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RESPONSE OF *Solanum melongena* L. SEEDLINGS GROWN UNDER SALINE CALCAREOUS SOIL CONDITIONS TO A NEW ORGANO-MINERAL FERTILIZER

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ABSTRACT

This study was planned to investigate the effect of soil application with an *organo-mineral fertilizer* [OMF; a 5:2:1 (w/w/w) mixture of green waste compost, elemental sulphur (S) and humic acid (HA), respectively] on physical and chemical characteristics of a reclaimed saline calcareous soil (ECe = 6.47 dS m^{-1} and $CaCO_3$ = 15.63%). In addition, growth, physiological and anatomical characters of eggplant (*Solanum melongena* L.) seedlings grown under the tested soil were investigated. The experiments were arranged in a completely randomized design with 4 experimental OMF treatments (i.e., 0, 10, 20, or 30 g kg^{-1} soil) with 10 replicates. The OMF-treated plants showed increased growth, concentrations of total soluble sugars, free proline, anthocyanin, K and Ca, ratios of Ca:Na and K:Na, and photosynthetic efficiency. An enhanced seedling anatomy was also observed with soil amendment by OMF. On the other hand, the OMF application led to a substantial reduction in soil salinity (ECe) and pH and an increase in field capacity and available water. The tested *organo-mineral fertilizer* could be recommended as a soil amendment for vegetable crops, including eggplant to overcome the adverse effects of salinity stress in newly-reclaimed soils.

Keywords: *Organo-mineral fertilizer*, Saline calcareous soil, Eggplant, Photosynthetic apparatus, Soil characteristics.

INTODUCTION

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* L.) is one of the most important crops grown in the summer season of Egypt. Eggplant is widely cultivated on newly-reclaimed soils in the country. However, most of these newly-reclaimed soils are affected by salinity with low fertility and a poor soil structure, hence plant growth and development is affected resulting low yield. In recent years, a lot of attention has been paid to the development of sustainable agriculture. For alleviating the salt stress, some strategies have been used (Qadir *et al.*, 2000; Bacilio *et al.*, 2004), including soil amendments. *Organo-mineral fertilizers* (OMFs) have been applied as soil amendments to overcome the adverse effects of soil salinity, and to improve fertility and the structure of soil (Rady and Osman, 2011; Rady, 2012). 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 in 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). Therefore, HS are useful for reclaimed, saline soils because they help plants to resist salinity and drought, help to establish a desirable environment for the development of microorganisms (Salman *et al.*, 2005) and stimulate plant growth (Osman and Rady, 2012). Under different soil conditions, the application of HS has been reported to improve plant growth and chemical composition, which are positively reflected in higher crop yields and quality (Mahmoud and Hafez, 2010; Rady, 2012).

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; Osman and

Rady, 2012). 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 have been reported to reduce soil pH values by the oxidation of S to sulphate through various species of soil microorganisms (El-Eweddy *et al.*, 2005). Decreasing soil pH improved 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). Adequate S nutrition improved photosynthesis and growth of plants, and has regulatory interactions with N assimilation (Scherer, 2008). S is required for protein synthesis, N assimilation, and is a structural constituent of several co-enzymes and prosthetic groups (Marschner, 1995). It is incorporated into organic molecules in plants and is located in thiol (–SH) groups in proteins (e.g., cysteine residues) and non-protein thiols (e.g., glutathione). The pool size of some thiol-containing compounds, especially reduced glutathione which is sensitive to an oxidizing environment, represents a potential modulator of the stress response (Szalai *et al.*, 2009).

Owing to considerable evidence of the negative effects of the newly-reclaimed saline calcareous soil 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 such saline calcareous soil (ECe = 6.47 dS m^{-1} and $CaCO_3$ = 15.63%) on eggplant seedlings. 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 eggplant seedling growth by adjusting the soluble sugars and proline contents, nutritional status, photosynthetic efficiency and positively modifying seedling anatomy under aforementioned stress.

MATERIALS AND METHODS

Plant material, growth conditions, and organo-mineral fertilizer treatments: The *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). The major components of OMF were: net humic acid content, 12.37% (w/w) on a dry weight basis; total N, 2.87% (w/w); total P, 0.65% (w/w); total K, 3.02% (w/w); total Ca, 8.20% (w/w); Fe, 0.38% (w/w); Mn, 0.19% (w/w); Zn, 0.12% (w/w); total fibers 31.50% (w/w); and water holding capacity, 8.19% g g^{-1}. 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 tested soil were measured according to the methods described by Page *et al*. (1982) and Klute (1986), and the values were: sand, 72.50% (w/w) on a dry weight basis; silt, 12.90% (w/w); clay, 14.60% (w/w); field capacity, 18.51%; available water, 8.04%; pH (soil/water extract, 1:2.5), 7.86; ECe, 6.47 dS m^{-1}; K, 79.8 mg kg^{-1}; Ca, 83.9 mg kg^{-1}; $CaCO_3$, 15.63%; and organic content, 8.60 g kg^{-1}.

Two greenhouse experiments were conducting during the summer season of 2012 one in April and the other in June for six weeks in each time, 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: OMF mixtures per pot. All plants were maintained in a controlled greenhouse condition (daytime temperature averaged 30.5° ± 2.0°C and night temperature averaged 19.1° ± 1.4°C. Daily relative humidity averaged 56 ± 6.4%) 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 soluble sugars concentration was assessed by washing 0.2 g leaves with 5 ml 70% ethanol and homogenizing with 5 ml 96% ethanol. The extract was centrifuged at 3500 × *g* for 10 min. The supernatant was collected and stored at 4°C (Irigoyen *et al.,* 1992). Freshly prepared anthrone (3 ml) was added to 0.1 ml supernatant. This mixture was incubated in hot water bath for 10 min. The absorbance was recorded at 625 nm using a UV- 160A UV Visible Recording Spectrometer, Shimadzu, Japan.

Leaf free proline contents were estimated using the rapid colourimetric 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.

Anthocyanin concentration was analyzed using the method described by Mancinelli (1990) with some modifications. Leaf samples were extracted with 1% HCl–MeOH for 24 h at room temperature in darkness with occasional shaking. The extracts were carefully decanted and their absorbance was measured at 530 and 657 nm. The formula $A_{530} - 0.25\ A_{657}$ was used to compensate for the absorption of chlorophyll degradation products. Anthocyanin concentration was expressed as mg of cyaniding 3-glucoside in 100 g of dry matter, using 29,600 as molecular extinction coefficient, and finally calculated as mg g^{-1} DW.

Leaf potassium ion (K^+), calcium ion (Ca^{++}) and sodium ion (Na^+) concentrations (in mg g^{-1} DW) were assessed using a Perkin-Elmer Model 52-A Flame Photometer (Page *et al.*, 1982), and their relations were calculated.

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 again at the end of experiment according to the methods and procedures outlined and described by Klute (1986) and Page *et al.* (1982).

Anatomical study: Stem and leaf samples of 6-week-old plants after transplanting were chosen for anatomical study. Samples were taken from the middle of fourth leaf from apex. They were killed and fixed in FAA solution (50 ml 95% ethyl alcohol + 10ml formalin + 5 ml glacial acetic acid + 35 ml distilled water) for 48 h. Thereafter, they were washed in 50% ethyl alcohol, dehydrated and cleared in tertiary butyl alcohol series, embedded in paraffin wax of 54–56 °C m.p. Cross sections, 20 µ thick, were cut by a rotary microtome, adhesived by Haupt's adhesive and stained with the crystal violet-erythrosin combination (Sass, 1961), cleared in carbol xylene and mounted in Canada balsam. The sections observed and documented using an upright light microscope (AxioPlan, Zeiss, Jena, Germany). Measurements were done, using a micrometer eyepiece and average of five readings were calculated.

Determination of soil physical and chemical properties: Soil field capacity (FC) and available water were conducted according to the procedures outlined and described by Klute (1986). Field capacity (water content at ⅓ bar) was measured and calculated using tension table when applied tension of ⅓ bar, and permanent welting point (PWP; water content at 15 bar) was measured using the pressure membrane device in the laboratory. The available water capacity (AWC) of a soil is the amount of water retained in the soil reservoir that can be removed by plants. This was estimated by the difference in water content between field capacity and permanent welting point as follows:

AWC = FC – PWP

Soil pH and electrical conductivity (ECe) were measured as outlined by Page *et al.* (1982). Soil pH values were estimated in the saturated paste of the tested soil using Beckman pH meter. Total soluble salts as ECe (dS m^{-1}) were measured in the soil extract using Metler EC meter.

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

Plant growth and physiological measurements: The organo-mineral fertilizer (OMF) used in the current study showed a positive effect on growth and physiological parameters (Tables 1 and 2). Leaf number, shoot length, shoot and root dry weight (DW) $plant^{-1}$ and leaf area were significantly increased by the application of the OMF compared to those in the control plants which not received OMF (Table 1). In addition, leaf concentration of free proline, leaf chlorophyll fluorescence (Fv/Fm) and performance index were significantly increased, however, leaf total soluble sugars and anthocyanin concentrations decreased as a result of OMF application compared to those in the control plants which not received OMF (Table 2). The OMF level of 20 g kg^{-1} was the most effective when compared to all other levels.

Nutritional status of the plants: The concentrations of K, Ca, Na and their relations are presented in table 3. Statistically significant differences between the OMF-treated plants were noted for K, Ca and Na

concentrations, and the ratios of Ca:Na, K/Na and Ca+K:Na. The highest K and Ca concentrations, and K:Na and Ca+K:Na were observed in plants treated with 30 g OMF kg^{-1} soil, while the highest Ca:Na ratio was noted with plants treated by 20 g OMF kg^{-1} soil compared to the control plants. On the other hand, the lowest Na concentration was obtained from plants treated with 20 g OMF kg^{-1} soil.

Stem and leaf anatomy: Table 4 and Figure 1 showed that, application of OMF to *Solanum melongena* in the growth medium with at 10, 20 and 30 g kg^{-1} soil increased stem diameter by 9.26%, 20.00% and 7.04%, respectively compared to the control plants, which grown in soil without OMF application. The increase in stem diameter was due to the substantial increase in cortex thickness (5.26%, 13.16% and 5.26%, respectively), diameter of vascular cylinder (8.51%, 26.33% and 12.50%, respectively) and pith diameter (1.07%, 15.79% and 14.04%, respectively) compared to those in untreated plants. The thickness of leaf blade, including both palisade and spongy tissues, was markedly increased by 53.85%, 38.46% and 23.08% as a result in soil application with OMF at 10, 20 and 30 g kg^{-1} soil, respectively (Table 5 and Figure 2). In addition, midvein dimension increased by 26.34 × 13.64%, 11.03 × 3.79% and 14.85 × 6.06%, respectively. This increase was accompanied by an increase in vascular bundle dimension by 19.23 × 17.65%, 7.69 × 3.53% and 7.69 × 3.53%, respectively compared to those in the control plants grown in untreated soil.

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. The reductions in ECe and pH were 11.6% and 1.5%, respectively with the application of OMF level at 30g kg^{-1} soil. On the other hand, the variations in soil field capacity (FC%) and available water content (AWC%) among the different levels of OMF, data showed a gradual increase in their values occurred with increasing OMF level, where the highest levels (30g kg^{-1} soil) gave the highest FC% and AWC% (43.9% and 59.2%) compared to the control.

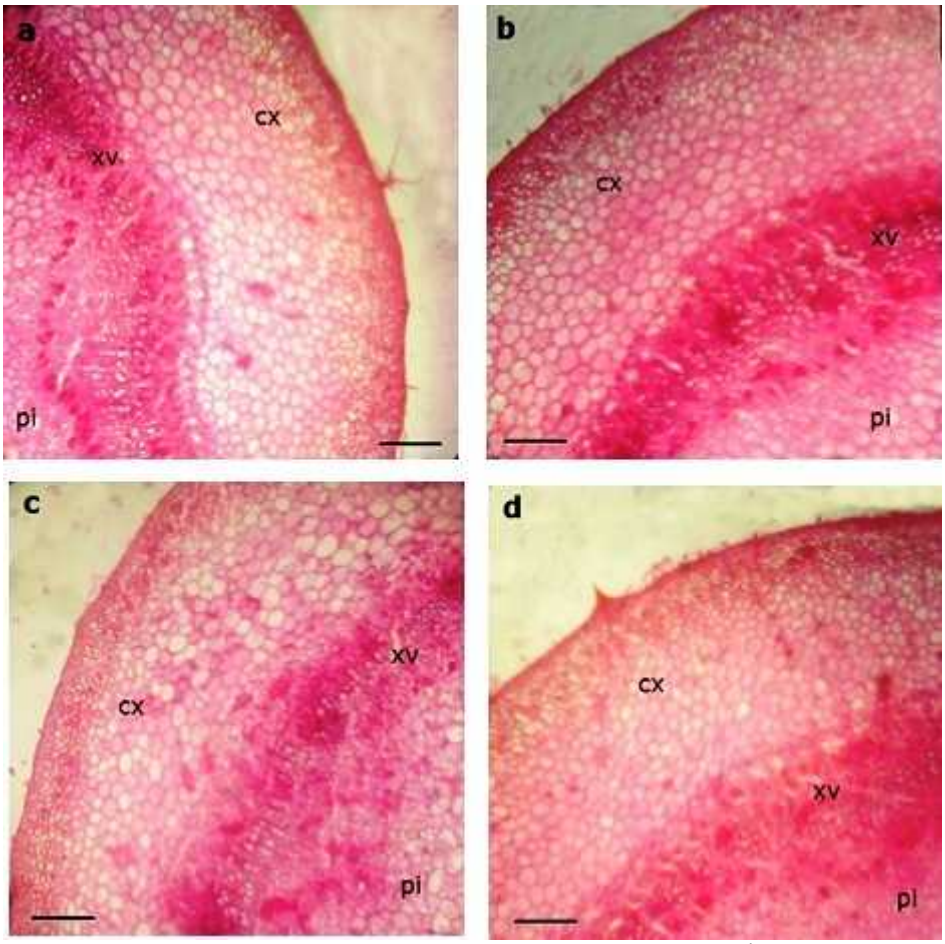

Fig. 1. Transverse section of *S. melongena* stem treated with: control (b) 10 g kg^{-1} OMF. (c) 20 g kg^{-1} OMF. (d) 30 g kg^{-1} OMF. (mv= medvien, vb= vascular bundle, pa= palisade tissue and sp= spongy tissue). Scale bars = 100 µm

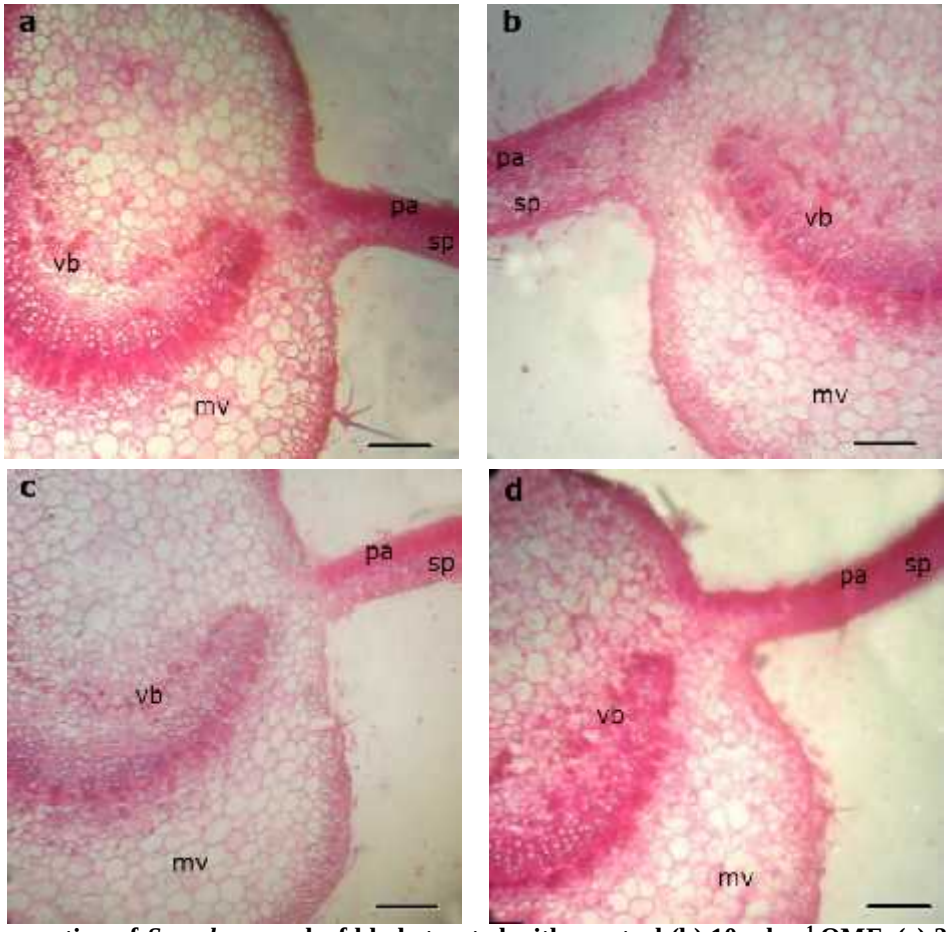

Fig. 2. Transverse section of *S. melongena* leaf blade treated with: control (b) 10 g kg $^{-1}$ OMF. (c) 20 g kg $^{-1}$ OMF. (d) 30 g kg $^{-1}$ OMF. (mv= medvien, vb= vascular bundle, pa= palisade tissue and sp= spongy tissue). Scale bars = 100 µm.

Table 1. Effect of the 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 = 20).

OMF level (g kg^{-1} soil)	Leaf No.	Leaf area (cm2)	Shoot length (cm)	Shoot DW plant^{-1} (g)	Root DW plant^{-1} (g)
0	7.2 ± 0.49b	35.7 ± 4.10c	9.5 ± 0.50c	3.03 ± 0.47c	1.30 ± 0.17d
10	8.4 ± 0.40ab	53.4 ± 3.17ab	11.5 ± 0.65a	5.39 ± 0.50b	2.87 ± 0.13c
20	9.4 ± 0.51a	57.5 ± 4.45a	11.6 ± 0.29a	6.00 ± 0.18ab	4.48 ± 0.17a
30	9.6 ± 0.75a	52.3 ± 4.02b	10.4 ± 0.29b	6.53 ± 0.30a	3.84 ± 0.29b

Different letters within columns indicate significant differences between entries ($P \leq 0.05$).

Table 2. Effect of the OMF application rate on leaf concentrations of total soluble sugars (TSS), free proline and anthocyanin, leaf chlorophyll fluorescence ratio (Fv/Fm), and performance index on absorption basis (PI_{ABS}) PI of 6-week-old eggplant (n = 20).

OMF level (g kg^{-1} soil)	TSS (mg g^{-1} DW)	Free proline (µg g^{-1} DW)	Anthocyanin (mg g^{-1} DW)	Fv/Fm	PI
0	2.24 ± 0.11a	1.65 ± 0.14c	0.28 ± 0.01a	0.74 ± 0.02c	3.40 ± 0.34cd
10	1.76 ± 0.15b	2.20 ± 0.24b	0.25 ± 0.01b	0.77 ± 0.01b	4.37 ± 0.52bc
20	1.70 ± 0.15b	3.03 ± 0.07a	0.20 ± 0.01c	0.78 ± 0.01b	5.01 ± 0.46b
30	1.65 ± 0.06b	3.06 ± 0.08a	0.21 ± 0.01d	0.80 ± 0.01a	8.04 ± 0.91a

Different letters within columns indicate significant differences between entries ($P \leq 0.05$).

Table 3. Effect of the OMF application rate on leaf concentrations of K, Ca and Na, and the ratios of Ca:Na, K:Na and Ca+K:Na of 6-week-old eggplant (n = 20).

OMF level (g kg^{-1} soil)	K (mg g^{-1} DW)	Ca (mg g^{-1} DW)	Na (mg g^{-1} DW)	Ca:Na ratio	K:Na ratio	K+Ca:Na
0	4.47 ± 0.25b	13.89 ± 2.42c	3.06 ± 0.77a	5.15 ± 1.67c	1.65 ± 0.40d	6.80 ± 2.04c
10	4.74 ± 0.90b	22.78 ± 0.68b	2.34 ± 0.21b	9.96 ± 1.23b	1.99 ± 0.21c	11.95 ± 1.08b
20	4.74 ± 0.71b	25.33 ± 2.22b	1.93 ± 0.14c	13.39 ± 2.06a	2.48 ± 0.43b	15.87 ± 2.26a
30	7.18 ± 0.52a	29.89 ± 0.78a	2.26 ± 0.08b	13.26 ± 0.42a	3.18 ± 0.19a	16.44 ± 0.29a

Different letters within columns indicate significant differences between entries ($P \leq 0.05$).

Table 4. Effect of the OMF application rate on the stem anatomy of 6-week-old eggplant.

OMF level (g kg^{-1} soil)	stem diameter (µ)	Cortex thickness (µ)	Vascular cylinder diameter (µ)	Pith diameter (µ)
0	6750	950	4700	3563
10	7375	1000	5100	3600
20	8100	1075	5938	4125
30	7225	1000	5288	4063

Table 5. Effect of the OMF application rate on the leaf anatomy of 6-week-old eggplant

OMF level (g kg^{-1} soil)	leaf blade thickness (µ)	midvein length (µ)	midvein width (µ)	Vascular bundle length (µ)	Vascular bundle width (µ)
0	325	3265	3300	650	2125
10	500	4125	3750	775	2500
20	450	3625	3425	700	2200
30	400	3750	3500	700	2125

Table 6. Effect of the OMF application rate on ECe, pH, field capacity (FC%), and available water % of the studied soil (n = 20).

OMF level (g kg^{-1} soil)	ECe dS m^{-1}	pH	FC%	Available water %
0	6.47 ± 0.06a	7.86 ± 0.01a	18.51 ± 3.98c	8.04 ± 1.48c
10	6.21 ± 0.01b	7.83 ± 0.01a	21.31 ± 0.06b	9.02 ± 0.23c
20	5.89 ± 0.06c	7.77 ± 0.00b	23.70 ± 1.83b	10.48 ± 0.93b
30	5.72 ± 0.03d	7.74 ± 0.01b	26.63 ± 0.91a	12.80 ± 0.67a

Different letters within columns indicate significant differences between entries ($P \leq 0.05$).

DISCUSSION

This study introduces a potential useful soil amendment, a new fertilizer (*organo-mineral fertilizer*; OMF) consisting of a 5:2:1 mixture of green waste compost, elemental S and humic acid, respectively. Positive results were obtained as a result in the application of OMF, which may be attributed to the fact that the added humic acid (HA) and S ameliorated the harmful effects of soil salinity (Table 4). These results reflected improvement in leaf concentrations of total soluble sugars, anthocyanin, free proline and photosynthetic efficiency (Table 2). Soluble sugars increased in seedlings subjected to the adverse conditions of untreated soil, but decreased when soil received the OMF. This result may be attributed to the reduction in the ECe of the soil solution (Table 6) and consequently the reduction in osmotic stress. Soluble sugars contribute to osmotic adjustment and can directly or indirectly modulate the expression of genes involved in metabolic processes, storage functions, and defence (Hebers and Sonnewald, 1998). In addition, the accumulation of proline is one of the most frequent changes induced by salinity, although there is controversy concerning whether its accumulation is a stress resistance mechanism or a mere indicator of the existence of stress (Thakur and

Sharma, 2005). Proline accumulation, apart from being important in osmoregulation and acting as a nitrogen reserve, may reduce stress in itself, acting as a substrate for respiration and generating energy that could be inverted for plant recovery from stress (Tarakcioglu and Inal, 2002). Moreover, 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 treatment of plant medium with the OMF enhanced the level of proline under its adverse condition. The acceleration of increased pool of proline resulted in an increase in the capacity of tolerance to the adverse conditions of the soil under study. Anthocyanin is utilized as a precursor with cytoprotective function in the secondary metabolism (El-Saht, 2001). Thus, the increased level of anthocyanin in untreated plants indicates an index for a good mechanism of plant resistance towards the changes in the environmental conditions (Winkel-Shirley, 2001). Winkel-Shirley (2002) reported that anthocyanin localizes in root, stem and leaf tissues to allow the plant to develop resistance to a number of environmental stresses. The 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 the OMF-treated plants compared to the control plants (Table 2). 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. However, the increased ratios of K:Na, Ca:Na and Ca+K:Na 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, thus, the OMF led to higher concentrations of nutrients, including elemental K. This element leads to a corresponding increase in chlorophyll fluorescence (Table 2) which can serve as an indicator of the stress induced by alterations in the balance of endogenous hormones (Marschner, 1995). Humic acid, a component of the OMF, improved chemical properties of the soil by increasing the soil microorganisms which enhanced nutrient status of the plants. Humic acid promoted 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. It also enhanced 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 (Rady, 2011). 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 Rady, 2012).

The adverse conditions of the OMF-untreated soil were negatively reflected in the anatomical structure of the eggplant leaves and stems. Stem diameter and blade thickness of plants obtained from this soil were greatly reduced. This may have resulted in a reduction in cortex and mesophyll cells in stem and leaf, respectively. A thinner leaf blade resulting from smaller palisade parenchyma cells and a reduction in the number and size of the spongy parenchyma cells also represents the main anatomical changes observed in OMF-untreated plants. However, OMF-treated plants had a considerable improvement in the anatomical attributes and showed the highest stem diameter and leaf blade thickness under stress conditions of the tested soil. The OMF played a prominent role in enhancing plant growth, some osmoprotectants and nutritional status (Tables 1-3). It could also play an indirect role in various physiological processes, including cell division and expansion, xylem differentiation and stem elongation. Disturbances in cell division and elongation could occur due to disturbances in plant water relations and mineral nutrition and also slowing down of basic metabolic processes (Seregin and Ivanov, 2001).

Soil ECe and pH values decreased 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. Soil field capacity (FC%) and available water content (AWC%) increased with increasing OMF level, where the highest levels (30g kg^{-1} soil) gave the maximum FC% and AWC%. These findings are confirmed by Askar *et al.* (1994) who found that the addition of organic materials

to soil significantly 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. The OMF has high percentage of fibers, which improved water retention through their high water holding capacity (8.19 g g^{-1}), and can bind organic compounds. 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.

Conclusion: It may be concluded that reclaimed saline calcareous soil treated with the OMF [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 total soluble sugars, anthocyanin and proline. The OMF-treated plants had higher levels of K^+ and Ca^{2+}, lower levels of Na^+ and higher ratios of their relations in leaf tissues. The enhanced levels of total soluble sugars, proline and anthocyanin indicate indices for good mechanisms of plant resistance, thus protection of the photosynthetic machinery under salinity and calcareous stress conditions.

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