Available online at www.sciencedirect.com

SciVerse ScienceDirect

South African Journal of Botany 81 (2012) 8 – 14

SOUTH AFRICAN JOURNAL OF BOTANY

www.elsevier.com/locate/sajb

A novel *organo-mineral fertilizer* can mitigate salinity stress effects for tomato production on reclaimed saline soil

M.M. Rady *

Agricultural Botany Department, Faculty of Agriculture, Fayoum University, 63514-Fayoum, Egypt

Received 30 September 2011; received in revised form 16 March 2012; accepted 24 March 2012
Available online 25 April 2012

Abstract

A novel *organo-mineral fertilizer* [a 2:10:1 (w/w/w) mixture of calcium sulphate, ground rice bran and humic acid] was used as a soil amendment to study its effect on the growth, fruit yield, leaf nutrient status and antioxidant enzymes activities of tomato (*Solanum lycopersicum* L.) plants grown in reclaimed saline soil (EC = 8.9 dS m^{-1}). The *organo-mineral fertilizer*-treated plants showed increased growth, proline, chlorophyll and nutrient contents. They also revealed increased fruit yield and quality, and increased activity of antioxidant enzymes when compared to the control plants. Therefore, the tested *organo-mineral fertilizer* may be recommended as a soil amendment for vegetables such as tomato to overcome the adverse effects of salinity stress in newly-reclaimed soils.

Keywords: Antioxidants; Growth; *Organo-mineral fertilizer*; Salinity; Tomato

1. Introduction

Tomato (*Solanum lycopersicum* L.) production has a major role in global horticulture, ranking only second in importance to potato in many countries. Tomato is widely cultivated on newly-reclaimed soils in Egypt. However, most of these newly-reclaimed soils are affected by salinity with low fertility and a poor soil structure. Salinity is a major limiting factor in agricultural production and exerts unfavorable influence on various physiological and biochemical processes associated with plant growth and development (Greenway and Munns, 1980; Pitman and Läuchli, 2002). The negative impact of salinity on plant growth and metabolism has been attributed, principally, to enhanced Na^+ ion uptake, which causes an excess of Na^+ ions in plant tissues (Abbas et al., 1991). One of the primary effects of increasing the salinity of the growth medium is the inhibition of K^+, Ca^{2+} and NO_3^- ion uptake by plant roots (Maas, 1986). In addition, it is well-established that salinity stress damages plant cells through the production of reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, hydroxyl anions, and singlet oxygen (Scandalios, 1997). Soil salinity inhibits the activities of the key enzymes of photosynthesis namely rubisco and PEP carboxylase (Soussi et al., 1998). Moreover, salinity induces the closure of stomata (Bethkey and Drew, 1992) and damages photosynthesis and photosynthetic electron transport chain. All these impaired events finally culminate into a severe loss in the rate of photosynthesis (Sudhir and Murthy, 2004).

Efforts have been made to control salinity by various technological means including soil reclamation, drainage, the use of high leaching fractions, and the application of soil amendments (Abdel-Naby et al., 2001). In recent years, much attention has been paid to the development of sustainable agriculture; hence, several materials have been applied as soil amendments to overcome the adverse effects of soil salinity, to improve the physical and chemical properties of soils, to increase their water retention, and to provide the nutrients required during plant growth.

* Tel.: +20 2107302668, +20 2119092061; fax: +20 2846343970, +20 2846334964.
E-mail address: mrady2009@yahoo.com.

0254-6299/$ -see front matter © 2012 SAAB. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.sajb.2012.03.013

The application of humic acid as an organic soil amendment, individually or in combination with other materials, resulted in significant increases in plant growth and crop yields in sandy soils by improving the hydrophysical properties and nutrient availability of such soils (Osman and Ewees, 2008). Humic acids enable growing plants to overcome the adverse effects of moderate soil salinity by improving the soil properties such as aggregation, aeration, permeability, water holding capacity, micronutrient uptake and availability, and by the decrease in the uptake of some toxic elements (Tan, 2003).

Calcium is considered as an important factor for the maintenance of cell membrane integrity and the regulation of ion-transport. Ca^{+} is essential for K^{+} vs Na^{+} ion selectivity and membrane integrity (Hanson, 1984). Elevated concentrations of Ca^{2+} in the nutrient solution mitigated the adverse effects of salinity by inhibiting the uptake of Na^{+} (Greenway and Munns, 1980). In addition, Ca^{2+} ions reduce ion leakage through membranes (Leopold and Willing, 1984). LaHaye and Epstein (1969) confirmed that Ca^{2+}/Na^{+} ion interactions took place at the plasmalemma. They suggested that Na^{+} acted by displacing Ca^{2+} ions from membranes, leading to increased membrane permeability and higher intracellular Na^{+} ion concentrations.

Owing to considerable evidence of the adverse effects of soil salinity on plant growth, it was hypothesized that the novel *organo-mineral fertilizer* used in this study as a soil amendment can overcome the injurious effects of soil salinity (EC = 8.9 dS m^{-1}) on tomato plants. Thus, the primary objective of this work was to examine whether or not the *organo-mineral fertilizer* could mitigate the effects of soil salinity and regulate tomato plant growth by adjusting the proline content, nutritional status and antioxidant enzymes activities involved in stress tolerance.

2. Materials and methods

2.1. Plant material and growth conditions

The novel *organo-mineral fertilizer* used in this research was generated by mixing calcium sulphate ($CaSO_4$), ground rice bran (a by-product of rice milling process), and humic acid (Alpha Chemika, Mumbai, India) at a ratio of 2:10:1 (w/w/w). These proportions of the *organo-mineral fertilizer* used gave the best results among several proportions examined in preliminary studies (data not shown). Therefore, they were selected. Table 1 summarizes the major components of the novel *organo-mineral fertilizer* used in these experiments. The soil used in this research was obtained from the Experimental Farm (a newly-reclaimed saline soil with EC = 8.9 dS m^{-1}) of the Faculty of Agriculture in South-east Fayoum (29° 17′N; 30° 53′E), Egypt. The main characteristics of the soil according to Wilde et al. (1985) are given in Table 2.

Two greenhouse experiments were initiated on 1 September 2009 and 2010 in which pots were filled with various soil: *organo-mineral fertilizer* mixtures, with the portion of the *organo-mineral fertilizer* ranging from 0 (control) to 25 g kg^{-1} soil (i.e., 0, 5, 10, 15, 20, or 25 g kg^{-1} soil). The experiments were arranged in a completely randomized design with these six experimental *organo-mineral fertilizer* treatments, 20 replications (20 pots) of each. Five-week-old tomato seedlings (cv. Saria), obtained from the Ministry of Agriculture Nurseries, Cairo, Egypt, were transplanted separately in 6 kg of each of the various soil:*organo-mineral fertilizer* mixtures per pot. All plants were maintained in a greenhouse at 25° ± 2 °C under a natural photoperiod. Irrigation was applied twice a week and the pots were irrigated every 2 weeks with a nutrient solution containing 200 mg l^{-1} nitrogen (N), 100 mg l^{-1} phosphorus (P), 200 mg l^{-1} potassium (K), 2.0 mg l^{-1} iron (Fe), 1.0 mg l^{-1} manganese (Mn), 0.5 mg l^{-1} boron (B), 0.1 mg l^{-1} copper (Cu), 0.1 mg l^{-1} zinc (Zn), and 0.05 mg l^{-1} molybdenum (Mo).

2.2. Determination of plant growth and preparation for other estimations

Seven-week-old tomato plants from transplanting were used for the determinations of shoot dry weight (DW) $plant^{-1}$ and root DW $plant^{-1}$. The fourth true leaf on each plant was used for determining the activity of antioxidant enzymes, total chlorophyll and free proline contents, as well as leaf N, P, K, Ca and Na contents. In addition, ripe tomato fruit were used to determine fruit quality including vitamin C and total soluble solids (TSS) contents. Four individual plants were randomly selected from each experimental treatment to determine plant growth and other 4 plants for chemical determinations. Shoot and root DWs $plant^{-1}$ (in g) were estimated after drying the appropriate tissue to constant weight at 70 °C using a forced air-oven for 48 h.

2.3. Determination of pigment and proline contents

Total chlorophyll (in mg g^{-1} FW) was estimated adopting the procedure given by Arnon (1949). Leaf discs were homogenized with 80% acetone and centrifuged; the optical density of the acetone extract was measured at 663, 645 and 470 nm using a UV-160A UV Visible Recording Spectrometer, Shimadzu, Japan.

Leaf free proline contents (in μg g^{-1} DW) were measured using the rapid colourimetric method, as suggested by Bates et al. (1973). Proline was extracted from 0.5 g of each leaf sample by grinding in 10 ml 3% (v/v) sulphosalicylic acid and the mixture was then centrifuged at 10,000 ×*g* for 10 min. Two ml of the supernatant was added to a test-tube, to which 2 ml of a freshly prepared acid-ninhydrin solution was then added. The tubes were incubated in a water-bath at 90 °C for 30 min, and the reaction

Table 1
Major components of the novel *organo-mineral fertilizer* * used in these experiments.

Component	N	P	K	Ca	Fe	Mn	Zn	Humic acid	Total fiber	Water holding capacity (g g^{-1})
% (w/w)	2.81	0.71	3.02	7.98	0.31	0.17	0.10	12.49	32.46	7.33

* *Organo-mineral fertilizer* = 2:10:1 (w/w/w) calcium suphate, ground rice bran and humic acid.

Table 2
Some of the physical and chemical characteristics of the reclaimed saline soil used in these experiments.

Composition [% (w/w)]			pH	EC (dS m^{-1})	OC [a] (g kg^{-1})	N (g kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})
Clay	Loam	Sand										
11.9	16.6	71.5	7.7	8.9	8.4	0.7	18.6	81.7	85.1	6.4	4.0	2.1

[a] OC, organic content.

was terminated in an ice-bath. The reaction mixture was extracted with 5 ml toluene and vortex mixed for 15 s. The tubes were allowed to stand for ≥20 min in the dark at room temperature to separate the toluene and aqueous phases. Each toluene phase was then carefully collected into a clean test-tube and its absorbance was read at 520 nm. The concentration of free proline in each sample was determined using a standard curve prepared using analytical grade proline, and was calculated on % DW basis.

2.4. Determinations of N, P, K, Ca and Na contents

Leaf nitrogen contents (in mg g^{-1} DW) were determined according to Hafez and Mikkelsen (1981). An Orange-G dye solution was prepared by dissolving 1.0 g of 96% (w/w) assay-dye in 1.0 l of distilled water with 21.0 g citric acid, which acted as a buffer to maintain the correct pH, and 2.5 ml 10% (v/v) thymol in alcohol as an inhibitor of microbial growth. Ground plant leaf material (0.2 g) was placed in a centrifuge tube and 20 ml of the dye reagent solution was added. The contents of the tube were shaken on auto-shaker at 300 rpm for 15 min. After filtration, the solution was diluted 100-times with distilled water and its absorbance was measured at 482 nm. N contents were calculated using the formulae:

$$N(\%) = 0.39 + 0.954 \times \text{Dye absorbed } (g/100g),$$

and

$$\text{Dye absorbed } (g/100g) = \frac{a-b}{a} \times \frac{cfv}{w} \times 100$$

where, a was the absorbance of the dye reagent solution at 482 nm without any plant material (blank), b was the absorbance of the dye reagent solution at 482 nm with plant material, c was the concentration of the dye reagent (1.0 g l^{-1} distilled water), f was the purity factor of the dye reagent (96%), v was the volume of the dye reagent solution used per sample (20 ml), and w was the weight of ground dry material in g (0.2).

The molybdenum-reduced molybdophosphoric blue colour method (Jackson, 1967), in sulphuric acid, was the method used for phosphorus determinations (in mg g^{-1} DW) in leaf tissue. In addition, diluted sulphomolybdic acid, and 8% (w/v) sodium bisulphite-H_2SO_4 solution were used as reagents. Leaf potassium ion (K^+), calcium ion (Ca^{2+}) and sodium ion (Na^+) contents (in mg g^{-1} DW) were assessed using a Perkin-Elmer Model 52-A Flame Photometer (Page et al., 1982).

2.5. Determinations of fruit yield, vitamin C and TSS contents

The number of fruit $plant^{-1}$ and fruit yield $plant^{-1}$ were recorded, using the remaining 12 pots, at the end of the experiment on 11 November. Ripe fruit were used for determining vitamin C and TSS contents.

The vitamin C contents of fruit (mg 100 g^{-1} juice) were determined using the 2,6-dichloro-indophenol method (Helrich, 1990). Frozen samples were pulverised in a domestic grinder (Magefesa, Spain) and triplicate 10 g aliquots of each sample were immediately homogenised in 50 ml (w/v) of metaphosphoric acid/acetic acid solution. The extracts were centrifuged for 15 min at 7000 ×g, filtered through six layers of cheese-cloth, and made up to 100 ml (v/v) with metaphosphoric acid/acetic acid solution. Triplicate aliquots of each sample were titrated with 2,6-dichloro-indophenol solution. Ascorbic acid reduced the 2,6-dichloro-indophenol to a colourless solution and a slight excess of unreduced dye, resulting in a characteristic light-pink colour, indicated the end point of the reaction (Helrich, 1990). Total soluble solids (TSS) contents [in % (w/v)] of tomato ripe fruit were measured at 20 °C using an ATC-1E hand-held refractometer (Atago, Kyoto, Japan).

2.6. Determination of antioxidant enzymes activities

Peroxidase (POX) and polyphenol oxidase (PPO) activity was assayed in fresh leaf by the method of Kumar and Khan (1982). POX activity was expressed in Unit mg^{-1} protein. One Unit is defined as the change in the absorbance by 0.1 min^{-1} mg^{-1} protein. PPO activity was expressed in Unit mg^{-1} protein (Unit=Change in 0.1 absorbance min^{-1} mg^{-1} protein). Catalase (CAT) activity was measured according to Chandlee and Scandalios (1984). CAT activity was expressed in Unit mg^{-1} protein (Unit=1 mM of H_2O_2 reduction min^{-1} mg^{-1} protein).

2.7. Statistical analysis

All data were subjected to ANOVA using SAS software (1996), and means comparisons between the different treatments were performed using the Least Significant Differences (LSD) procedure at the $P=0.05$ level, as illustrated by Snedecor and Cochran (1980).

3. Results

3.1. Shoot and root dry weights (DWs), free proline and chlorophyll contents

All levels of the *organo-mineral fertilizer* increased shoot and root DWs in tomato plants (Table 3). An *organo-mineral fertilizer* level of 25 g kg^{-1} soil was more effective and significantly increased shoot and root DWs as compared to the control. The application of the *organo-mineral fertilizer* also

Table 3
Effect of the novel *organo-mineral fertilizer* * application rate on shoot dry weight (DW) plant^{-1}, root DW plant^{-1}, leaf free proline and chlorophyll contents [means (n=4)±standard deviations] of 7-week-old tomato plants in both 2009 and 2010 seasons.

Organo-mineral fertilizer level (g kg^{-1} soil)	Shoot DW plant^{-1} (g)	Root DW plant^{-1} (g)	Proline content (μg g^{-1} DW)	Chlorophyll content (mg g^{-1} FW)
2009 season				
0	6.34±0.56f	3.05±0.27e	25.12±0.58c	0.70±0.03e
5	8.84±0.69e	4.38±0.40d	24.98±0.49c	0.85±0.05d
10	11.01±0.87d	5.64±0.48c	24.84±0.47c	0.90±0.04d
15	12.95±0.96c	6.15±0.47c	26.87±0.50c	1.14±0.07c
20	15.87±1.29b	7.78±0.62b	31.80±0.56b	1.31±0.05b
25	18.59±1.18a	9.04±0.69a	39.99±0.62a	1.52±0.07a
2010 season				
0	7.01±0.65e	3.21±0.31e	27.45±0.49c	0.68±0.05e
5	8.78±0.81e	5.00±0.47d	28.15±0.56c	0.82±0.05d
10	10.89±0.89d	6.03±0.49c	26.88±0.53c	0.88±0.07d
15	13.03±1.22c	6.86±0.54c	26.93±0.54c	1.12±0.06c
20	16.12±1.46b	7.98±0.72b	33.56±0.49b	1.42±0.08b
25	18.73±1.58a	9.41±0.78a	40.89±0.55a	1.61±0.08a

In a column, treatment means having a common letter(s) are not significantly different at the 5% level.
* *Organo-mineral fertilizer*=2:10:1 (w/w/w) calcium suphate, ground rice bran and humic acid.

significantly increased leaf free proline and chlorophyll contents, especially at the rate of 25 g kg^{-1} soil as compared to the control (Table 3). Similar trends were observed in both the 2009 and 2010 seasons.

3.2. Fruit yield and quality

Results of this study showed that all levels of *organo-mineral fertilizer* significantly increased the average number of fruit plant^{-1} and fruit yield pot^{-1}. However, the *organo-mineral fertilizer* rates of 20 or 25 g kg^{-1} soil were more effective than all others (Table 4). Using the *organo-mineral fertilizer* as a soil conditioner led to a significant increase in the yield of tomato fruit as compared to the control over both 2009 and 2010 growing seasons. The application of the *organo-mineral fertilizer* also significantly increased vitamin C content, especially at the rate of 25 g kg^{-1} soil (Table 4), whereas TSS% content showed no significant differences between any level of the *organo-mineral fertilizer* and the control. The same trends were seen in both 2009 and 2010 seasons.

3.3. Nutritional status of the tomato plants

The nutrient content, Na content, and Ca:Na ratio of the tomato leaf are presented in Table 5. Statistically significant differences between the *organo-mineral fertilizer* treatments were noted for N, K and Ca contents, and Ca:Na ratio. The highest N, K and Ca contents, and Ca:Na ratio were obtained from plants amended with 25 g *organo-mineral fertilizer* kg^{-1} soil compared to the control plants. Use of the *organo-mineral*

Table 4
Effect of the novel *organo-mineral fertilizer* * application rate on fruit number plant^{-1}, fruit yield plant^{-1}, vitamin C and total soluble solids (TSS%) contents [means (n=4)±standard deviations] of 10-week-old tomato plants in both 2009 and 2010 seasons.

Organo-mineral fertilizer level (g kg^{-1} soil)	Fruit number plant^{-1}	Fruit yield plant^{-1} (kg)	Vitamin C (mg 100 g^{-1} juice)	TSS (%)
2009 season				
0	11.25±0.94f	0.51±0.04e	18.03±0.09e	4.14±0.06a
5	13.21±0.98e	0.73±0.05e	22.46±0.12d	4.16±0.05a
10	15.81±1.29d	1.03±0.08d	23.72±0.11 cd	4.19±0.04a
15	18.59±1.25c	1.39±0.08c	25.26±0.14c	4.16±0.05a
20	21.12±1.64b	1.80±0.12b	28.45±0.14b	4.14±0.04a
25	24.43±1.78a	2.07±0.15a	32.66±0.16a	4.22±0.05a
2010 season				
0	10.96±0.89e	0.52±0.06f	17.89±0.06e	4.26±0.04a
5	12.65±0.93e	0.77±0.05e	21.32±0.09d	4.22±0.05a
10	15.33±0.99d	1.12±0.09d	24.69±0.08c	4.28±0.05a
15	19.00±1.23c	1.50±0.09c	26.98±016bc	4.32±0.04a
20	20.95±1.56b	1.92±0.11b	29.94±0.15b	4.29±0.05a
25	23.90±1.49a	2.21±0.16a	34.78±0.15a	4.36±0.05a

In a column, treatment means having a common letter(s) are not significantly different at the 5% level.
* *Organo-mineral fertilizer*=2:10:1 (w/w/w) calcium suphate, ground rice bran and humic acid.

Table 5
Effect of the novel *organo-mineral fertilizer* application rate on leaf nutrient and Na contents, and Ca:Na ratio [means (n=4)±standard deviations] in 7-week-old tomato plants in both 2009 and 2010 seasons.

Organo-mineral fertilizer level (g kg^{-1} soil)	N (mg g^{-1} DW)	P (mg g^{-1} DW)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Na (mg g^{-1} DW)	Ca:Na ratio
2009 season						
0	10.24±0.56d	0.15±0.01a	11.32±0.66e	5.32±0.18d	21.87±0.88a	0.24±0.01e
5	10.66±0.54 cd	0.15±0.01a	12.00±0.59de	5.50±0.16d	18.23±0.89b	0.30±0.01de
10	11.15±0.58bc	0.14±0.01a	12.90±0.62c	6.83±0.21c	14.02±0.34c	0.49±0.02d
15	11.67±0.53ab	0.16±0.02a	13.46±0.57bc	7.45±0.19b	8.87±0.32d	0.84±0.03c
20	11.77±0.60a	0.15±0.01a	13.93±0.59ab	8.39±0.16a	6.76±0.22e	1.24±0.05b
25	12.09±0.58a	0.16±0.01a	14.48±0.58a	8.66±0.14a	4.06±0.19f	2.13±0.08a
2010 season						
0	11.14±0.61f	0.16±0.02a	11.26±0.58f	6.02±0.14f	22.13±0.96a	0.27±0.02f
5	11.68±0.55ef	0.16±0.01a	11.98±0.61ef	6.27±0.12e	19.12±0.75b	0.33±0.02ef
10	12.44±0.63d	0.17±0.01a	13.08±0.59d	6.89±0.15d	15.21±0.42c	0.45±0.03de
15	12.69±0.58 cd	0.17±0.02a	13.56±0.57 cd	7.38±0.15c	9.14±0.29d	0.81±0.03c
20	13.08±0.65bc	0.17±0.01a	14.00±0.59bc	8.24±0.14b	6.33±0.18e	1.30±0.06b
25	14.12±0.56a	0.17±0.01a	14.98±0.55a	9.12±0.15a	3.94±0.11e	2.31±0.08a

In a column, treatment means having a common letter(s) are not significantly different at the 5% level.
**Organo-mineral fertilizer*=2:10:1 (w/w/w) calcium suphate, ground rice bran and humic acid.

fertilizer had no effects on the contents of P (Table 5). All levels of the *organo-mineral fertilizer* significantly reduced the Na contents in the tomato leaf when compared to the control (Table 5). This reflected in the increase in the ratio of Ca:Na in the *organo-mineral fertilized*-plants compared to the control ones. The same trend was observed in results of both 2009 and 2010 seasons.

3.4. Antioxidant enzyme activity

All levels of the *organo-mineral fertilizer* increased the activity of catalase (CAT) and polyphenol oxidase (PPO) in tomato leaves (Table 6). An *organo-mineral fertilizer* level of 25 g kg^{-1} soil significantly increased CAT and PPO activities as compared to the control. In contrast, the activity of peroxidase (POX) enzyme reduced with the application of all levels of the *organo-mineral fertilizer*. The same trend was observed over both 2009 and 2010 seasons.

4. Discussion

The *organo-mineral fertilizer* used in the current study generated positive findings as a result of overcoming the harmful effects of soil salinity by the Ca^{2+} (Greenway and Munns, 1980) and humic acid (Osman and Ewees, 2008) presented in this *organo-mineral fertilizer*. The healthy metabolic status of the stressed plants grown in saline soil applied with the *organo-mineral fertilizer* resulted in the healthy plant growth, in terms of increased shoot and root dry weights (DWs) (Table 3). The mechanisms by which humic acid stimulated plant growth may

Table 6
Effect of the novel *organo-mineral fertilizer* application rate on the activity of catalase (CAT), polyphenol oxidase (PPO) and peroxidase (POX) in leaves [means (n=4)±standard deviations] of 7-week-old tomato plants in both 2009 and 2010 seasons.

Organo-mineral fertilizer level (g kg^{-1} soil)	CAT (Unit mg^{-1} protein)	PPO (Unit mg^{-1} protein)	POX (Unit mg^{-1} protein)
2009 season			
0	4.3±0.03d	2.4±0.02e	2.3±0.03a
5	4.6±0.02c	2.7±0.01d	2.1±0.03b
10	4.6±0.02c	2.9±0.02c	2.1±0.02b
15	4.7±0.03c	2.9±0.02c	2.0±0.03c
20	4.9±0.03b	3.0±0.01b	1.9±0.02d
25	5.2±0.02a	3.2±0.02a	1.7±0.02e
2010 season			
0	3.9±0.02e	2.6±0.02d	2.1±0.04a
5	4.3±0.02d	3.0±0.02c	1.8±0.03b
10	4.5±0.03c	3.0±0.03c	1.7±0.03c
15	4.6±0.03c	3.1±0.02c	1.7±0.03c
20	4.8±0.03b	3.3±0.03b	1.5±0.02d
25	5.1±0.03a	3.5±0.02a	1.4±0.02e

In a column, treatment means having a common letter(s) are not significantly different at the 5% level.
**Organo-mineral fertilizer*=2:10:1 (w/w/w) calcium suphate, ground rice bran and humic acid.

be similar to that of other plant growth regulators such as auxins, gibberellins and cytokinins that affect plant metabolism in a positive manner. This may explain the positive results of the *organo-mineral fertilizer* on proline and chlorophyll contents under saline soil conditions that were then positively reflected in the growth of tomato plants. Humic acid leads to higher rates of uptake of elemental K (Table 5), thus leads to a corresponding increase in chlorophyll fluorescence which can serve as an indicator of the stress induced by alterations in the balance of endogenous hormones (Marschner, 1995). Proline accumulation under stress conditions may either be caused by induction or activation of enzymes of proline biosynthesis or a decreased proline oxidation to glutamate, decreased utilization of proline in protein synthesis, and enhanced protein turnover (Delauney and Verna, 1993). The increased content of proline has been shown to alleviate salinity-induced oxidative stress by scavenging some of harmful reactive oxygen species (ROS). Therefore, being a hydroxyl and singlet oxygen scavenger, proline has efficiently reduced the threat of ROS in the salts-excess tomato leaves under salinity stress (Rady, 2011). However, the interesting thing that emerged in the present study is that the indirect treatment of plants with the *organo-mineral fertilizer* (as soil amendment) enhanced the level of proline (Table 3) under salt-stress condition. Therefore, maximum values were recorded in the plants grown in the saline soil amended with the highest levels of the *organo-mineral fertilizer* (20 and 25 g kg^{-1} soil). The acceleration of increased pool of proline resulted in an increase in the capacity of tolerance to salinity in the present study.

The increased tolerance to the salt-stress was manifested in terms of improved growth and photosynthetic pigments (total chlorophyll; Table 3) and the subsequent fruit yield (Table 4). The present investigation also shows that salinity stress caused a significant reduction in the chlorophyll concentration (in the control; Table 3). The decrease in chlorophyll content may be attributed to increased activity of chlorophyll-degrading enzyme chlorophyllase, under stress conditions (Reddy and Vora, 1986) and may by the inhibition of their biosynthesis and consequently may disturb the photosynthetic process. While, soil application with the *organo-mineral fertilizer* enables plants to overcome the adverse effects of salinity stress and consequently the increase in the content of total chlorophyll positively reflecting in the plant growth (Table 3). All levels of the *organo-mineral fertilizer* significantly increased the fruit yield of tomato plants due to the higher shoot and root DWs (Table 3), nutrient status of plants (Table 5) and activity of antioxidant enzymes (Table 6).

The favourable tomato yield obtained in our experiments may be due to the positive combined effect of calcium, rice bran and humic acid (the components of the novel *organo-mineral fertilizer*). Calcium has an antagonistic effect to the harmful effects of Na^+, whilst rice bran has high percentage of fibers (Table 1) which improves water retention through their high water holding capacity [7.33 (g g^{-1})], and can bind organic compounds (Schneeman, 1986). Humic acid (a component of the *organo-mineral fertilizer*) improves chemical properties of the soil by increasing the soil microorganisms which enhance nutrient status of the tomato plants (Table 5). It also promotes plant growth by its effects on ion transfer at the root level by activating the oxidation-reduction state of the plant growth medium and so increased absorption of nutrients by preventing precipitation in the nutrient solution. Furthermore, it enhances cell permeability, which in turn made for a more rapid entry of nutrients into root cells and so resulted in higher uptake of plant nutrients (Sayed et al., 2007). Jianguo et al. (1998) found that humic acid application improved the nutritional regulation of plants as indicated by changes in various physiological and biochemical indexes. These effects were associated with the function of hydroxyls and carboxyls in these compounds (Osman and Ewees, 2008). Taken together, these amendments enable plants to overcome the adverse effects of soil salinity.

An *organo-mineral fertilizer* level of 20 or 25 g kg^{-1} soil significantly reduced the Na content. This, increased the ratio of Ca:Na, thus generated more antagonistic effects to the harmful effects of Na^+ ions. The *organo-mineral fertilizer* may acts as a reservoir for nutrients, ensuring slow release to the substrate solution or directly to plant roots. It is a relatively abundant mineral resource (Table 1).

According to our results, antioxidant enzymes activities (Table 6) found to be identical with those reported by Lin and Kao (1999) on rice seedling, Sulochana et al. (2002) on groundnut, Ozturk and Demir (2003) on spinach and Jaleel et al. (2008) on *Dioscorea rotundata*. The decreased POX seems to indicate that this enzyme does not play a crucial role in defense mechanisms against oxidative stress, or that cooperation is activated between different antioxidant enzymes to establish a proper H_2O_2 balance when POX activity is reduced by salt toxicity (Chaparzadeh et al., 2004). Reduction of catalase (CAT) activity under salt stress may result in H_2O_2 accumulation and may be associated with its tolerance mechanism through signal transduction (Shim et al., 2003). The *organo-mineral fertilizer*, in this study, played an important role in increasing the activity of CAT and polyphenol oxidase (PPO) (Table 6) and consequently a weighty role in defense mechanisms against oxidative stress, especially salinity. This may be due to the fact that this novel *fertilizer* contains calcium, humic acid and rice bran which have many properties for overcoming the salt-stress effects.

5. Conclusion

Our results have shown that reclaimed saline soil (EC = 8.9 dS m^{-1}) treated with the novel *organo-mineral fertilizer* [a 2:10:1 (w/w/w) mixture of calcium sulphate, ground rice bran, and humic acid] significantly increased the growth and fruit yield of tomato plants grown in such soil. The *organo-mineral fertilizer*-treated plants had higher levels of N^+, K^+, and Ca^{2+}, and lower levels of Na^+ in their leaf tissues. In addition, it enhanced the levels of proline and chlorophyll, and the activities of antioxidant enzymes (CAT and PPO) under salinity stress conditions. The influence of the *organo-mineral fertilizer* on proline, chlorophyll and antioxidant enzymes activities was more pronounced under stress situation, suggesting that these parameters, at least in part, increased the tolerance of tomato plants to salinity stress, thus protected the photosynthetic machinery and plant growth.

References

Abbas, M.A., Younis, M.E., Shukry, W.M., 1991. Plant growth, metabolism and adaptation in relation to stress conditions. III. Effect of salinity on the internal solute concentrations in *Phaseolus vulgaris*. Journal of Plant Physiology 138, 722–729.

Abdel-Naby, A., El-Beltagy, M.S., Abou-Hadid, A.F., Helmy, Y.I., El-Abd, S.O., 2001. Effect of salinity stress modified by calcium application on potato plants. Egyptian Journal of Horticulture 28, 519–530.

Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenol-oxidase in *Beta vulgaris* L. Plant Physiology 24, 1–5.

Bates, L.S., Waldem, R.P., Teare, I.D., 1973. Rapid determination of free proline for water-stress studies. Plant and Soil 39, 205–207.

Bethkey, P.C., Drew, M.C., 1992. Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of Capsicum annum during progressive exposure to NaCl salinity. Plant Physiology 99, 219–226.

Chandlee, J.M., Scandalios, J.G., 1984. Analysis of variants affecting the catalase development program in maize scutellum. Theoretical and Applied Genetics 69, 71–77.

Chaparzadeh, N., Amico, M.L., Nejad, R.K., Izzo, R., Izzo, F.N., 2004. Antioxidative responses of *Calendula officinalis* under salinity conditions. Plant Physiology and Biochemistry 42, 695–701.

Delauney, A.J., Verna, D.P.S., 1993. Proline biosynthesis and osmoregulation in plants. The Plant Journal 4, 215–223.

Greenway, H.R., Munns, R., 1980. Mechanisms of salt tolerance in non-halophytes. Annual Reviews of Plant Physiology 31, 149–190.

Hafez, A.R., Mikkelsen, D.S., 1981. Colorimetric determination of nitrogen for evaluating the nutritional status of rice. Communications in Soil Science and Plant Analysis 12, 61–69.

Hanson, J.B., 1984. The function of calcium in plant nutrition. In: Tinker, P.B., Lauchi, A. (Eds.), Adv. Plant Nutr., vol. 1. Praeger, NY, USA, pp. 149–208.

Helrich, K., 1990. Official Methods of Analysis. Vitamin C (Ascorbic Acid), 15th ed. The Association of Official Analytical Chemists, Benjamin Franklin Station, Washington, DC, USA, pp. 1058–1059.

Jackson, M.L., 1967. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd, New Delhi, India. 144–197 and 326–338.

Jaleel, C.A., Gopi, R., Gomathinayagam, M., Panneerselvam, R., 2008. Effects of calcium chloride on metabolism of salt-stressed *Dioscorea rotundata*. Acta Biologica Cracoviensia Botanica 50, 63–67.

Jianguo, Y., Shuiying, Y., Yujuan, Z., Yingchang, S., Tiezhang, W., 1998. Influence of biochemical-humic acid on the physiological and biochemical indexes of apple trees infected with *Physalospora piricola*. Forest Research 11, 623–628.

Kumar, K.B., Khan, P.A., 1982. Peroxidase and polyphenol oxidase in excised ragi *Eleusine coracana* (V. PR 202) leaves during senescence. Indian Journal of Experimental Botany 20, 412–416.

LaHaye, P.A., Epstein, E., 1969. Salt toleration by plants: enhancement with calcium. Science 166, 395–396.

Leopold, A.C., Willing, R.P., 1984. Evidence for toxicity effects of salt on membranes. In: Staples, R.C., Toenniesen, E.H. (Eds.), Salinity Tolerance in Plants: Strategies for Crop Improvement. John Wiley and Sons, NY, USA, pp. 37–66.

Lin, C.C., Kao, C.H., 1999. NaCl induced changes in ionically bound peroxidases activity in roots of rice seedlings. Plant and Soil 216, 147–153.

Maas, E.V., 1986. Crop tolerance to saline soil and water. Proc. of the US Pak. Biosal. Res. Workshop. Bot. Dept., Karachi Univ., Pak, pp. 205–219.

Marschner, H., 1995. Mineral Nutrition of Higher Plants, 2nd ed. Acad. Press Publ, NY, USA, pp. 559–579.

Osman, A.S., Ewees, M.S.A., 2008. The possible use of humic acid incorporated with drip irrigation system to alleviate the harmful effects of saline water on tomato plants. Fayoum Journal of Agricultural Research and Development 22, 52–70.

Ozturk, L., Demir, Y., 2003. Effects of putrescine and ethephon on some oxidative stress enzyme activities and proline content in salt stressed spinach leaves. Plant Growth Regulation 40, 89–95.

Page, A.I., Miller, R.H., Keeny, D.R., 1982. Methods of Soil Analysis. Part II. Chemical and Microbiological Methods, 2nd ed. Amer. Soc. Agron, Madison, WI, USA, pp. 225–246.

Pitman, M.G., Läuchli, A., 2002. Global impacts of salinity and agricultural ecosystem. In: Läuchli, A., Lüttge, U. (Eds.), Salinity: environment-plants-molecules. Kluwer Academic, Dordrecht, The Netherlands, pp. 3–20.

Rady, M.M., 2011. Effect of 24-epibrassinolide on growth, yield, antioxidant system and cadmium content of bean (*Phaseolus vulgaris* L.) plants under salinity and cadmium stress. Scientia Horticulturae 129, 232–237.

Reddy, M.P., Vora, A.B., 1986. Changes in pigment composition, hill reaction activity and saccharide metabolism in bajra (*Pennisetum typhoides* S&H) leaves under NaCl salinity. Photosynthesis 20, 50–55.

SAS, 1996. SAS/STAT User's Guide. Version 6.12. SAS Institute, Cary, NC, USA.

Sayed, R.A., Ibrahim, M.A., Solaiman, B.M., 2007. Response of Valencia orange trees to foliar and soil application of humic acids under new reclaimed land conditions. The 3rd Conf. Sustain. Agric. Dev., Fac. Agric., Fayoum Univ., Egypt, pp. 259–274.

Scandalios, J.G., 1997. Molecular genetics of superoxide dismutases in plants. In: Scandalios, J.G. (Ed.), Oxidative Stress and the Molecular Biology of Antioxidant Defenses. Cold Spring Harbor Laboratory Press, Plainview, NY, USA, pp. 527–568.

Schneeman, B.O., 1986. Dietary fiber. Physical and chemical properties, methods of analysis, and physiological effects. Food Technology 40, 104–110.

Shim, I.S., Momose, H.Y., Yamamoto, A.A., 2003. Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plant. Plant Growth Regulation 39, 285–292.

Snedecor, G.W., Cochran, W.G., 1980. Statistical Methods, 7th ed. Iowa State Press, Ames, IA, USA, pp. 393–413.

Soussi, M., Ocand, A., Lluch, C., 1998. Effect of salt stress on growth, photosynthesis and nitrogen fixation in chickpea (*Cicer arietinum* L.). Journal of Experimental Botany 49, 1329–1337.

Sudhir, P., Murthy, S.D.S., 2004. Effect of salt stress on basic process of photosynthesis. Photosynthesis 42, 481–486.

Sulochana, C.H., Sreenivasa Rao, T.J.V., Savithramma, N., 2002. Effect of calcium on water stress amelioration through calmodulin and scavenging enzymes in groundnut. Indian Journal of Plant Physiology 7, 151–158.

Tan, K.H., 2003. Humic Matter in Soil and Environment, Principles and Controversies. Marcel Dekker, Inc, Madison, New York. ISBN: 0–8247–4272–9.

Wilde, S.A., Corey, R.B., Lyer, J.G., Voigt, G.K., 1985. Soil and Plant Analysis for Tree Culture, 3rd ed. Oxford and IBM Publishers, New Delhi, India, pp. 93–106.

Edited by AK Cowan