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Effects of the application of 'cash' on the growth, fruit yield, and nutrient status of tomato (*Solanum lycopersicum* L.) grown in reclaimed saline soil

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SUMMARY
The effects of 'cash' [a novel 2:5:1 (w/w/w) mixture of calcium sulphate, ground sunflower heads, and humic acid] as a soil amendment on the growth, fruit yield, and leaf nutrient status of tomato (*Solanum lycopersicum* L.) grown on reclaimed saline soil (EC = 9.4 dS m^{-1}) were investigated. A glasshouse experiment was performed in a randomised complete block design with six treatments (0, 10, 20, 30, 40, or 50 g 'cash' kg^{-1} soil) each with four replicates. The results indicated that 'cash' increased both the shoot dry weight (DW) $plant^{-1}$ and the root DW $plant^{-1}$, the free proline contents of leaves, and leaf chlorophyll contents. The use of 'cash' also increased the number of fruit $plant^{-1}$, fruit yield pot^{-1}, and fruit vitamin C contents, although total soluble solids (TSS) contents were not affected. The contents of nitrogen (N), potassium (K), and calcium (Ca), and the Ca:Na ratios of leaf tissues increased with all application rates of 'cash'. Leaf phosphorus (P) contents showed no response to any applied level of 'cash'. Leaf sodium (Na) contents declined gradually with an increase in the level of 'cash' applied to the soil. We concluded that 'cash' has a pronounced positive effect on the growth and fruit yield of tomato plants grown in reclaimed saline soil. 'Cash' therefore has the potential to be used as a soil amendment for vegetable crops such as tomato to overcome the adverse effects of salinity in newly-reclaimed soils.

Tomato (*Solanum lycopersicum* L.) production has a major role in global horticulture, ranking second only 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, have low fertility, and a poor soil structure. Saline conditions disrupt several physiological processes in plants, leading to a general reduction in growth and yield (Greenway and Munns, 1980). 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 an 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). 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, natural 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.

The application of humic acid as an organic soil amendment, alone 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 soil properties such as aggregation, aeration, permeability, water-holding capacity, micronutrient uptake and availability, and by decreasing the uptake of some toxic elements (Tan, 2003).

Calcium is considered to be an important factor for the maintenance of cell membrane integrity and the regulation of ion-transport. Ca^{2+} ions are essential for K^+ *vs.* Na^+ ion selectivity and membrane integrity (Hanson, 1984). Elevated concentrations of Ca^{2+} ions in the nutrient solution mitigated the adverse effects of salinity by inhibiting the uptake of Na^+ ions (Greenway and Munns, 1980) and by reducing ion leakage through membranes (Leopold and Willing, 1984). LaHaye and Epstein (1969) postulated that Ca^{2+}/Na^+ ion interactions took place at the plasmalemma. They suggested that Na^+ ions acted by displacing Ca_2^+ ions from membranes, leading to increased membrane permeability and higher intracellular Na^+ ion concentrations.

The objective of this investigation was to assess the effects of using different amounts of 'cash' [a novel 2:5:1

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TABLE I
Major components of the 'cash'‡ used in these experiments

Major component	Content [% (w/w)]
N	2.72
P	0.63
K	2.98
Ca	8.16
Fe	0.33
Mn	0.19
Zn	0.12
Humic acid	12.50
Total fibre	31.78
Water holding capacity (g g^{-1})	7.45

‡'Cash' = 2:5:1 (w/w/w) calcium sulphate, ground sunflower heads, and humic acid.

(w/w/w) mixture of calcium sulphate, ground sunflower heads, and humic acid] on the growth, fruit yield, and nutrient uptake of tomato plants grown in reclaimed saline soil (EC = 9.4 dS m^{-1}).

MATERIALS AND METHODS

The 'cash' used in this research was generated by mixing calcium sulphate ($CaSO_4$), ground sunflower heads (a waste by-product of the sunflower crop), and humic acid (Alpha Chemika, Mumbai, India) at a ratio of 2:5:1 (w/w/w). These proportions of ingredients gave the best results among several proportions that were examined in preliminary studies on 'cash' (data not shown) and were therefore selected for this study. Table I summarises the major mineral and organic components of the 'cash' used in these experiments. The soil used in this research was obtained from the Experimental Farm (a newly-reclaimed saline soil with an EC = 9.4 dS m^{-1}) of the Faculty of Agriculture in Southeast Fayoum (29° 17'N; 30° 53'E), Egypt. The main characteristics of the soil according to Wilde *et al*. (1985) are given in Table II.

A greenhouse experiment was initiated on 6 and 1 September 2009 and 2010, respectively. Pots were filled with various soil: 'cash' mixtures, with the portion of 'cash' ranging from 0 (control) to 50 g kg^{-1} soil (i.e., 0, 10, 20, 30, 40, or 50 g kg^{-1} soil). The experiment was arranged in a randomised complete block design with four replicates for each of these six experimental 'cash' treatments and five pots per replication. Five-week-old tomato seedlings (cv. Saria), obtained from the Ministry of Agriculture Nurseries, Cairo, Egypt, were transplanted separately in 12 kg pot^{-1} of each of the various soil: 'cash' mixtures. 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 also 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).

Seven-week-old tomato plants were used for determinations of shoot dry weight (DW) $plant^{-1}$ and root DW $plant^{-1}$. The fourth true-leaf on each plant was used to determine 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 tested from each experimental treatment. 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.

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 was terminated in an ice-bath. Each 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 a % DW basis.

Leaf chlorophyll contents (in mg g^{-1} FW) were determined using the colorimetric method of Arnon (1949). 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 then shaken 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

TABLE II
Some physical and chemical characteristics of the reclaimed saline soil used in these experiments

Composition [% (w/w)]												
Clay	Loam	Sand	pH	EC (dS m^{-1})	OC‡ (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})
12.3	16.2	71.5	7.8	9.4	8.6	0.7	16.8	79.8	83.9	5.8	3.2	1.8

‡OC, organic content.

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 (0.2 g).

The molybdenum-reduced molybdophosphoric blue colour method (Jackson 1967) in sulphuric acid (with reduction to exclude arsenate), was the method used for phosphorus determinations (in mg g^{-1} DW) in leaf tissue. In addition, sulphomolybdic acid (molybdenum blue), diluted sulphomolybdic acid, and 8% (w/v) sodium bisulphite-H_2SO_4 solution were used as reagents.

Leaf potassium ion (K^+) 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).

The number of fruit $plant^{-1}$ and the fruit yield pot^{-1} were recorded at the end of the experiment. Ripe fruit were used for determining vitamin C and TSS contents.

The vitamin C contents of fruit (in 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 of 0.3 M metaphosphoric acid/1.4 M acetic acid solution. The extracts were centrifuged for 15 min at 7,000 × *g*, filtered through six layers of cheese-cloth, and made up to 100 ml with the 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 [expressed in % (w/v)] of ripe tomato fruit were measured using an ATC-1E hand-held refractometer (Atago, Kyoto, Japan) at 20°C.

All data were subjected to ANOVA using MSTAT-C. Combined analysis of the data of two seasons (2009 and 2010) was conducted and significant differences between means were compared using the Least Significant Differences (LSD) procedure at the $P = 0.05$ level, as illustrated by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

All six levels of 'cash' application increased shoot and root DWs in tomato (Table III). A 'cash' level of 50 g kg^{-1} soil increased mean shoot DWs by 218% to 19.50 g and root DWs by 187% to 9.24 g, compared to 6.13 g and 3.22 g in the controls, respectively. These positive results were obtained as a result of 'cash' overcoming the harmful effects of soil salinity by virtue of the added Ca^{2+} ions (Greenway and Munns, 1980) and the humic acid (Osman and Ewees, 2008) present in 'cash'. The application of 'cash' also increased leaf free proline and chlorophyll contents significantly, especially at a rate of 50 g 'cash' kg^{-1} soil. Proline and chlorophyll contents increased to 45.54 µg g^{-1} DW and 1.75 mg g^{-1} FW compared to 26.32 µg g^{-1} DW and 0.66 mg g^{-1} FW in the non-amended controls, respectively (Table III). The mechanism by which humic acid (a component of 'cash') stimulated plant growth may be similar to that of plant growth regulators. Humic substances include auxins, or can function as auxins, and thus affect plant metabolism in a positive manner (Osman and Ewees, 2008). This may explain the positive effect of 'cash' on leaf proline and chlorophyll contents under saline soil conditions, which were then positively reflected in the growth of the tomato plants. Humic acids lead to higher rates of uptake of elemental K and therefore 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).

Our results showed that all levels of 'cash' increased the average number of fruit $plant^{-1}$ and the fruit yield pot^{-1}. However, 'cash' rates of 40 or 50 g kg^{-1} soil were more effective than all others (Table IV). Using 'cash' as a soil conditioner led to a significant increase in the yield of tomato fruit. Compared to the non-amended control, the addition of 'cash' in our greenhouse experiment resulted in 131% and 318% increases in fruit number $plant^{-1}$ and fruit yield pot^{-1}, respectively. The application of cash also significantly increased vitamin C contents, especially at the rate of 50 g kg^{-1} soil (Table IV), whereas TSS contents showed no significant differences between any level of 'cash' and the non-amended controls. All levels of 'cash' significantly increased the yields of tomato plants. This may be due to the higher shoot and root DWs (Table III), which positively affected tomato yield.

The favourable tomato yields obtained in our experiment may be due to the positive combined effects of Ca^{2+} ions, ground sunflower heads, and humic acid. Calcium ions have an antagonistic effect on the harmful effects of Na^+ ions, while ground sunflower heads have a high percentage of fibre (Table I) which improves water retention through their high water-holding capacity (7.45 g g^{-1}), and which can also bind organic compounds (Schneeman, 1986). Humic acid improves the chemical properties of soil because it increases the level of soil microorganisms which enhances nutrient cycling and has

TABLE III

Effect of the application rate of 'cash'‡ on shoot dry weight (DW) $plant^{-1}$, root DW $plant^{-1}$, leaf free proline and leaf chlorophyll contents of 7-week-old tomato plants

'Cash' level (g kg^{-1} soil)	Shoot DW (g $plant^{-1}$)	Root DW (g $plant^{-1}$)	Free proline content (µg g^{-1} DW)	Chlorophyll content (mg g^{-1} FW)
0	6.13†	3.22	26.32	0.66
10	10.74	5.98	24.92	0.96
20	12.26	6.64	24.44	1.10
30	12.65	6.85	24.87	1.14
40	14.68	9.18	35.30	1.32
50	19.50	9.24	45.54	1.75
LSD ($P = 0.05$)	1.54	1.09	6.85	0.14

†Values are means (n = 8).
‡'Cash' = 2:5:1 (w/w/w) calcium sulphate, ground sunflower heads, and humic acid.

TABLE IV

Effect of the application rate of 'cash'‡ on fruit number $plant^{-1}$, fruit yield pot^{-1}, and vitamin C and TSS contents of 7-week-old tomato plants

'Cash' level (g kg^{-1} soil)	Fruit number $plant^{-1}$	Fruit yield pot^{-1} (kg)	Vitamin C (mg 100 g^{-1} juice)	TSS (%)
0	10.65‡	0.49	17.16	4.12
10	17.21	1.52	24.81	4.08
20	20.11	1.56	26.70	4.09
30	20.51	1.55	25.19	4.16
40	23.09	1.89	27.89	4.16
50	24.60	2.05	35.43	4.21
LSD ($P = 0.05$)	1.74	0.70	3.16	NS§

‡'Cash' = 2:5:1 (w/w/w) calcium sulphate, ground sunflower heads, and humic acid.
†Values are means (n = 12).
§NS, not significant.

TABLE V
Effect of the application rate of 'cash'[‡] on leaf nutrient contents, Na contents, and Ca:Na ratios in 7-week-old tomato plants

'Cash' 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	10.24[†]	0.15	11.32	5.32	21.87	0.24
10	10.66	0.15	12.00	5.50	18.23	0.30
20	11.15	0.14	12.90	6.83	14.02	0.49
30	11.67	0.16	13.46	7.45	8.87	0.84
40	11.77	0.15	13.93	8.39	6.76	1.24
50	12.09	0.16	14.48	8.66	4.06	2.13
LSD (P = 0.05)	0.62	NS[§]	0.90	0.29	2.04	0.18

[‡]'Cash' = 2:5:1 (w/w/w) calcium sulphate, ground sunflower heads, and humic acid.
[†]Values are means (n = 8).
[§]NS, not significant.

a positive effect on photosynthesis and growth (Sayed *et al.*, 2007). Taken together, these combined soil amendments ('cash') enable plants to overcome many of the adverse effects of soil salinity.

The nutrient contents, Na^+ ion contents, and Ca:Na ratios of leaves are presented in Table V. Statistically significant differences between the various 'cash' treatments were noted for N^+, K^+, and Ca_2^+ ion contents, and Ca:Na ratios. The highest N^+, K^+, and Ca_2^+ ion contents, and Ca:Na ratio (12.09 mg g^{-1} DW, 14.48 mg g^{-1} DW, 8.66 mg g^{-1} DW, and 2.13, respectively) were obtained from the 50 g 'cash' kg^{-1} soil treatment compared to the corresponding values in the controls (10.24 mg g^{-1} DW, 11.32 mg g^{-1} DW, 5.32 mg g^{-1} DW, and 0.24, respectively). The use of 'cash' had no effect on P contents (Table V). All levels of 'cash' significantly reduced the Na^+ ion contents of tomato leaves (Table V). 'Cash' at 50 g kg^{-1} soil reduced Na^+ ion contents by 81% to 4.06 mg g^{-1} DW, compared to 21.87 mg g^{-1} DW in the controls. This increased the Ca:Na ratio by 788% to 2.13, compared to 0.24 in the controls, and thus mitigated the harmful effects of Na^+ ions.

'Cash' may act as a reservoir for nutrients, ensuring their slow release into the substrate solution or directly to plant roots. It also represents a relatively abundant source of minerals (Table I).

Humic acid (a component of 'cash') improves the chemical properties of soil because it increases soil microorganisms which enhance nutrient cycling (Sayed *et al.*, 2007). It also promotes plant growth through its effects on ion transfer at the root level by activating the oxidation-reduction state of the medium and increasing the absorption of nutrients by preventing their precipitation in the nutrient solution. Furthermore, humic acid enhances cell permeability, which facilitates the entry of nutrients into root cells and results in a higher uptake. Jianguo *et al.* (1998) found that the application of humic acid improved nutritional regulation in plants, as indicated by changes in various physiological and biochemical indices. These effects were associated with the roles of hydroxyl and carboxyl compounds (Osman and Ewees, 2008).

Our results have shown that reclaimed saline soil (EC = 9.4 dS m^{-1}) treated with 'cash' [a 2:5:1 (w/w/w) mixture of calcium sulphate, ground sunflower heads, and humic acid] significantly increased the growth and yield of tomato plants. In addition, treated plants had higher levels of N^+, K^+, and Ca_2^+ ions, and lower levels of Na^+ in their leaf tissues.

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