A Novel Organo-Mineral Fertilizer Can Alleviate Negative Effects of Salinity Stress for Eggplant Production on Reclaimed Saline Calcareous Soil

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Abstract

A novel organo-mineral fertilizer [a 5:2:1 (w/w/w) mixture of green waste compost, elemental sulphur (S) and humic acid (HA), respectively] was used as a soil amendment to study its effect on the growth, chlorophyll contents, chlorophyll fluorescence, and leaf nutrient status of eggplant (*Solanum melongena* L.) grown in reclaimed saline calcareous soil (ECe=6.47 dS m^{-1} and $CaCO_3$=15.63%). The organo-mineral fertilizer-treated plants showed increased growth, proline, chlorophyll and nutrient contents. A significant decrease in photosynthetic efficiency was observed on non-treated plants. The use of organo-mineral fertilizer led to an apparent decrease in soil salinity and soil bulk density. Total porosity, field capacity, and useful pores were greatly increased by organo-mineral fertilizer application. Therefore, we recommend using the tested organo-mineral fertilizer as a soil amendment for vegetables such as eggplant to overcome the adverse effects of salinity stress in newly reclaimed soils.

INTRODUCTION

Approximately one-third of the 260 million hectares of irrigated land worldwide, land that provides 40% of the global food production, is affected by salinization (United Nations, 2011). Countries such as Australia, Egypt, India, Pakistan, and the United States, all of which have substantial salinity and drainage problems affecting between 15 and 36% of their irrigated lands, are devoting substantial resources toward this problem (Schwabe et al., 2006).

Eggplant (*Solanum melongena*) is one of the most important crops grown in the summer season of Egypt. Eggplant 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. Strategies for alleviation of salt stress involve developing salt-resistant cultivars, leaching excess soluble salts from upper to lower soil depths, flushing soils that contain salt crusts at the surface, inoculating crops seeds and seedlings with various plant growth-promoting bacteria, and the application of soil amendments (Qadir et al., 2000; Bacilio et al., 2004 ; Rady, 2012). In recent years, lots of attention has been paid to the development of sustainable agriculture. Organo-mineral fertilizers have been applied as soil amendments to overcome the adverse effects of soil salinity, and to improve fertility and the structure of soil. They provide a simultaneous incorporation of all substances necessary to the soil and the plants in a single fertilizing step.

The application of humic substances (HS), the major component of soil organic matter, has been reported to have a positive effect on plant growth (Nardi et al., 2002; Arancon et al., 2006). The beneficial effects of HS on plant growth may be related to their indirect (increase of fertilizer efficiency or reducing soil compaction), or direct (improvement of the overall plant biomass) effects. Particularly, the increase of root growth is generally more apparent than that of the shoot (Vaughan and Malcom, 1985). Moreover, 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).

Sulphur (S) has a variety of vital functions within the plant, not only in the growth and development of higher plants, but also as it is associated with increased stress tolerance in plants (Nazar et al., 2011). Sulphur has been applied to many agricultural areas in order to improve the properties of saline and alkaline soils. Egyptian soils, characterized by a rise in pH, S has been reported to reduced soil pH values by the oxidation of S to sulphate through various species of soil microorganisms (El-Eweddy et al., 2005). Decreasing soil pH improves the availability of microelements (e.g., Fe, Zn, Mn, and Cu; Hetter, 1985) and positively modified the chemical properties of alkaline soils as well as increasing yields and related characteristics (Kineber et al., 2004). The main interest of the present study was to examine whether or not the organo-mineral fertilizer could counteract soil salinity effects and regulate growth by adjusting endogenous proline, nutritional status and photosynthetic apparatus.

MATERIALS AND METHODS

Plant Material, Growth Conditions, and Organo-Mineral Fertilizer Treatments

The novel organo-mineral fertilizer (OMF) used in the present study was generated by mixing green waste compost, elemental sulphur (S) and humic acid (HA) (Alpha Chemika, Mumbai, India) at a ratio of 5:2:1 (w/w/w). Table 1 summarizes the major components of the novel OMF used in these experiments. Saline calcareous soil was obtained from the Experimental Farm (a newly reclaimed saline soil with EC=6.47 dS m^{-1} and $CaCO_3$=15.63%) of the Faculty of Agriculture in South-east Fayoum (29°17'N; 30°53'E), Egypt. The main characteristics of the soil are given in Table 2.

Two greenhouse experiments were conducting during the summer season of 2012 one in April and the other in June, in which pots were filled with various soil: OMF mixtures, with the portion of the OMF ranging from 0 (control) to 30 g kg^{-1} soil (i.e., 0, 10, 20, or 30 g kg^{-1} soil). The experiments were arranged in a completely randomized design with these four experimental OMF treatments, 10 replications (10 pots) of each. Six-week-old eggplant seedlings (cv. 'Anan'), obtained from the Ministry of Agriculture Nurseries, Fayoum, Egypt, were transplanted separately, one transplant per pot, in 4 kg of each of the various soil: organo-mineral fertilizer OMF mixtures per pot. All plants were maintained in a greenhouse condition under a natural photoperiod. Initially transplants were watered with tap water for one week; thereafter a half-strength Hoagland's nutrient solution was applied every 2-4 days until the soil substrate was saturated, depending on the size of the plant. Six weeks after transplanting, various analyses were performed.

Growth and Physiological Measurements

Five individual plants were randomly chosen from each experimental treatment and evaluated for growth measurements. Leaf number, shoot length, shoot and root dry weight (DW) $plant^{-1}$, and leaf area were recorded. Dry weight measurements were carried out after drying to constant weight in a ventilated oven at 70°C. The actual leaf areas of the plants were measured by a hand-held digital planimeter (Planinx7, Tamaya Technics Inc., Tokyo, Japan).

Total chlorophyll was evaluated according to 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 were estimated using the rapid colorimetric method, as described 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 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.

Determination of leaf nitrogen contents in dry leaf samples was done 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:

$$N(\%) = 0{:}39 + 0{:}954 \times \text{Dye absorbed (g/100 g), and}$$

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

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^{++}) 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).

Chlorophyll fluorescence was measured on two different sunny days using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd., Kings Lynn, UK). One leaf (the same age) was chosen per plant from five plants from each treatment. A total of 10 measurements per treatment were made. Fluorescence measurements included: Maximum quantum yield of PS II F_v / F_m was calculated as $F_v / F_m = (F_m - F_o)/F_m$ (Maxwell and Johnson, 2000). Performance index of photosynthesis based on the equal absorption (PI_{ABS}) was calculated as reported by Clark et al. (2000). Physical and chemical properties, of the studied soil were conducted according to the methods and procedures outlined and described by Klute (1986) and Page et al. (1982).

Statistical Analysis

The data collected were of two experiments and subjected to a combined analysis using ANOVA procedures in GENSTAT statistical package (version 11) (VSN International Ltd, Oxford, UK). Difference between means was compared using least significant difference test (LSD) at 5% level.

RESULTS AND DISCUSSION

Plant Growth and Physiological Measurements

The organo-mineral fertilizer (OMF) used in the current study showed a positive effect on growth physiological parameters. Leaf number, shoot length, shoot and root dry weight (DW) $plant^{-1}$, and leaf area were significantly increased by the application of the OMF. The OMF level of 20 g kg^{-1} was more effective when compared to all other treatments (Table 3). These positive results may be attributed to the fact that the added HA and S significantly improved leaf contents of chlorophyll, free proline, and photosynthetic efficiency. OMF-treated plants had Fv/Fm values above 0.75, showing no stress, while the

control plants had lower Fv/Fm values. Significant increases were also observed in PI of OMF-treated plants as compared to the control (Table 4). The healthy metabolic status of the OMF-treated plants resulted in the healthy plant growth, in terms of increased shoot and root dry weight. This agreed with the earlier work done by Rady (2012). Mechanisms suggested to the stimulatory effect of HA hypothesize a 'direct' action on the plants, which is hormonal in nature, together with a positive 'indirect action' on the metabolism of soil microorganisms, the dynamics of uptake of soil nutrients, and soil physical condition (Arancon et al., 2006). A combined positive effect of HA and S were reported by Osman and Rady (2012), attributed to their effects on the soil, which led to an increase in organic matter content and bio-available nutrients, as a result of a reduction in soil pH.

Nutritional Status of the Plants

Nutrient content, Na content, and Ca:Na ratio are presented in Table 5. Statistically significant differences between the OMF-treated plants were noted for N, K and Ca contents, and Ca:Na ratio. The highest N, K and Ca contents, were obtained from plants treated with 30 g organo-mineral fertilizer kg^{-1} soil compared to the control plants. An OMF level of 20 g kg^{-1} soil reduced the Na content. This, increased the ratio of Ca:Na, thus generated more antagonistic effects to the harmful effects of Na^+ ions. The OMF may act as a reservoir for nutrient supplement, ensuring slow release to the substrate solution or directly to plant roots (Table 5).

Soil Physical and Chemical Properties

The effects of OMF on soil physical and chemical properties are illustrated in Table 6. Soil ECe and pH values tended to decrease with increasing the OMF levels. This could be attributed to the accumulation of active organic acids in soil and the cation exchange capacity of humic acid (200 to 500 milliequivalent per 100 grams at pH 7) which led to a reduction in pH values. The values of soil ECe tended to decrease probably due to the occurrence of the charged sites (i.e., COO^-) accounts for the ability of humic acid to chelate and retain cation in non-active forms.

Concerning the variations in soil bulk density among the different levels of OMF, data showed a gradual decrease in its values occurred with increasing OMF level, where the highest level (30 g kg^{-1} soil) gave the lowest soil bulk density values. This positive effect could be attributed to the pronounced content of organic colloidal particles, which plays an important role for modifying distribution pattern of pore spaces in soil. These findings are in agreement with those obtained by Batey (1990) who reported that soil bulk density was closely related to solid phase properties and pore spaces. Since the applied OMF possesses a positive effect for soil bulk density (i.e., reduced its value), hence it leads to increase total porosity of the soil. However the addition of OMF to saline calcareous soil encouraged the creation of medium and micro pores (i.e., water holding capacity and useful pores) between simple packing sand particles, and in turn increasing capillary potential. The abovementioned case is more attributed to an increase in soil moisture content at field capacity and then available water content. These findings are confirmed by Askar et al. (1994) who found that the addition of organic materials to soil greatly increased the water holding pores and decreased the area between the boundary lines (drying and wetting curve) of the hysteresis loops. In addition, such organic substances of humic acid have high ability to retain a pronounced content of water. These results are emphasized by Cheng et al. (1998) who reported that active organic acids decreased the loss of soil moisture, and in turn enhanced the water retention.

CONCLUSIONS

The current study has shown that reclaimed saline calcareous soil treated with the novel organo-mineral fertilizer [a 5:2:1 (w/w/w) mixture of green waste compost, elemental sulphur (S) and humic acid (HA), respectively] enhanced salinity tolerance in terms of increased growth and endogenous proline. The OMF -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 protected the photosynthetic machinery under salinity stress conditions.

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Tables

Table 1. Major components of the novel OMF.

Component	N	P	K	Ca	Fe	Mn	Zn	Humic acid	Total fiber	WHC[z] ($g\ g^{-1}$)
% (w/w)	2.87	0.65	3.02	8.20	0.38	0.19	0.12	12.37	31.50	8.19

[z] WHC= Water holding capacity.

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

Physical properties							
Sand	Silt	Clay	Texture	Bulk	FCz	WPy	Available
72.50	12.9	14.60	S. loam	1.53	18.51	10.47	8.04

Chemical properties								
pH (Soil/water extract 1:2.5)	ECe ($dS\ m^{-1}$)	N ($g\ kg^{-1}$)	P ($mg\ kg^{-1}$)	K ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	$CaCO_3$ %	OC* ($g\ kg^{-1}$)
7.86	6.47	0.7	16.8	79.8	83.9	6.4	15.63	8.6

*OC, organic content, [z]FC, Field capacity and [y]WP, Wilting point.

Table 3. Effect of the novel OMF application rate on leaf No., shoot length, shoot dry weight (DW) $plant^{-1}$, root DW $plant^{-1}$ and leaf area of 6-week-old eggplant. (n = 5).

OMF level ($g\ kg^{-1}$ soil)	Leaf No.	Shoot length (cm)	Shoot DW $plant^{-1}$ (g)	Root DW $plant^{-1}$ (g)	Leaf area (cm^2)
0	7.2b	9.5b	3.03c	1.30d	35.7b
10	8.4ab	11.5a	5.39ab	2.87c	53.4a
20	9.4a	11.6a	6.00a	4.48a	57.5a
30	9.6a	10.4ab	6.53a	3.84b	52.3a
LSD ($P = 0.05$)	1.9	1.36	1.06	0.64	11.9

Table 4. Effect of the novel OMF application rate on chlorophyll contents, free proline content in the leaf, leaf chlorophyll fluorescence ratio Fv/Fm, and performance index on absorption basis (PI_{ABS}) PI of 6-week-old eggplant. (n = 5).

OMF level (g kg^{-1} soil)	Chlorophyll content (mg g^{-1} FW)	Free proline content (µg g^{-1} DW)	Fv/Fm	PI
0	4.39a	20.62c	0.74c	3.40cd
10	5.42a	27.55b	0.77b	4.37bc
20	5.58a	37.89a	0.78b	5.01b
30	5.34a	38.26a	0.80a	8.04a
LSD (*P* = 0.05)	1.34	5.42	0.02	1.73

Table 5. Effect of the novel OMF application rate on several leaf nutrient contents, Na contents, and Ca:Na ratios of 6-week-old eggplant. (n = 5).

OMF 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
0	45.46b	0.08ab	4.47b	13.89c	3.06a	5.15b
10	59.37a	0.14a	4.74b	22.78b	2.34a	9.96ab
20	59.31a	0.06b	4.74b	25.33b	1.93a	13.39a
30	60.17a	0.08b	7.18a	29.89a	2.26a	13.26a
LSD (*P* = 0.05)	13.03	0.07	1.24	3.92	1.53	4.99

Table 6. Effect of the novel OMF application rate on ECe, Soil pH, bulk density, field capacity, available water, total porosity, and useful pores in the studied soil. (n = 5).

OMF level (g kg^{-1} soil)	ECe (dS m^{-1})	Soil pH	Bulk density (g cm^{-3})	FC %	Available water %	Total porosity %	Useful pores (28.8-0.19 u)
0	6.47a	7.86a	1.53a	18.51b	8.04b	33.35c	11.01a
10	6.21b	7.83b	1.50a	21.31ab	9.02b	35.76b	11.92a
20	5.89c	7.77c	1.46b	23.70ab	10.48ab	37.22a	13.11a
30	5.72d	7.74d	1.41c	26.63a	12.80a	38.06a	15.63a
LSD (*P* = 0.05)	0.12	0.01	0.04	7.70	3.28	1.29	6.03