Integrative Potassium Humate and Biochar Application Reduces Salinity Effects and Contaminants, And Improves Growth and Yield of Eggplant Grown Under Saline Conditions

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Abstract

In agriculture sector, soil salinity is one of the major problems that limit plant performance, particularly in arid and semiarid regions, including Egypt. The effect of potassium humate (KH) and casuarina biochar (Bch), applied singly or in integration, on plant performance, physio-biochemical attributes and antioxidant, and contents of contaminants of *Solanum melongena* plants grown under salt stress (EC = 6.96 – 7.08 dS m^{-1}) was investigated. Results showed that, soil treatment with KH significantly improved plant growth and productivity, physio-biochemical attributes, and contents of K^+, osmoprotectants and antioxidants (soluble sugars, proline and ascorbic acid), and significantly lowered plant contents of contaminants (NO_3^-, NO_2^- and Cd^{2+}) and Na^+ ion compared to the untreated controls. The same results trend was obtained with soil treatment with Bch. Integrative application of KH + Bch was most effective compared to the single KH or Bch treatment. The above results recommended benefits of the integrative treatment KH+TOC to soil for the possibility of sustainable agronomic performance of eggplant grown on saline soils.

Keywords: Organic Substances; Pollutants; Eggplant Performance; Salinity.

1. Introduction

Worldwide, particularly in arid and semi-arid regions, salinization of irrigated lands is approximately steadily increasing year after year due to many factors. Poor irrigation water that contains massive amounts of salts with poor management, low rainfall, high evaporation rate, accumulation of salts in the top layer of the soil due to over-irrigation, proximity to the sea, and the capillarity rise of salts from underground water into the root zone due to excessive evaporation are of factor examples that could cause salinity related problems in these regions [1]. More than one-third of the irrigated land, worldwide, which provides approximately 40% of the global food production, is affected by salinization. Among many countries, Australia, Egypt, India, Pakistan, and the United States, all of which have authentic problems of salinity and drainage, affecting between 15 and 36% of their irrigated lands, are devoting substantial resources toward this problem [2].

In many tropical, subtropical and Mediterranean countries including Egypt, *Solanum melongena* L.; Eggplant is one of the most important traditional vegetable crops. Eggplant is classified as a salt sensitive [3] or as a moderately sensitive vegetable crop [4]. The difference in eggplant tolerance to salinity in the two works could be attributed to the used variety or cultivar, and also to the different environmental conditions under which studies were conducted. In Egypt, eggplant is widely cultivated on newly-reclaimed soils, however, most of these soils are salt-affected with low fertility and a poor structure. Plant growth and development is, therefore, affected resulting low fruit yield.

Mineral fertilizers are important for plant nutrition; however, they are also a potential source of environmental pollution, particularly mineral-N and mineral-P fertilizers when they are used without using organic fertilizers [5]. The extensive use of mineral-N fertilizers increases NO_3^- and NO_2^- ions contents in the edible parts of plant [6]. In addition, the extensive use of mineral-P fertilizers (the major anthropogenic sources of Cd^{2+} ions) increases Cd^{2+} ion (a heavy metal that causes loss in agricultural productivity and hazardous human health effects) content in the edible parts of plant [7] due to its easily absorption by roots and translocation to other plant parts [8].

In recent years, a lot of attention has been paid to the development of sustainable agriculture. To mitigate salt stress effects in plant, some strategies have been used, including soil amendments [9, 10, 11]. As a fertilizer/a soil conditioner in agriculture, supplementation of humic substances was attempted and their positive impacts on saline soil structure, and plant growth and yield were reported [6, 9, 12, 13, 14]. In these reports, it has been concluded that application of humic acid (HA) or potassium humate (KH) in proper concentrations can overcome the adverse effects of soil salinity, improve fertility and the structure of soil, and enhance plant and root growth and plant productivity under normal or soil salinity stress conditions.

Another soil amendment, biochar (Bch) is used to increase plant productivity or even to ameliorate soil properties [15]. Bch potential to increase plant biomass and productivity has been demonstrated in a number of tropical agricultural studies, finding that Bch treatments increased crop yields, with effects observed on acidic and coarse-textured soils. It has also been demonstrated that increased crop yields over several years can result in a single Bch treatment [16]. The favorable effects of Bch on productivity are thought to include high specific surface area, CEC, and, depending on pyrolysis conditions, micro porosity, although detailed physiological mechanisms of Bch remain unclear [17]. In addition to enhancing water and nutrient retention in soils, these properties also enable Bch to adsorb a wide range of potentially toxic materials, including heavy metals and other contaminants [18]. The biochar longevity in the soil presents more advantages for bioremediation than other organic materials that break down more quickly [19].

Accordingly, the current work was designed with the objective to evaluate the potential utilization effects of KH and/or Bch on the changes in growth, yields, endogenous physio-biochemical attributes, and leaf and fruit contaminant contents of *Solanum melongena* plants grown under saline soil conditions (EC = 6.96–7.08 dS m^{-1}). The objective also aimed to establish a relationship between the changes in physio-biochemical attributes and the degree of plant tolerance, in terms of improvement in plant performance (growth and yields). The hypothesis tested is that integrative application of KH + Bch to soil will elevate the level of some antioxidants and osmoprotectants that will protect plants against salt stress.

2. Materials and Methods

2.1. Location of Experiments, Soil Analyses, Materials and Treatments

Two pot experiments were conducted during summer seasons of 2015 and 2016 in an open greenhouse. The experiments were located at the Experimental Farm of the Faculty of Agriculture, Fayoum University, Southeast Fayoum (29º 17'N; 30º 53'E), Egypt. The initial soil chemical and physical characteristics (**Table 1**) were assessed [20, 21], and soil EC_e values classed the soil as being moderately saline [22].

Table 1. Physico-chemical properties of the soil used for experiments

Particle size distribution				FC	pH	EC_e dS/m	$CaCO_3$ %	OM %	Available macro-nutrients (ppm)		
Sand %	Silt	Clay	Texture class						N	P	K
Season of 2015											
28.9	27.0	44.1	CL	27.2	7.62	7.08	3.68	0.96	198	1.92	162
Season of 2016											

25.7	28.3	46.0	CL	29.2	7.45	6.96	3.85	1.02	242	4.26	266

CL means clay loam, FC means field capacity, WP means wilting point, AW means available water, and OM means organic matter.

Potassium humate (KH) used was purchased (Alpha Chemika, Mumbai, India) and found to contain approximately 60% humic acid (HA) and 15% potassium oxide (K_2O), besides traces of other elements. It was used at a level of 0.2 g per kg soil as a single treatment, however, it was used as 0.1 g per kg soil when it was used in combination with Bch.

Casuarina trees wastes (e.g., branches and leaves) were collected from a major farm in Abshwai District and allowed to dry thoroughly under the sun. Bch was produced from the casuarina wastes using a top-lit-updraft stove. Casuarina wastes were placed in the large Elsa burner and ignited. The hot Bch produced after pyrolysis was quenched with distilled water, collected and sun-dried, weighed and stored. Casuarina wastes Bch was analyzed for bulk density, pH, cation exchange capacity (CEC), and exchangeable cations (Na, K, Mg and Ca) [20, 21]. All of Bch characteristics are shown in **Table 2**. Bch was used at a level of 20 g per kg soil as a single treatment, however, it was used as 10 g per kg soil when it was used in combination with KH.

Table 2. Physico-chemical properties of the biochar used for experiments

pH	Ash (%)	Bulk density (g cm^{-3})	C (%)	P (%)	CEC* (cmol+/kg)	Exchangeable Cations (cmol+/kg biochar)			
						K	Ca	Mg	Na
2015 season									
8.9	35.7	0.81	44.9	0.08	45.5	40.9	11.9	6.2	7.1
2016 season									
8.7	34.9	0.80	45.3	0.09	46.4	42.1	12.2	7.0	6.8

*CEC means cation exchange capacity

Healthy and uniform eggplant (*Solanum melongena* L., cv. "Blackberry") transplants (30 days old) were obtained from the Ministry of Agriculture Nurseries, Fayoum, Egypt. Transplants were transplanted separately, one transplant per pot with 10 kg soil of each (35 cm in diameter, 32 cm depth). All pots were arranged in a completely randomized design for 4 treatments; a control (without any of KH or Bch), 0.2g KH per kg soil, 20g Bch per kg soil, and a combination of 0.1g KH + 10g Bch per kg soil, each with 20 replicates where each pot was represented a replicate.

Fertilization program was as follows: at transplanting, each pot was applied with 2g calcium superphosphate (15.5%, w/w, P_2O_5), 0.5g ammonium nitrate (33.5%, w/w, N), and 0.25g potassium sulphate (48%, w/w, K_2O). At a month after transplanting, 1g ammonium nitrate and 0.5g potassium sulphate was applied for each pot. Finally, 1g ammonium nitrate and 0.75g potassium sulphate was applied for each pot at 2 months after transplanting. Pots were irrigated with an equal volume of tap water day after day or once every 3 days according to the climate to maintain 100% of field capacity.

2.2. Plant Growth and Yield Assessments

From each of the 4 treatments, 60-day-old eggplant plants (n = 3) were removed gently by assist of tap water and the number and area (using a LI-COR 3100C leaf area meter; LI-COR, Inc., Lincoln, NE, USA) of leaves plant^{-1} was recorded. Plants were separated to shoots and roots. Lengths of shoots and roots were measured using a meter scale and were then weighed for fresh weight (FW). For dry weight (DW), shoots and roots were placed in an oven at 70 °C until constant weight.

At the marketable fruit stage of both experiments, fruits on 12 plants (n = 12) from each treatment were collected several times, counted and weighed individually and per plant.

2.3. Determination of Leaf Pigment Contents, Chlorophyll Fluorescence and Tissue Water Content and its Health

Contents of total chlorophylls and carotenoids (mg g^{-1} FW) were determined [23]. On two different sunny days, chlorophyll fluorescence was measured using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK) [24], and performance index of photosynthesis was calculated [25].

Relative water content [26], membrane stability index [27] and electrolyte leakage [28] were assessed using fresh fully-expanded leaves after excluding the midrib.

2.4. Determinations of Osmoprotectants and Non-Enzymatic Antioxidants Contents

Total soluble sugars were extracted and determined [29] using leaf samples that were homogenized in 96% (v/v) ethanol and washed with 70% (v/v) ethanol. Homogenizations were centrifuged at 3500 × g for 10 min and the supernatant was stored at 4 °C for measurement. Soluble sugars were determined by reacting 0.1 ml of the ethanolic extract with 3 ml of freshly prepared anthrone reagent [150 mg anthrone plus 100 ml of 72% (v/v) sulphuric acid] and then placed in a boiling water bath for 10 min. After cooling, samples were read at 625 nm using a Bausch and Lomb-2000Spectronic Spectrophotometer.

Leaf free proline content was determined [30]. Samples were extracted in 3% (v/v) sulphosalicylic acid and the mixtures were then centrifuged at 10,000 × g for 10 min. Two ml of a freshly prepared acid ninhydrin solution was added to 2 ml of supernatant and incubated in a water bath at 90 °C for 30 min. Reaction was terminated in an ice bath, and mixtures were extracted with 5 ml toluene and vortex-mixed for 15 s.

Separation of toluene and aqueous phases was allowed and each toluene phase was then carefully collected into a clean test-tube and was read at 520 nm.

Content of ascorbic acid (AsA) was determined [31]. Samples were extracted in 6% (w/v) TCA. The extracts were mixed with 2 ml of 2% (w/v) dinitrophenyl hydrazine (in acidic medium), then 1 drop of 10% (w/v) thiourea in 70% (v/v) ethanol was added and the mixtures were boiled for 15 min in a water bath. After cooling, 5 ml of 80% (v/v) H_2SO_4 was added at 0 °C and samples were read at 530 nm.

2.5. Determinations of Leaf and Fruit Contaminants Contents

Nitrate (NO_3^-) and nitrite (NO_2^-) contents in eggplant leaf and fruit were determined [32]. For extraction, samples (10 g) were well homogenized and blended for 5 min with 70 ml of distilled water and 12 ml of 2% NaOH solution. The pH of the suspension was adjusted to 8, and then heating in an oven (50–60°C) with occasional stirring was done. ZnSO solution (10 ml) was added and temperature was maintained at 50 °C for additional 10 min. NaOH solution (2%) was added until appearance of white precipitate. Thereafter, solution was cooled and diluted, and then transferred to 200 ml measuring flask and the volume was completed with distilled water. Solution was filtered through filter paper No.1. Filtrate was collected for NO_3^- and NO_2^- analyses in the same day of filtrate obtaining. For determining the NO_3^- content, 10 ml of filtrate was mixed with 5 ml of NH_4Cl buffer solution (pH 5), and the mixture was then passed through cadmium column with 15 ml distilled water and th combined effluent was collected and washed in 50 ml volumetric flask. Acetic acid (5 ml, 60%) and 10 ml of colour reagent (prepared by mixing equal volumes of sulfuric acid solution and N-(1-naphthyl) ethylene-diamine reagent, just before use) were added. The mixture was then diluted with distilled water and left to stand for 25 min in the dark, and the absorbance was read at 550 nm. For determining the NO_2^- content, 10 ml of the filtrate was transferred to 50 ml volumetric flask and 9 ml of NH Cl buffer (pH 5), 5 ml of 60% acetic acid and 10 ml of colour reagent (prepared by mixing equal volumes of sulfuric acid solution and N-(1-naphthyl) ethylene-diamine reagent, just before use) were added. The mixture was then diluted to an appropriate volume and left to stand for 25 min in the dark, and then the absorbance was read at 550 nm.

Leaf and fruit contents of Cd^{2+} were determined using a Perkin-Elmer Model 3300 Atomic Absorption Spectrophotometer [33].

2.6. Determinations of Leaf and Root Potassium (K) and Sodium (Na) Contents

The contents of K^+ (%) and Na^+ (%) were determined using 0.2 g of dried leaves that was digested with sulphuric acid in the presence of H_2O_2 [34]. The mixture was then diluted with distilled water and the contents of Na^+ and K^+ were measured using Flame Spectrophotometry [35].

2.7. Statistical Analysis

All data were subjected to an analysis of variance for a completed randomized design. Significant differences between means were compared at $P \leq 0.05$ using Duncan's multiple range test. The statistical analysis was carried out using COSTAT computer software (CoHort Software version 6.303, Berkeley, CA, USA).

3. Results

Data in **Table 3** show that growth traits [e.g., number and area of leaves plant^{-1}, and length, fresh weight (FW) and dry weight (DW) of shoot and roots plant^{-1}] of salt-stressed eggplant plants were significantly increased by either potassium humate (KH) or biochar (Bch) application compared to the untreated controls over both growing seasons; 2015 and 2016. In addition, integrative application of KH + Bch further increased growth characteristics in both growing seasons by 39.6 and 36.5% for number of leaves plant^{-1}, 47.3 and 62.2% for leaves area plant^{-1}, 27.0 and 28.3% for shoot length, 34.4 and 28.9% for shoot FW, 43.2 and 36.2% for shoot DW, 32.0 and 33.1% for root length, 21.8 and 24.9% for root FW, and 38.2 and 36.5% for root DW, respectively compared to the untreated controls.

Eggplant yield components (e.g., number of fruits plant^{-1}, average fruit weight, and fruit weight plant^{-1}) showed the same trend of growth traits (**Table 4**). Either KH or Bch application significantly increased yield components of salt-stressed eggplants by 9.8 or 6.6% for number of fruits plant^{-1}, 26.4 or 23.0% for average fruit weight, and 40.4 or 32.7% for fruit weight plant^{-1}, respectively in 2015 season, and by 17.4 or 14.2%, 39.1 or 38.0%, and 65.8 or 58.7%, respectively in 2016 season compared to the untreated controls. Moreover, integrative application of KH + Bch further increased these yield components in both seasons by 25.1 and 27.0%, 52.7 and 60.9%, and 92.3 and 104.8%, respectively compared to the untreated controls.

Table 3. Effect of soil application with potassium humate (KH) and biochar (Bch) on growth traits of *Solanum melongena* plants grown under saline conditions

Treatment	Parameters							
	Leaf		Shoot			Root		
	Number plant^{-1}	area plant^{-1} (dm^2)	Length (cm)	FW (g)	DW (g)	Length (cm)	FW (g)	DW (g)
2015 season								
Control	17.7c	11.2c	60.1c	69.8c	9.13c	29.7c	40.4c	6.68c
KH	20.7b	13.7b	67.2b	83.6b	11.39b	34.3b	45.8b	7.78b
Bch	20.7b	13.3b	66.8b	82.0b	11.16b	33.9b	44.8b	7.54b
KH+Bch	24.7a	16.5a	76.3a	93.8a	13.07a	39.2a	49.2a	9.23a
KH+Bch % of control	**+ 39.55**	**+ 47.32**	**+ 26.96**	**+ 34.38**	**+ 43.15**	**+ 31.99**	**+ 21.78**	**+ 38.17**
2016 season								
Control	20.3c	11.9c	63.0c	76.8c	9.83c	31.4c	48.2c	8.32c
KH	24.3b	16.5b	73.8b	90.2b	11.84b	37.4b	54.4b	9.94b

Bch	23.7b	16.4b	72.5b	88.4b	11.66b	36.8b	53.2b	9.76b
KH+Bch	27.7a	19.3a	80.8a	99.0a	13.39a	41.8a	60.2a	11.36a
KH+Bch % of control	**+ 36.45**	**+ 62.18**	**+ 28.25**	**+ 28.91**	**+ 36.22**	**+ 33.12**	**+ 24.90**	**+ 36.54**

Values are means (n = 3), and mean values in each column followed by different lower-case letters are significantly different by LSD test at $P \leq 0.05$.

Table 4. Effect of soil application with potassium humate (KH) and biochar (Bch) on yield components of *Solanum melongena* plants grown under saline conditions

Treatment	Parameters					
	Number of fruits plant^{-1}	**% of control**	Average fruit weight (g)	**% of control**	Fruit weight plant^{-1} (kg)	**% of control**
2015 season						
Control	7.02c	-	74.7c	-	0.52c	-
KH	7.71b	**+ 9.83**	94.4b	**+ 26.37**	0.73b	**+ 40.38**
Bch	7.48bc	**+ 6.55**	91.9b	**+ 23.03**	0.69b	**+ 32.69**
KH+Bch	8.78a	**+ 25.07**	114.1a	**+ 52.74**	1.00a	**+ 92.31**
2016 season						
Control	7.70c	-	82.3c	-	0.63c	-
KH	9.04b	**+ 17.40**	114.5b	**+ 39.13**	1.04b	**+ 65.80**
Bch	8.79b	**+ 14.16**	113.6b	**+ 38.03**	1.00b	**+ 58.73**
KH+Bch	9.78a	**+ 27.01**	132.4a	**+ 60.87**	1.29a	**+ 104.76**

Values are means (n = 12), and mean values in each column followed by different lower-case letters are significantly different by LSD test at $P \leq 0.05$.

Similarly, data in **Table 5** exhibit significant increases over both seasons in the contents of total chlorophylls (39.1 and 35.5%, respectively) and total carotenoids (37.9 and 39.4%, respectively), and in chlorophyll fluorescence measured in terms of Fv/Fm (5.1 and 7.7%, respectively) and performance index (PI; 33.4 and 31.5%, respectively), and also in relative water content (RWC; 19.7 and 19.5%, respectively) and membrane stability index (MSI; 18.0 and 18.9%, respectively) by the integrative application of KH + Bch compared to the untreated controls.

Table 5. Effect of soil application with potassium humate (KH) and biochar (Bch) on leaf photosynthetic pigments contents (mg g^{-1} FW) and their efficiency, and tissue health in *Solanum melongena* plants grown under saline conditions

Treatment	Parameters						
	Total chlorophylls	Total carotenoids	Fv/Fm	PI	RWC (%)	MSI (%)	EL (%)
2015 season							
Control	1.38c	0.29c	0.79b	7.25c	72.2c	63.4c	17.2a
KH	1.62b	0.35b	0.82ab	8.52b	79.6b	69.4b	14.5b
Bch	1.55b	0.33b	0.81ab	8.10b	78.9b	68.7b	14.8b
KH+Bch	1.92a	0.40a	0.83a	9.67a	86.4a	74.8a	11.5c
KH+Bch % of control	**+ 39.13**	**+ 37.93**	**+ 5.06**	**+33.38**	**+19.67**	**+17.98**	**- 33.14**

2016 season							
Control	1.43c	0.33c	0.78b	7.60c	74.0c	65.2c	16.8a
KH	1.71b	0.38b	0.82a	8.95b	80.2b	71.9b	13.4b
Bch	1.68b	0.38b	0.82a	8.88b	79.3b	69.9bc	13.7b
KH+Bch	1.98a	0.46a	0.84a	9.99a	88.4a	77.5a	11.1c
KH+Bch % of control	**+ 38.46**	**+ 39.39**	**+ 7.69**	**+31.45**	**+19.46**	**+18.87**	**- 33.93**

Values are means (n = 3), and mean values in each column followed by different lower-case letters are significantly different by LSD test at $P \leq 0.05$. Fv/Fm means maximum quantum yield of PSII photochemistry (Fm = maximum fluorescence, and Fv = variable fluorescence), PI means performance index of PSII, RWC means relative water content, MSI means membrane stability index, and El means electrolyte leakage.

Osmoprotectants and antioxidants contents (**Table 6**) were recorded the same trends with the integrative application of KH + Bch in both seasons in leaves (34.4 and 38.4% increases for soluble sugars content, 46.2 and 60.0% increases for free proline content, and 39.4 and 52.8% increases for ascorbic acid content, respectively) and in roots (30.3 and 33.5% increases for soluble sugars content, 38.9 and 38.1% increases for free proline content, and 50.0 and 51.5% increases for ascorbic acid content, respectively) compared to the untreated controls. The content of K and K/Na ratio (**Table 8**) were recorded the same trends and were significantly increased with the integrative application of KH + Bch over both experimental seasons by 51.6 and 57.9%, and 120.1 and 151.6%, respectively in leaves, and by 52.4 and 55.0%, and 130.3 and 155.3%, respectively in roots compared to the untreated controls.

Table 6. Effect of soil application with potassium humate (KH) and biochar (Bch) on leaf and root contents of osmoprotectants (total soluble sugars and free proline; mg g^{-1} DW) and antioxidants (ascorbic acid – AsA; µmol g^{-1} DW) of *Solanum melongena* plants grown under saline conditions

Treatment	Parameters					
	Leaf			Root		
	Soluble sugars	Free proline	AsA	Soluble sugars	Free proline	AsA
2015 season						
Control	2.62c	0.13c	1.32c	3.24c	0.18c	1.62c
KH	2.98b	0.16b	1.50b	3.72b	0.22b	1.98b
Bch	2.93b	0.16b	1.48b	3.65b	0.22b	1.94b
KH+Bch	3.52a	0.19a	1.84a	4.22a	0.25a	2.43a
KH+Bch % of control	**+ 34.35**	**+ 46.15**	**+ 39.39**	**+ 30.25**	**+ 38.89**	**+ 50.00**
2016 season						
Control	3.02c	0.15d	1.27c	3.34c	0.21c	1.73c
KH	3.81b	0.20b	1.55b	3.96b	0.25b	2.12b
Bch	3.72b	0.18c	1.52b	3.84b	0.24b	2.06b
KH+Bch	4.18a	0.24a	1.94a	4.46a	0.29a	2.62a
KH+Bch % of control	**+ 38.41**	**+ 60.00**	**+ 52.76**	**+ 33.53**	**+ 38.10**	**+ 51.45**

Values are means (n = 3), and mean values in each column followed by different lower-case letters are significantly different by LSD test at $P \leq 0.05$.

Table 7. Effect of soil application with potassium humate (KH) and biochar (Bch) on leaf and fruit contents of contaminants; NO_3^-, NO_2^- (mg g^{-1} DW), and Cd^{2+} (mg kg^{-1} DW) in *Solanum melongena* plants grown under saline conditions

Treatments	Parameters					
	Leaf content of			Fruit content of		
	NO_3^-	NO_2^-	Cd^{2+}	NO_3^-	NO_2^-	Cd^{2+}
2015 season						
Control	3.22a	0.122a	2.18a	2.24a	0.078a	1.52a
KH	2.72b	0.092b	1.04b	1.84b	0.062b	0.91b
Bch	2.72b	0.095b	1.16b	1.82b	0.060b	0.92b
KH+Bch	2.44c	0.076c	0.90c	1.53c	0.048c	0.79c
KH+Bch % of control	**- 24.22**	**- 37.70**	**- 58.72**	**- 31.70**	**- 38.46**	**- 48.03**
2016 season						
Control	3.42a	0.130a	1.94a	2.18a	0.069a	1.13a
KH	2.95b	0.098b	0.94b	1.81b	0.054b	0.72b
Bch	2.96b	0.101b	0.98b	1.85b	0.056b	0.72b
KH+Bch	2.80c	0.082c	0.80c	1.46c	0.044c	0.59c
KH+Bch % of control	**- 18.13**	**- 36.92**	**- 58.76**	**- 33.03**	**- 36.23**	**- 47.79**

Values are means (n = 3), and mean values in each column followed by different lower-case letters are significantly different by LSD test at $P \leq 0.05$. NO_3^- means nitrate, and NO_2^- means nitrite.

In contrast, either KH or Bch application significantly reduced electrolyte leakage (EL, **Table 5**), the contents of contaminants measured as nitrate (NO_3^-), nitrite (NO_2^-) and cadmium (Cd^{2+}) (**Table 7**) in both leaves and fruits, and sodium content (Na^+, **Table 8**) in both leaves and roots over two growing seasons; 2015 and 2016. In addition, the integrative application of KH + Bch further decreased the above parameters in both seasons (33.1 and 33.9% decreases for leaf EL, 24.2 and 18.1% decreases for leaf NO_3^- content, 37.7 and 36.9% decreases for leaf NO_2^- content, 58.7 and 58.8% decreases for leaf Cd^{2+} content, 31.7 and 33.0% decreases for fruit NO_3^- content, 38.5 and 36.2% decreases for fruit NO_2^- content, 48.0 and 47.8% decreases for fruit Cd^{2+} content, 31.2 and 37.3% decreases for leaf Na^+ content, and 33.8 and 39.4% decreases for root Na^+ content, respectively) compared to untreated controls.

Table 8. Effect of soil application with potassium humate (KH) and biochar (Bch) on leaf and root K^+ and Na^+ contents (mg g^{-1} DW) and K^+/ Na^+ ratio of *Solanum melongena* plants grown under saline conditions

Treatments	Parameters					
	Leaf content of			Root content of		
	K^+	Na^+	K^+/Na^+ ratio	K^+	Na^+	K^+/Na^+ ratio
2015 season						
Control	19.4c	6.41a	3.03c	24.6c	9.81a	2.51c
KH	26.2b	5.72b	4.58b	32.3b	8.15b	3.96b
Bch	25.9b	5.65b	4.58b	32.5b	8.06b	4.03b
KH+Bch	29.4a	4.41c	6.67a	37.5a	6.49c	5.78a
KH+Bch % of control	**+ 51.55**	**- 31.20**	**+ 120.13**	**+ 52.44**	**- 33.84**	**+ 130.28**
2016 season						
Control	20.2c	6.52a	3.10c	25.1c	9.94a	2.53c
KH	28.2b	5.25b	5.37b	33.4b	7.48b	4.47b
Bch	27.8b	5.12b	5.43b	32.8b	7.36b	4.46b
KH+Bch	31.9a	4.09c	7.80a	38.9a	6.02c	6.46a
KH+Bch % of control	**+ 57.92**	**- 37.27**	**+ 151.61**	**+ 54.98**	**- 39.44**	**+ 155.34**

Values are means (n = 3), and mean values in each column followed by different lower-case letters are significantly different by LSD test at $P \leq 0.05$. K^+ means potassium ions, and Na^+ means sodium ions.

4. Discussion

Irrigated land productivities face a considerable problem, particularly in arid and semi-arid regions (dry environments), due to increasing salinity that accumulates by several factors. Low rainfall and high rate of evaporation, poor irrigation water and its management, and over-irrigation led accumulation of salts in the top layer of soil are some of these factors [1], which expose plants to osmotic stress [11]. Salt stress suppresses plant performance due to stimulation of reactive oxygen species (ROS) overproduction through various organelles and enzymes. In the current study, the decrease in eggplant growth and productivity under saline conditions (control of Tables) could be attributed to the osmotic stress effect that probably increase growth inhibitors contents, decrease growth promoters' contents and disrupt water balance in stressed plants. These effects lead to an ionic imbalance, a reduction in photosynthesis efficiency, an accumulation of toxic ions, and consequently an inhibition of plant growth [1, 36].

Osmotic adjustment and improving the antioxidative defense systems (**Table 6**), and ion homeostasis (**Table 8**) are of strategies adopted by plants to avoid these salt stress effects [37]. Several reports have shown to use soil amendments, as indirect exogenous supports, to alleviate plant cytotoxicity stimulated by salt stress conditions [9, 10, 11, 12, 13, 14]. These soil applications have proved to improve soil characteristics [11], conferring the opportunity for in-field protection against this dangerous environmental stress conditions.

Results of the current study indicate that addition of potassium humate (KH) and/or casuarina biochar (Bch) to soil can alleviate the adverse effects of saline conditions on plant performance (i.e., plant growth and yield). Amelioration of salt stress effects was evident at the integrative supplement rates of 0.1g KH + 10 g Bch per kg soil. This integrative supplement had a pronounced fertilization effect under greenhouse or open field conditions generally favorable to plant growth and productivity, especially eggplant plants. Finding out a suitable alleviant to ameliorate stress effects is one of the plant biologist tasks. In recent decades, exogenous protectants applied for growing media (i.e., humic substances, organo-mineral fertilizers, minerals such as K, S and P, etc) have an effective role in alleviating the salt stress induced damages in plants [5, 6, 10, 11, 12, 13, 14, 38, 39]. These soil amendments conferred the capacity, in different degrees, to improve the plant's growth and productivity, and stress tolerance under salt stress conditions.

Soil supplementation of KH with or without Bch significantly improved growth characteristics and yield components of eggplant plants grown under saline soil conditions (6.96 – 7.08 dS m^{-1}) conditions (**Tables 3 and 4**). Soil treatment with KH significantly improved plant growth and productivity through improving osmoprotectants and antioxidants (**Table 6**), tissue health and water availability (**Table 5**) and nutrient uptake (**Table 8**) [9, 11, 12], and through reducing plant contents of pollutants (**Table 7**) [6, 14]. In integration with KH, Bch acts to mitigate the impacts of plant stress, either by reducing exposure of plants to stress factors, or by ameliorating the stress responses of plants [40]. Biochar can also substantially increase the water holding capacity of soils [41], and therefore improve the water status of plants (**Table 5**), particularly during drought periods existed due to salt stress. Salts seriously impact plants through both osmotic effects and ionic toxicity [42], and enhanced water availability is expected to mitigate both of these effects as shown from results of the current study. Thus, Bch's capacity to increase water availability may explain, in part, the alleviation of salt impacts observed in the present study. These results suggest that the main mechanism for mitigation was sorption of NaCl resulting in reduced exposure [40]. In the present experiment, the application of Bch as incorporation into the soil is likely to have enhanced its capacity to sorb salts. The largest growth responses of eggplant plants to biochar additions are expected in situations of poor soil quality and nutrient status, high soil acidity, or low water holding capacity [17]. The recent literature supports, in general, this expectation, with larger benefits observed on relatively nutrient-poor, acidic, and coarse-textured soils [43]. Alleviation of salt stress effects provides another example of this general trend. However, plant growth benefits of Bch have also been documented in relatively rich soils under favorable conditions [44].

Salt stress partially inhibited photosynthesis by a reduction in leaf photosynthetic pigments and chlorophyll fluorescence (Fv/Fm and PI); **Table 5**. However, KH application to soil had increased these attributes that were further increased by integrated KH + Bch, protecting photosynthetic machinery from salt-induced ROS by acting as a free radical scavenger. The few existing studies on photosynthetic responses to Bch additions have emphasized increased leaf-level or whole-plant water-use efficiency [18], but the mechanism responsible for this effect is unclear. Similar to other non-nutrient soil amendments, elucidation of the mechanisms for growth responses to Bch will likely require a range of techniques. The similarity of Bch to lime additions is informative and suggests use of vector nutrient analysis or alternative approaches to deduce the relative importance of specific plant nutrient resources in driving observed responses [40]. Bch deactivates the photosynthesis-derived reactive oxygen species (ROS; mainly O_2^- and OH^-), in an indirect manner. The Fv/Fm and PI are used as a noninvasive method to determine the photosynthetic machinery functional state. These physiological attributes were reduced significantly by salt stress, while in integration with KH, Bch application significantly improved these attributes in leaves of salt-stressed eggplant plants [45]. The RWC, EL and MSI are other tested physiological attributes that were affected seriously by salt stress, however, they affected positively by KH and/or Bch applications (**Table 5**). Under salt stress, soil salts trigger the osmotic stress, and the over-accumulation of salts in plant cells causes ionic stress. These stresses individually affect the physiological status of plant [46]. Soil application with KH played an important role in water relations of plants under salt stress and helped plants to absorb more water to attain turgidity [38]. In the present study, addition of K to the soil in the form of KH significantly increased leaf turgor and RWC under salt stress. Production of ROS resulted in a reduction of MSI in the present study (**Table 5**), however, KH application increased MSI and reduced EL. The increase in available water content of the tested saline soil by KH application helped plant tissues to maintain more water, increasing RWC and consequently sustaining the stability of cell membranes. In integration with KH, Bch application to soil enabled plant to maintain high levels of RWC by regulating leaf osmolality (e.g., soluble sugars and proline contents; **Table 6**), alleviating the effects of salt stress and maintaining cells turgid for healthy metabolic processes and membranes integrity. The increase in water and osmotic potentials might help stabilization of protein and increase photosynthesis **[47]**. Enhanced plant nutrient status by Bch application, particularly increased K^+ uptake (**Table 8**), can result in increased growth through positive changes in photosynthetic pigments (**Table 5**), osmoprotectants and antioxidants (**Table 6**), and positively reflected in eggplant productivity (**Table 4**).

Osmoprotectants and antioxidants measured herein as endogenous soluble sugars, free proline and AsA (**Table 6**) were positively affected by the integrated application of KH + Bch. Application of KH contributed in increasing these attributes by alleviating the adverse effects of salt stress and through availability of water and nutrient absorption [12], especially the osmolyte K^+. Addition of KH showed increases in the endogenous contents of soluble sugars, free proline and AsA. This probably due to that humic substances are capable of stimulating the genetic pathways leading to improve plant defense mechanisms evidenced by the improved antioxidants [48]. In conjunction with KH, Bch application stimulated the accumulation of soluble sugars, free proline and AsA contents in salt-stressed plant either via increasing endogenous levels of certain phytohormones or by acting as activators of carbohydrates synthesis and generally of photosynthetic systems [11]. The accumulation of soluble carbohydrates is found to play a key role in alleviating salinity stress, either via osmotic adjustment or by conferring some desiccation resistance to plant cells [45]. It has been reported that proline protects the macromolecules by stabilizing protein structure and sub-cellular structures that could be considered as a sink for energy as well even a stress-related signal. It also sustains the osmotic adjustment and/or scavenges the ROS produced under stress conditions [49]. These results are accompanied by the increased content of AsA in plant tissues (**Table 6**), which enable eggplant plants to alleviate salt stress effects by limiting the ROS damages. As substrates of the Halliwell–Asada cycle, AsA act as an antioxidant in an isolated way on being involved in the direct reduction of ROS during different types of stress [50]. It can directly eliminate $O_2^{\bullet -}$ and H_2O_2 in a non-enzymatic way [51]. It has been found that further increased contents of osmoprotectants (soluble sugars and proline) and antioxidants (proline and AsA) were conferred by the integrative treatment of KH + Bch that may contribute to advantages to plants and help to perform better in various aspects of growth and metabolism as they defend against the harmful effect of salt stress effects.

Results of this study have found that leaf and fruit contents of NO_3^-, NO_2^- and Cd^{2+} were significantly reduced by soil application of KH or Bch. However, the integrative treatment of KH + Bch was most effective in decreasing the contents of these contaminants (**Table 7**). It has been reported that addition of OMF compost (containing KH) to soil exhibited significant decreases in plant (leaf, pod, and seed of common bean) contents of NO_3^- and Cd^{2+} [10]. In addition, soil application with a mixture of KH and farmyard manure (FYM) resulted in tomato plants with lower NO_3^- and NO_2^- contents that were positively reflected in these pollutant contents of the fruit for human nutrition and health. Increasing the available N in the soil by applying the recommended dose of mineral-N in the control fertilization regime led to a significant increase in the NO_3^- and NO_2^- contents of plant leaves that was also negatively reflected in the edible parts (i.e., fruits) [6]. The accumulation of NO_3^- and NO_2^- ions in edible plant parts poses a problem which can be attributed to the supply of readily available NO_3^- and NO_2^- to plants from mineral-N fertilizer and available Cd^{2+} to plants from mineral-P fertilizer. In contrast, the release of these pollutants (NO_3^-, NO_2^- and Cd^{2+}) was comparatively slow in the organic fertilizer treated soil. In addition, an increase in the percentage of organic matter (OM) in the soil treated with organic fertilizers may control the release and transformation of N-fertilizer to NO_3^- and NO_2^-, and also control the release of Cd^{2+} from P-fertilizer [10]. It has been found that the addition of FYM to cultivated soil was effective in minimizing NO_3^- and NO_2^-

toxicity in beet leaves. This result is attributed to the incorporation of OM, in the form of FYM, which enhanced the organic carbon content of the soil and had direct and indirect effects on many soil properties and processes [52]. Contents of NO_3^- and NO_2^- in NPK-fertilized crop plants were significantly higher than in organic crops. When organic based fertilizer was used, the NO_3^- and NO_2^- contents were lower even at higher fertilizer levels as shown in our results (**Table 7**). Fertilizers used in organic farms contain N bound to organic material from which it is slowly released [53]. It would be best to use organic fertilizers for crop productions to minimize the harmful effects of NO_3^-, NO_2^- and Cd^{2+} to humans from the use of higher levels of NPK fertilizers. Although Cd^{2+} is a non-essential element for crop plants, it is easily taken up by plants growing on Cd^{2+}-supplemented or Cd^{2+}-contaminated soils, entering food chain and causing damage to plant and human health [54]. It has been reported that the mean concentration of Cd^{2+} ranges from 0.013 to 0.22 mg kg^{-1} for cereal grains and from 0.08 to 0.28 mg kg^{-1} for legumes [54]. A number of approaches are being used to minimize the entry of Cd^{2+} into the plants. Soil application of organic fertilizers is one of the good strategies to alleviate the damaging effects of Cd^{2+} on plants and to avoid its entry into plants, and subsequently the food chain as shown in the present study. Cd^{2+} was found to bind to the organic matter existing in soil, and to rule out away from the plant roots. The results of hundreds of works indicated that organic crops have a higher antioxidant activity and a lower concentration of Cd^{2+} compared to inorganic crops [55]. This is in accordance with our results (**Table 7**), which showed that plants of the control (received the full recommended NPK fertilizer dose) had Cd^{2+} content above the limits determined in plants, while those that received KH + Bch had low Cd^{2+} content.

The integrative supplementation of KH+Bch to soil significantly increased plant content of K^+ and ratio of K^+/Na^+, and significantly decreased plant content of Na^+ (**Table 8**). In this connection, it has been suggested that increased accumulation of Na^+ and Cl^- ions in the tissues under salt stress inhibits biochemical processes related to photosynthesis through direct toxicity, leading to low water potential. The promotion of Na^+ ion uptake under salt stress conditions was accompanied by a corresponding decline in K^+ content, showing an antagonism between K^+ and Na^+ [56]. The selectivity of high K^+/Na^+ ratio in plants is considered an important mechanism and criterion selection for salt tolerance. Better plant tolerance to salt stress is primarily due to better K^+ assimilation, resulting in higher K^+/Na^+ ratio [57]. In integrative with the benefits of KH for K^+ content and K^+/Na^+ ratio in plants, results of this study show a promotion in Na^+ uptake under salt stress (control) that was accompanied by corresponding decline of K^+ content, showing an apparent antagonism between K^+ and Na^+. In contrast, the application of Bch reversed the status of these ions, where increased K^+ content, reduced Na^+ content, and increased the ratio of K^+/Na^+, which positively reflected in plant growth and yield. This promotion in nutrient contents may be attributed to the role of soil amendments; Bch and KH in increasing osmotolerance and/or regulating various processes, including absorption of nutrients from soil solution and improving membrane permeability. The antagonistic relation between Na^+ and K^+ may be taken as an indication of the role played by Bch or KH in modifying K^+/Na^+ selectivity under salt stress conditions. The integrative treatment of KH+Bch was most effective in this concern.

All examined parameters (i.e., growth traits, photosynthesis efficiency, plant water relations, osmoprotectants and antioxidants, content of K^+ and its relation with Na^+ and final yield) under salt stress were significantly improved, and plant contents of contaminants (NO_3^-, NO_2^- and Cd^{2+}) were significantly lowered by supplementation of KH or Bch, however, these effects were more pronounced with the integrative application of KH+Bch to soil.

5. Conclusion

Application of KH and/or Bch to soil has been shown to improve plant salt stress-defence responses, to act indirectly at improving total plant performances under salt stress, and to increase the activity of the antioxidative defense system including osmoprotectants (soluble sugars and proline) and non-enzymatic antioxidants (proline and AsA), leading to an increase in photosynthesis efficiency and, subsequently, to increase plant performances. Thus, the integrative supplement of KH+Bch to soil may provide an effective strategy to alleviate the adverse effects of salt stress through increased N-utilization and the synthesis of antioxidant compounds. Increased contents of soluble sugars, proline and AsA, resulted in less damage to photosynthesis and greater protection of dangerous effects of salt stress. Therefore, the application of KH in integration with Bch may act to alleviate the severity of the effects of salt stress on *Solanum melongena* plants grown on saline soils.

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