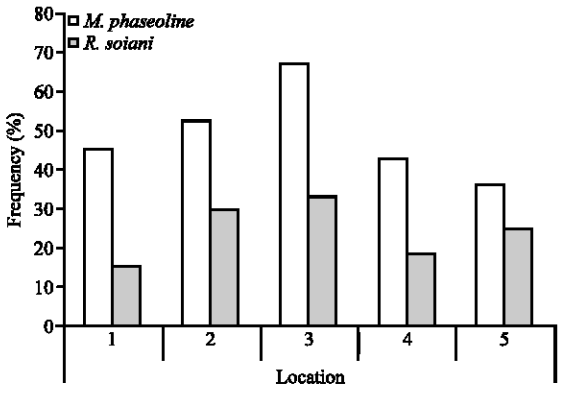

Fig. 1: Frequency of the causal pathogens of damping-off and charcoal diseases isolated from sunflower plant at different Maryout locations

Greenhouse experiments

Pathogenicity test: Results illustrated in Fig. 2 show the effect of fungal isolates on the percentages of pre- and post-emergence damping-off as well as on the survival plants, data show that both tested fungi were pathogenic and cause typical symptoms of damping-off. *M. phaseolina* showed to be highly virulent fungus. It gave the highest values of pre- and post-emergence damping-off (42.59 and 26.23%, respectively) followed by *R. solani* (18.28 and 16.49%, respectively).

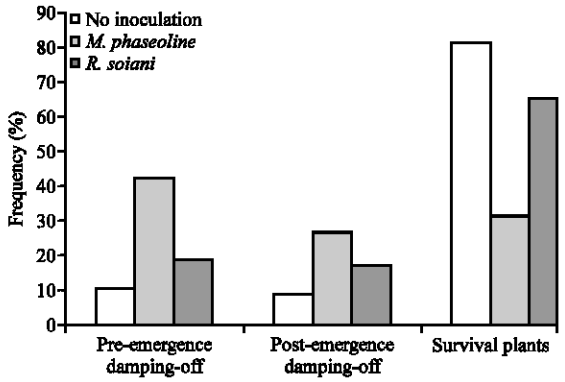

Fig. 2: Pathogenicity test of pre- and post-emergence damping-off and survival of sunflower plants infected with isolated fungi under greenhouse conditions

Germination and damping-off disease of sunflower as affected by different combinations of SP, K and Zn: The effect of SP, K and Zn as well as their combinations on germination and damping-off of sunflower plants under greenhouse conditions are shown in Table 2. Data show that the highest germination percentage and the lowest damping-off of sunflower occurred when SP+Zn+K treatment was allocated followed by K and SP+Zn treatments for both *M. phaseolina* and *R. solani*.

Field evaluation of seed soaking in SP and/or foliar spraying of K and/or Zn on sunflower

Effect on germination and damping-off disease caused by *R. solani*: Data presented in Table 3 show the effect of SP, K and Mn as well as their combinations on germination and damping-off of sunflower plants under field conditions. In general, all combinations of SP, K and Zn, significantly reduced damping-off disease incidence of sunflower compared with control treatment in both seasons. The highest germination percentage which corresponding the lowest damping-off disease occurred by SP+Zn+K (96.34 and 3.66%), followed by K (89.56 and 10.44%) and SP+Zn (86.93 and 13.07) treatments, respectively. These data were previously confirmed under greenhouse conditions (Table 2).

Effect on disease incidence of charcoal rot caused by *M. phaseolina*: Regarding to charcoal rot disease, data

Table 2: Effect of seed soaking with SP and/or foliar application with K and/or Zn on germination percentage and damping-off disease of sunflower plant under greenhouse conditions

	M phaseolina		*R. solani*	
Treatment	Germination (%)	Damping-off	Germination (%)	Damping-off
Control	50.41h	49.59a	60.20h	39.80a
Potassium, KCl (K) (2%)	74.63b	25.37g	81.56b	18.44g
Zinc, $ZnSO_4$ (Zn) (0.01%)	51.02g	48.98b	60.75g	39.25 b
Spermine (SP) (10 mg L^{-1})	57.18f	42.82c	65.90f	34.10c
K+Zn	64.21e	35.79d	71.78e	28.22d
SP+K	64.54d	35.46e	72.08d	27.92e
SP+Zn	72.44c	27.56f	78.70c	21.30f
SP+K+Zn	79.26a	20.74h	94.56a	5.44h

Values with different letter are significantly different ($p \leq 0.05$)

Table 3: Effect of seed soaking in SP and/or foliar application with K and/or Zn on germination percentage and damping-off disease (*R. solani*) of sunflower plant after 30 days from sowing under field conditions during the two growing seasons of 2007 and 2008

	2007		2008	
Treatment	Germination (%)	Damping-off	Germination (%)	Damping-off
Control	60.49h	39.51a	70.23h	29.77a
Potassium, KCl (K) (2%)	89.56b	10.44g	95.15b	4.85g
Zinc, $ZnSO_4$ (Zn) (0.01%)	61.22g	38.78b	70.88g	29.12b
Spermine (SP) (10 mg L^{-1})	68.62f	31.38c	76.88f	23.12c
K+Zn	77.05e	22.95d	83.74e	16.26d
SP+K	77.45d	22.55e	84.09d	15.91e
SP+Zn	86.93c	13.07f	91.82c	8.18f
SP+K+Zn	96.34a	3.66h	99.3a	0.7h

Values with different letter are significantly different ($p \leq 0.05$)

Table 4: Effect of seed soaking in SP and/or foliar spraying with K and Zn on disease incidence of charcoal rot caused by *M. phaseoliana* at the 90th day from sowing under field conditions during the two growing seasons of 2007 and 2008

	2007		2008	
Treatment	Disease incidence (%)	Reduction (%)	Disease incidence (%)	Reduction (%)
Control	20.30a	0.00	24.42a	0.00
Potassium, KCl (K) (2%)	5.24g	74.19	6.41g	73.75
Zinc, $ZnSO_4$ (Zn) (0.01%)	8.07b	60.25	10.01b	59.01
Spermine (SP) (10 mg L^{-1})	7.64c	62.36	9.27c	62.04
K+Zn	7.16d	64.73	8.69d	64.41
SP+K	6.90e	66.01	8.13e	66.71
SP+Zn	5.81f	71.38	7.11f	70.88
SP+K+Zn	3.39h	83.30	4.14h	83.05

Values with different letter are significantly different ($p \leq 0.05$)

Table 5: Effect of seed soaking in SP and/or foliar spraying with K and Zn on growth parameters of sunflower plants at the 50th day after planting during the two growing seasons of 2007 and 2008

	2007					2008				
Treatment	Plant height (cm)	Stem diameter (cm)	Shoot fresh wt. (g)	Shoot dry wt. (g)	No. of leaves $plant^{-1}$	Plant height (cm)	Stem diameter (cm)	Shoot fresh wt. (g)	Shoot dry wt. (g)	No. of leaves $plant^{-1}$
Control	122a	1.8a	683a	76a	23a	124a	2.0a	742a	93a	22a
Potassium, KCl (K) (2%)	125b	2.2c	932d	105e	23a	130b	2.5b	914c	112b	22a
Zinc, $ZnSO_4$ (Zn) (0.01%)	127bc	2.0b	794b	87b	23a	133b	2.5b	885b	109b	22a
Spermine (SP) (10 mg L^{-1})	120a	2.0b	841c	93c	22a	124a	2.5b	929d	114b	22a
K+Zn	126bc	2.2c	850c	95c	23a	135c	2.5b	890b	110b	22a
SP+K	125b	2.0b	840c	93c	23a	130b	2.0a	930d	114b	22a
SP+Zn	125b	2.2c	930d	101d	23a	124a	2.8c	1017e	127c	22a
SP+K+Zn	129d	2.3c	940d	110e	23a	132b	2.9c	935d	130c	22a

Values with different letter are significantly different ($p \leq 0.05$)

Table 6: Effect of seed soaking in SP and/or foliar spraying with K and Zn on yield and yield components of sunflower plants at the 90th day after planting during the two growing seasons of 2007 and 2008

	2007					2008				
Treatment	Head diameter (cm)	No. of seed $head^{-1}$	Seed wt. $head^{-1}$ (g)	100 Seeds wt. (g)	Seed yield (kg ha^{-1})	Head diameter (cm)	No. of seed $head^{-1}$	Seed wt. $head^{-1}$ (g)	100 seeds wt. (g)	Seed yield (kg ha^{-1})
Control	19.0a	867a	86a	7.2a	2277a	21.8a	1010a	85a	6.1a	2258a
Potassium, KCl (K) (2%)	21.0b	1191b	108b	9.2c	3729b	23.5c	1381b	106b	8.3bc	3679b
Zinc, $ZnSO_4$ (Zn) (0.01%)	21.3b	1219c	115c	9.3c	3993b	23.5c	1404c	113c	8.8bc	3905c
Spermine (SP) (10 mg L^{-1})	20.1a	1201c	107b	8.7b	3831b	23.9c	1382b	104b	7.9b	3617b
K+Zn	20.2ab	1220b	120c	9.2c	3998b	23.5c	1410c	115c	8.9bc	3927c
SP+K	20.6b	1170b	111b	9.3c	3831b	23.9c	1374b	108b	8.2bc	3705b
SP+Zn	19.9a	1447d	129d	9.6c	4479c	22.8bc	1550d	125d	9.0c	4333d
SP+K+Zn	21.9b	1460d	135e	9.9d	4522c	24.1d	1560d	130d	9.8d	4379d

Values with different letter are significantly different ($p \leq 0.05$)

presented in Table 4, show marked differences among treatments. All individuals and combinations of SP, K and Zn significantly reduced charcoal rot disease incidence compared with control treatment. SP+Zn+K was the most effective treatment followed by K then SP+Zn in both seasons.

Effect on growth parameters: Data in Table 5 show that soaking in SP combined with foliar application of K or Zn under salt soil stress increased most of growth parameters of sunflower. Treatment with K+Zn, SP+K, SP+Zn and SP+Zn+K increased all growth parameters except, leaves number of sunflower. During the two growing seasons, SP+Zn and SP+Zn+K treatments were the most effective in this respect. Application of SP+Zn+K showed the best results in enhancing growth parameters and counteracting the harmful effects of calcareous saline soil and improving the tolerance of sunflower plants to these adverse conditions.

Effect on yield and yield components: Data of Table 6 show that, all individual and combinations of SP, K and/or Zn increased head diameter, number of seeds/head, seeds weight/head, seed index and seeds yield during the seasons of 2007 and 2008. The application of individual Zn was more effective than K in this respect. Seed soaking in SP significantly enhanced seeds number/head, seeds weight/head, 100 seeds weight and seeds yield in

Table 7: Effect of seed soaking in SP and foliar spraying with K and Zn as well as their combinations on minerals content of sunflower plants at the 50th day after planting during the two growing seasons of 2007 and 2008

	2007						2008					
Treatment	Na	K	Ca	K/Na ratio	P (mg g^{-1} dry wt.)	Zn (mg 100^{-1} g dry wt.)	Na	K	Ca	K/Na ratio	P (mg g^{-1} dry wt.)	Zn (mg 100^{-1} g dry wt.)
	---(mg g^{-1} dry wt.)---						---(mg g^{-1} dry wt.)---					
Control	18.4c	44.4a	79.0a	2.41a	0.28a	1.16a	15.8d	46.5a	66.6a	2.94a	0.22a	0.91a
Potassium, KCl (K) (2%)	16.1b	47.1b	82.9b	2.93b	0.40c	1.55b	13.9bc	54.9b	69.0c	3.94b	0.40c	1.10b
Zinc, $ZnSO_4$ (Zn) (0.01%)	16.4b	46.9b	81.3b	2.86b	0.36b	1.54b	14.4c	52.2b	67.5b	3.62b	0.33b	1.10b
Spermine (SP) (10 mg L^{-1})	15.9ab	47.3b	89.3c	2.97b	0.40c	1.65b	13.9b	53.2b	71.0cd	3.76b	0.40c	1.30c
K+Zn	16.5b	48.0c	83.0b	2.90b	0.40c	1.60b	14.5c	55.0b	70.0c	3.80b	0.40c	1.10b
SP+K	16.0b	47.0b	90.0c	2.94b	0.40c	1.65b	13.7bc	53.0b	70.0c	3.89b	0.30b	0.90a
SP+Zn	14.0a	49.0cd	86.0d	3.50c	0.40c	1.80c	12.8a	60.0c	70.0c	4.69c	0.52d	0.95a
SP+K+Zn	13.5a	50.0de	92.0e	3.70c	0.45d	1.85c	12.0a	63.0c	72.0e	5.25d	0.55d	1.35c

Values with different letter are significantly different ($p \leq 0.05$)

Table 8: Effect of seed soaking in SP combined with foliar spraying of K and/or Zn on oil content of sunflower at the 50th day after planting during the two growing seasons of 2007 and 2008

	2007		2008	
Treatment	Oil (%)	Oil yield (kg ha^{-1})	Oil (%)	Oil yield (kg ha^{-1})
Control	34.3a	971a	34.7b	975a
Potassium, KCl (K) (2%)	35.1a	1306bc	35.8b	1318b
Zinc, $ZnSO_4$ (Zn) (0.01%)	37.7bc	1549d	37.9c	1561c
Spermine (SP) (10 mg L^{-1})	36.4b	1368c	36.2c	1347b
K+Zn	36.0bc	1380c	35.3bc	1368b
SP+K	36.6c	1287b	36.0c	1299b
SP+Zn	38.0d	1594e	32.0a	1606d
SP+K+Zn	38.2d	1606e	38.0d	1611d

Values with different letter are significantly different ($p \leq 0.05$)

both seasons. Head diameter, number of seeds per head, seeds number/head, seeds weight/head and seeds yield significantly increased by interaction treatments during the two seasons. Yield and yield components reached the highest values by the application of SP+Zn+K, (in particular, seeds number, seeds weight/head and seeds yield) as compared with the control plants. It could be concluded that SP+Zn+K treatment showed the best results in alleviating the harmful effects of calcareous soil and salinity stress on sunflower yield and its components.

Effects on chemical constituents: Data presented in Table 7 show that seed soaking in SP and foliar applications with K and Zn significantly decreased Na content and increased K, K/Na ratio, Ca, P and Zn during the two seasons. The interaction treatments reduced Na content, which recorded higher values in control plants grown under calcareous saline soil, application of SP+Zn+K 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.

Effect on oil content of sunflower seeds: Data in Table 8 clearly demonstrate that, seed soaking in SP and foliar applications with K and Zn significantly increased oil percent and oil yield of sunflower seeds in both seasons. Application of SP+Zn+K recorded the highest values of oil content (38.2 and 38.0%) and oil yield (1606 and 1611 kg ha^{-1}) comparing with all other treatments in both seasons.

Effect on enzymatic antioxidants activity: Data shown in Table 9 declare that application of SP and/or minerals as well as their combinations significantly increased enzymatic activities of total peroxidase (TPX), ascorbic peroxidase (APX), Super Oxide Dismutase (SOD) and catalase (CAT) throughout the two growing seasons. The highest values of TPX, APX, SOD and CAT were obtained in the case of SP+Zn+K treatment the corresponding values were 238, 187, 419 and 66, respectively, followed by potassium treatment (231, 153, 389 and 65, respectively) and SP+Zn treatment (226, 148, 384 and 64, respectively). All treatments of SP and nutrient minerals as well as their combinations increased significantly all enzymatic activities compared with control treatment in both seasons.

Effect on non-enzymatic antioxidant contents: As presented in Table 9, the tested treatments significantly increased non-enzymatic activity of chlorophyll A, chlorophyll B, carotenoids and total phenol throughout the two growing seasons. Results show that the highest values of non-enzymatic activity of chlorophyll A,

Table 9: Effect of seed soaking in SP combined with foliar spraying of K and/or Zn on endogenous enzymatic activities and non-enzymatic antioxidants in sunflower plant grown under calcareous-saline soil during the two growing seasons of 2007 and 2008

	Enzymatic antioxidants activity*				Non-enzymatic antioxidants contents			
					Chl A	Chl B	Caroteinoids	
Treatment	TPX	APX	SOD	CAT	(mg g^{-1} fresh wt.)			Total phenols (mg 100^{-1} g fresh wt.)
First season								
Control	189h	132f	368g	60e	1.46g	0.94e	0.71e	596.48h
Potassium, KCl (K) (2%)	231b	153b	389b	65ab	1.87b	1.15b	0.82b	895.03b
Zinc, $ZnSO_4$ (Zn) (0.01%)	203g	140e	372f	62d	1.52f	0.85g	0.72ed	673.19g
Spermine (SP) (10 mg L^{-1})	208f	142d	379e	63cd	1.53f	1.06c	0.68f	707.61f
K+Zn	217e	147c	379e	63cd	1.58e	0.87f	0.71e	725.34e
SP+K	223d	147c	381d	63cd	1.65d	1.03d	0.73d	836.97d
SP+Zn	226c	148c	384c	64bc	1.77c	1.02d	0.79c	861.30c
SP+K+Zn	238a	187a	419a	66a	2.07a	1.26a	0.85a	924.81a
Second season								
Control	196g	137f	375e	61d	1.45g	0.93e	0.71e	608.65h
Potassium, KCl (K) (2%)	234b	152b	387b	69b	1.87b	1.19b	0.82b	913.28b
Zinc, $ZnSO_4$ (Zn) (0.01%)	208f	137f	379d	63c	1.51f	0.85g	0.71e	722.03g
Spermine (SP) (10 mg L^{-1})	226e	143e	379d	63c	1.52f	1.07c	0.68f	740.09f
K+Zn	228d	145d	379d	63c	1.57e	0.87f	0.71e	744.08e
SP+K	231c	147c	381c	64c	1.65d	1.03d	0.73d	854.08d
SP+Zn	232c	147c	382c	64c	1.76c	1.02d	0.79c	878.88c
SP+K+Zn	267a	173a	442a	72a	2.06a	1.26a	0.85a	943.69a

*TPX: total peroxidase activity (units g^{-1} fresh weight). APX: ascorbic peroxidase activity (units g^{-1} fresh weight). SOD: superoxide dismutase activity (units mg^{-1} protein min^{-1}). CAT: catalase activity (µmol H_2O_2 red mg^{-1} protein min^{-1}). Values with different letter are significantly different ($p \leq 0.05$)

chlorophyll B, caroteinoids and total phenol occurred when sunflower plants treated with SP+Zn+K (2.07, 1.26, 0.85 and 924.81, respectively) followed by potassium treatment (1.87, 1.15, 0.82 and 895.03, respectively) and SP+Zn treatment (1.77, 1.02, 0.79 and 861.30, respectively). Generally, the treatment with SP, K and/or Zn significantly increased the non-enzymatic activities of chlorophyll A, chlorophyll B, caroteinoids and total phenol compared with control treatment in both seasons.

DISCUSSION

In the present study, it was planed to investigate the possibility of using foliar application of the antioxidant; SP alone or combined with K and/or Zn to reduce the harmful effects of charcoal rot and damping-off diseases on sunflower under calcareous-saline soil. In such soils, there is inhibitory effect on sunflower growth as in the present investigation, this may be due to a decrease in water absorption, metabolic processes, merestematic activity and/or cell enlargement (Sakr *et al*., 2008) or by damaging the grown cells so that they cannot perform their functions (Chen and Murata, 2002).

Plant resistance to pathogen requires the activation of complex metabolic pathways in the infected cells, aimed at recognizing pathogen presence and hindering its propagation within plant tissues (De Gara *et al*., 2003). The role of antioxidants on overcoming the injurious effects of both *M. phasolaina* and *R. solani* may be attributed to the regulation of plant development and chilling of disease resistance (Dmitrier *et al*., 2003; Achuo *et al*., 2004). In addition, antioxidants may neutralize the harmful oxygen radicals released during the infections (Shahda, 2002). Zinc is co-factors of SOD, which considered enzymatic antioxidant, hence alleviate the harmful effect of Reactive Oxygen Species (ROS) i.e., free radicals caused by fungal stress. Kostas and Christos (2006) found that the foliar application of microelements (Zn and Mn) could be used to reduce the severity of tan spot disease on durum wheat, however the physiological basis of this pattern still unknown. Zinc performs various important roles in protecting cells from the damaging reactions caused by ROS in plants grown in salt soil stress. Zinc is particularly needed within the environment of plasma membranes to maintain their structural and functional integrity. The Zn-deficiency-related disturbances in cellular metabolism are responsible for oxidative damage of membrane proteins, phospholipids, chlorophyll, nucleic acids, SH-containing enzymes and IAA and thus inhibition of plant growth. The shoot and root meristematic activities of plants are rapidly blocked under oxidative stress conditions because of DNA damage (Reichheld *et al*., 1999). Very high concentrations of Zn in meristematic plant cells demonstrate the crucial roles played by Zn in highly metabolically active differentiating cells (Kitagishi and Obata, 1986; Hossain *et al*., 1997). The positive effect of zinc on vegetative growth may be due to the role of zinc as essential constituent of three enzymes (Carbonic anhydrase, Alcohol dehydrogenase and superoxide dismutase). Also, zinc has marked effect on the level of auxin and appears to be required in the synthesis of intermediates in the metabolic pathway, through conversion of tryptophan to auxin, which encourage the meristematic activity (Ohki, 1978; Devlin and Witham, 1983).

The interpretation for the promotive effect of K on sunflower growth is that K increases the photosynthetic rates, 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 the osmotic adjustment for plant under saline conditions to maintain the selectivity and integrity of cell membrane (Satti and Lopez, 1994). K and Zn alleviated the harmful effect of calcareous-saline soil on sunflower plants. The application of antioxidant SP proved to be more effective in this respect. These results were confirmed by the findings of Mekki *et al.* (1999) on sunflower plants. However, K is required as a cofactor for many enzymes involved in respiration and photosynthesis. Thus, a gain in carbon fixation and energy production would be the result of K application that positively affect oil seed yield. Plants supplied with Zn showed significant increase in head diameter, number of seeds, seeds weight/head, 100 seeds weight and seeds yield during the seasons of 2007 and 2008. These results were in agreement with Hegazy (2004) on sunflower and Sawan *et al.* (2006) on seeds yield of Egyptian cotton. The efficiency of Zn on improving productivity of sunflower may be due to its effects on increasing auxin level and promoting hormonal balance within the plant tissues and the condensation of amino acids into protein (Jefferey, 1987). It has been suggested that Polyamines (PAs) may play a role in antioxidative system and protect membrane from peroxidation. The alleviating effect of polyamines on plants grown under saline stress may be due to one or more of the following factors: (1) Through activating antioxidative defense system (Chattopadhyay *et al*., 2002). (2) Suppressed the level of superoxide and H_2O_2 in leaf stressed plants (Hernandez *et al*., 1995). (3) Reduction in ROS through quenching of singlet oxygen and excited chlorophyll by elevating level of carotenoids thereby maintained chloroplastic membrane (Velikova *et al*., 2000). (4) Reduce membrane leakage and lipid peroxidation and decreased monodehydroascorbate contents in sugarcane leaves (Zhang and Kirkham, 1996). (5) Stabilization of membrane damage may be due to its polycationic nature (Tiburcio *et al*., 1994). (6) Increasing ascorbic peroxidase and glutathione reductase activity as well as carotenoids and reduced glutathione at all saline levels (Tiburcio *et al*., 1994). (7) Stimulation of chlorophylls synthesis and prevent chlorophyll degradation (Krishnamurthy, 1991). (8) Increasing all organic concentrations, which are involved in important biological processes, e.g., ionic balance and DNA, RNA and protein synthesis.

Obtaining disease control benefits from K can be complicated. There is little doubt that many plant species will be more susceptible to diseases if they are suffering from a K deficiency. Potassium alters the compatibility relationship of the host-parasite environment within the plant. The infection causes increased production of fungus inhibiting phenolic compounds and flavonoids at the site of infection and in other parts of the plant. However, K is especially critical in this role and a shortage of K reduces the amount of the plants natural antifungal compounds. Potassium plays a central role in the development of thick cuticles, a physical barrier to infection or penetration by sucking insects. Critical role in the balance among nutrients was reported by K, the balance between K and other elements is also important. For example, Stewart's wilt in corn is known to need or benefit from higher inorganic N in the tracheal sap of the plant. Potassium deficiency reduces the ability of corn to metabolize N, thus increasing the amount of inorganic N in the tracheal sap, this increases the susceptibility to Stewart's wilt, as a result of breaking down plant compounds that have fungicidal properties. However, other work has shown that a high K:Ca ratio in some plants can lead to more disease damage. Work with potatoes and citrus trees showed that infection by common scab (*Streptomyces scabies*) in potatoes and *Phytophthora* root rot (*P. parasitica*) in citrus was increased by high levels of K fertilization. It was concluded in both cases that higher K uptake probably caused a shortage of Ca. The Ca shortage apparently resulted in improper formation or function of the plant cell walls, leading to increased disease infection or spread within the plants (Jones *et al*., 1989). The fact that Zn is an active ingredient in some fungicides is evidence that it is directly toxic to some pathogens. Evidence also indicates that soils low in soluble Zn is more likely to support higher populations of some disease causing organisms. However, some species of *Fusarium* (*F. oxysporum* and *F. lycopersici*) have a higher demand for Zn than many crop species and exhibit less growth and virulence when deprived of Zn in isolated cultures. Shortages of K and Zn reduce the amount of the plants natural antifungal compounds at the site of infection. Zn aids the production and detoxification of oxygen radicals and hydrogen peroxide, thus limiting damage to plant cells. In summary, it can safely be concluded that improving the Zn nutrition of crops will be helpful against many, but not all diseases (Jones *et al*., 1989).

The lowest values of Na concentration comparing with control plants were obtained by K treatment in both growing seasons. The role of K in the osmotic adjustment of plants under saline conditions and consequently its

importance to the selectivity and integrity of cell membrane was reported (Satti and Lopez, 1994). Moreover, Liu *et al.* (1992) and Ismail (2005) reported that Zn concentrations and uptake increased in sorghum plant due to Zn application. It appears that trace elements play a role in the regulation of ions uptake in sunflower grown under salinity. Trace elements may also modify the movement of nutrients within the plants causing adaptation of the nutritional requirements under salinity (Hatung, 2004). Both Na and K will move along the electrochemical gradients of tissues but because of either discrimination of the cell membranes or Na extrusion, the ultimate concentration ratio may be 20 K+ to 1 Na+ (Ismail, 2005). In a saline environment, plants take up excessive amount of Na^+ and Cl^- as in halophytes resulted in high Na^+/Ca^{2+} and Na^+/k^+ ratios which may impair the selectivity of the root membrane (Khan *et al.*, 1997). The reduction of internal potassium concentration could be related to: (1) Increased potassium efflux into the growth medium (Cramer *et al.*, 1989) due to disrupt in membrane integrity caused by Na^+ and inhibit transport of this ion into root and up to the shoot. (2) The antagonism between K^+ and Na^+ cations, which increased considerable as salinity increased (Sairam and Srivastava, 2002). (3) Excess of Na^+ in the root media results in a passive accumulation of these ions in root and shoot lead to a high Na^+/K^+ ratio and reduced plant growth. Regarding phosphorus, the decrease associated with salinity conditions may be ascribed to (1) The high pH values (pH 8.7), which might hinder P availability to plants and/or (2) Decrease in the translocation of P upward through the stem because of the 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 high sodium levels in the external media which, reduced the activity of calcium in the solution and/or decrease the amount of calcium available for uptake by the plant and/or the antagonism of sodium and calcium at the site of uptake in roots and/or to the inhibition of uptake processes. In the present investigation, the promotion of growth and yield were concomitant with lower Na^+ content as well as higher K content and higher K/Na ratio as a result of SP treatment. In this context, many investigators reported that SP 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).

Regarding enzymatic antioxidant activity viz., TPX, APX, SOD and CAT, Dash and Panda (2001) reported that higher activity of antioxidant enzymes caused lower H_2O_2 production and lipid peroxidation and higher membrane stability. Beneficial effect of higher osmolyte concentration (soluble sugars, glycine-betaine, proline and potassium) is reflected in stabilization of essential enzyme proteins such as SOD and TPX resulting in higher activity under salinity stress. Plants posses antioxidant systems in the form of enzymes such as SOD, APX and metabolites viz., ascorbic acid, glutathione, α-tocopherol, carotenoid and flavonoids etc. These antioxidant enzymes and metabolites are reported to increase under various environmental stresses as well as comparatively higher activity has been reported in tolerant cultivars than the susceptible ones (Sairam and Srivastava, 2002).

The suggested treatments increased photosynthetic pigments which in turn increased carbohydrate content in plant tissues. Carbohydrates are the main repository of photosynthetic energy, they comprise structurally polysaccharide of plant cell walls, principally cellulose, hemicelluloses and pectin that consider a barrier against plant pathogens invasion and phenolic compounds are associated with structural carbohydrates, which play a major and important role in plant defense (Hahlbrock and Scheel, 1989). In addition, the enhancement in chlorophyll content is resulting from stimulating pigment formation and increasing the efficacy of photosynthetic apparatus with a better potential for resistance as well as decreasing photophosphorylation rate, which occurred after infection (Amaresh and Bhatt, 1988). In this connection, Rhodes and Wooltorton (1978) indicated that, the adaptation of plants to biotic and abiotic stress is due to the stimulation of protective biochemical systems and synthesis of secondary metabolites such as phenolics. The increase in seed oil content may be due to the improvement in photosynthetic pigments since there is a relationship between photosynthesis processes and oil biosynthesis during seed development in terms of inducing sucrose translocation (Smith *et al.*, 1989).

It was found that all tested chemicals decreased damping-off and charcoal rot diseases and at the same time enhanced the vegetative growth and increased the enzymatic activity, total phenols and chlorophyll contents. So, it is highly recommended to use a mixture of nutrient elements (K and Zn) and SP to alleviate the harmful effect of damping-off and charcoal rot diseases and maximizing the productivity of sunflower under calcareous-saline soil. Besides, these chemicals are save for both environment and public health.

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