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Humus Materials and Moringa (*Moringa oleifera* Lam.) Leaf Extract Modulate the Harmful Effect of Soil Salinity Stress in Sudan Grass (*Sorghum vulgare* L.)

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THE EFFECT of application different components of humus (humic acid and fulvic acid) and foliar spraying with Moringa leaf extract on Sudan grass vegetative criteria, some physiological and biochemical properties grown under three levels of soil salinity was conducted. Two pot experiments were carried out under the greenhouse conditions at the farm of Faculty of Agriculture, Zagazig University, Egypt, during the growth season 2014 and 2015. Data indicated that salinity stress condition significantly decreased plant height, shoot fresh and dry weight, chlorophyll a, b and caroteniods, photochemical activity, RNA, DNA, total carbohydrates and total sugars, while increment of antioxidant enzymes, i.e., peroxidase (POX) and catalase (CAT). Application of two humus component and foliar spraying with Moringa leaf extract overcome the harmful effects of salinity stress on the above mentioned criteria of Sudan grass compared with untreated plants. Humus components and Moringa leaf extract were helpful in improving these criteria at three cuts under different salinity levels. The highest value of the above mentioned criteria was observed with application of the humic acid + fulvic acid and spraying Moringa leaf extract, while the lowest one was recorded under the control treatment.

Keywords: Sudan grass (*Sorghum vulgare*), Humus, Moringa leaf extract, Soil salinity stress.

Introduction

Sudan grass (*Sorghum vulgare* var. *sudanense*) is the second biggest forage crop (next to alfalfa). The genus *Sorghum* consist of various differentiated yearly spring-sown species which bear a resemblance to maize inhabit but develop only tassels in which seeds are set (Józef & Szydełko, 2011). *Sorghum*, being a C_4 species, is an efficient employ of radiation and solar heat. It is resistant to drought, tolerates both alkaline and salinization soils (Śliwiński & Brzoska, 2008).

Salinity is an essential abiotic stress factor that causes counteractive effects on plant growth and productivity. It is appraise that about 7% of the world's land is affected by salinity stress which gravely impairs the plant productivity of at least 20% of irrigated land worldwide (Munns & Tester, 2008). High salinity causes disequilibrium ion, osmotic stress, as well as oxidative stress in tissues (Zhu, 2001) and activated many genes expression in tomato seedling after exposed to salinity (Ouyang et et al., 2007). The reduction in crop production under soil salinity condition may be due to of photosynthetic processes (Pitman & Läuchli, 2002). Actually, saltiness dishearten involved of specific enzymes for photosynthetic pigments synthesis, causing a decrease in chlorophyll content (Giri & Mukerji, 2004 and Sheng et al., 2008). Also, salt stress caused reducing photosynthetic rate which can stimulate an over-reduction of the reaction centers in photosystem II (PSII) and this may destruction the photosynthetic machinery if the plant that is unable to consume the excess energy (Baker,

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2008). The main effect of salinity has been attributed to the reduction water potential of soil or the enhance concentration of ion in plant cells to levels that interfere with metabolism (Kalaji & Pietkiewicz, 1993). Therefore, it is necessary to develop strategies to initiate salt resistance in plants against the adverse conditions. Numerous physiological and agronomic practices have been performed to enhance salinity stress resilience in various crops including breeding programs. However, commercial success has been limited. As alternatives, antioxidants substances plant and growth regulators (Desoky & Merwad, 2015; Rady & Hemida, 2016 and Desoky et al., 2017, 2018), plant biostimulants/extract (Howladar, 2014) and humic substances (Rady et al., 2016 a and Desoky et al., 2018) have been widely applied with agricultural crops to alleviate the adverse effects of salinity stress.

Humic substances are easily found in soils (Mackowiak et al., 2001 and Ulukan, 2008 b) and directly and indirectly improve plant growth (Nardi et al., 2002 and Cimrin & Yilmaz, 2005). They have indirect effects on plant development since they can enhance soil properties, for example, collection, air circulation, porousness, water holding limit, hormonal action, microbial development, natural issue (OM) mineralization, solubilization and accessibility of microelement and macroelements (Chen & Aviad, 1990; Ayuso et al., 1996 and Sharif et al., 2002). Directly, influence the procedures related with the take-up and humic substances transport into the plant tissues (Nardi et al., 2002).

Moringa leaf extract (MLE) are plant development improving capacities as it is rich in amino acid, phenols and essential elements. Moringa genuinely shows up an endowment of nature and a “Wonder” plant having incalculable advantages so that it can use to improve plants grown under a biotic stresses (Foidle et al., 2001). Leaf extract of Moringa have been showed to speed growth of wheat at seedling stage, improve resistance salinity and increase large and more fruits and generally increase yield by 20 to 35% (Fuglie, 2000). The objective of the current study is to evaluate the effect application of humus component (Humic acid and fulvic acid) with spraying Moringa leaf extract on growth characteristics, photosynthetic pigments, photochemical activity, some chemical constituents, by Sudan grass grown under salinity stress conditions.

Materials and Methods

Two pot experiments were conducted under the greenhouse conditions at the farm of Faculty of Agriculture, Zagazig University, Egypt. Sudan grass (*Sorghum vulgare* var. *sudanense*) seeds were sown during the growth season 2014 and 2015. It aims to evaluate the effect application of humus component (Humic acid and fulvic acid) with spraying Moringa leaf extract on growth characteristics, photosynthetic pigments, photochemical activity, some chemical constituents, by Sudan grass grown under salinity stress conditions. Three saline soils having almost the same near texture were collected from different location at El-Noubaria, near Alexandria, Egypt. Seeds were sterilized by using 1% (v/v) sodium hypochlorite for 2min then washed with distilled water and sown on the 20th of March. Closed bottom plastic pots of internal dimensions 25cm were filled with 10kg of each saline soil (3.01, 6.12, 12.33dSm^{-1}). The physical and chemical properties of the investigated soil were determined according to Piper (1950), Black (1968) and Jackson (1973) and are shown in Table 1.

Before planting, the treatments of humus materials, i.e., humic acid (HA) and fulvic acid (FA) were thoroughly mixed with the soil samples at the rate of 0.2g kg^{-1} soil. Mineral nitrogen was added as ammonium sulphate (1.8g/pot) three equal splits. The first was before the 1st irrigation while the second and third splits were added after the first and second cuts, respectively from the first splits. The recommended doses of phosphorus and potassium were added; for all experimental treatments as ordinary super phosphate (1.5g P /pot) before sowing. Potassium was added as potassium sulphate (1.8g/pot) were added before seeding.

An amount of 20g of young *Moringa oleifera* leaves was mixed with 675ml of distilled water and 80% ethanol as suggested by Makkar & Becker (1996). The suspension was stirred using a homogenizer to help maximize the amount of the extract. The solution was filtered using No.2 Whatman filter paper. Moringa leaves extract were used within five hours from cutting and extracting (if not ready to be used, the extract or the solution prepared was stored at 0^{o}C and only taken out when needed for use). The chemical composition of ethanolic extracts of *Moringa oleifera* leaves were investigated by Fuglie (2000) and Moyo et al. (2011) and are represented in Table 2. Foliar spraying of Moringa extract was done in each of 2 occasions (20 and 40 days) from seed germination, after 1st and 2nd cuts at the rate of 3%. Control plants were sprayed with distilled water and the spraying extract was maintained just to cover completely the plant foliage. Three cuts were taken from the sorghum and each cut was taken at age 60 day.

The experiment included 24 treatments in 6 replicates, so the experiment contained 144 pots. The experimental design was a split-split plot with three soil salinities (S1, S2 and S3) as main-plots, two HM levels (0 and 0.2g kg^{-1} soil) as subplots, and two MLE levels (0% and 3%) as sub-subplots.

TABLE 1. Some physical and chemical properties of the investigated soil.

Soil salinity		S1	S2	S3
Soil particles distribution	Sand %	41.52	46.57	45.36
	Silt %	37.42	29.56	29.13
	Clay %	21.06	23.87	24.51
	Textural class	Loam	Loam	Loam
Cations** (mmolc L^{-1})	Ca^{++}	8.54	17.09	46.56
	Mg^{++}	9.51	19.95	37.35
	Na^{+}	6.98	17.27	18.37
	K^{+}	5.12	6.95	21.12
Anions** ($mmol_c$ L^{-1})	CO_3^-	-	-	-
	HCO_3^-	7.82	20.38	37.75
	Cl^-	12.43	32.74	33.21
	SO_4^{--}	9.69	8.13	36.27
EC** (dSm^{-1})		3.01	6.12	12.33
pH*		7.89	7.83	8.02
Organic matter		6.42	7.41	8.63
$CaCO_3$ (g kg^{-1})		55.32	61.35	62.71
FC (%)		13.54	16.65	14.97
Available nutrient (mg kg^{-1} soil)	N	60.42	58.42	49.5
	P	10.95	8.6	6.8
	K	88.94	99	123

*: Soil paste; **: Soil paste extract; FC: Field capacity.

TABLE 2. Chemical composition of *Moringa oleifera* leaves (on basis of dry weight; DW).

Component	Unit	Value
Protein	g kg^{-1} DW	273
Phosphorus (P)		3.9
Potassium (K)		21.7
Calcium (Ca)		24.0
Magnesium (Mg)		4.5
Iron (Fe)		0.6
Vitamin A (β-carotene)	mg kg^{-1} DW	163
Vitamin B1(thiamine)		26
Vitamin B2 (riboflavin)		210
Vitamin B3(nicotinic acid)		800
Vitamin C (ascorbic acid)		1700
Vitamin E (tochopherol acetate)		1130

A random sample of three different cuts were taken from each treatment during the two growing seasons to record plant vegetative criteria, some physiological and biochemical properties of Sudan grass plants.

Plant vegetative criteria

Plant height (cm), fresh and dry weight of shoot systems (g) was determined. Plant samples were dried using an electric oven with drift fan at 70°C for 48h. till a constant weight was reached.

Physiological properties

Photosynthetic pigments

The photosynthetic pigments (chlorophyll *a, b* and carotenoids) were extracted from fresh leaf sample by pure acetone according to Fadeel's method (Fadeels, 1962). The pigments (as mg/g fresh weight) were calculated using the formula adapted by Von Wettestein (1957) as follows:-

Chl. a= (9.784 x E_{662}) – (0.99 x E_{644}) (mg/liter).

Chl. b= (21.426 x E_{644}) – (4.65 x E_{662}) (mg/liter).

Carot.= (4.695 x $E_{440.5}$) – (0.268 x Chl. a + Chl. b) (mg/liter).

where: E is the reading of the optical density at given wave length. The concentrations of pigments were then expressed in mg/g fresh weight of leaves.

Photochemical activity

Photochemical activity in fresh leaves of Sudan grass plants were determined according to Jagendorf (1956) and modified by Avron (1960) using Ferricyanide. About 0.2g of fresh leaf tissues was grind with 1.5ml phosphate buffer (pH 7.5) and then transferred to conical flasks.

Two ml of homogenate leaf tissues were transferred to a small beaker and 0.5ml of Ferricyanide was added and exposed to light for 10min, then centrifuged at 3000 4000 xg for 10min. The optical density of supernatant was determined spectrophotometrically at 420nm. At the same time, the optical density of supernatant resulted from exposure the suspention to dark for 10min was also spectrophotometrically determined. Chlorophylls concentration was also determined spectrophotometrically at 652nm. The photochemical activity was estimated as mM/gm Chl.

Catalase and peroxidase activities

Catalase activity: The method described by Feinstien (1949) was used for determining catalase activity as follows:

Second fresh leaf tissue 2.5g was ground with purified sand in a cooled mortar and 25ml of phosphate buffer pH 6.8 was added during grinding. The homogenate was filtered through cotton wool. One ml of enzyme extract was placed in a conical flask containing 8ml sodium perborate 1.5% and 1.5ml phosphate buffer pH 6.8. The mixture was incubated at 37°C for 5min after which the reaction was stopped by adding 10ml H_2SO_4 2N. One drop of manganese chloride 1% was added for each flask and titration was carried out by means of potassium permanganate 0.05 N using appropriate plank for each series.

Peroxidase activity: This determination was carried out following the method described by Purr (1950). Second fresh leaf tissue 2.5g was placed in a cooled mortar and carefully ground with purified sand, then transferred to a conical flask with the aid of 25ml distilled water (through a filtered cotton wool was squeezed after filtration). The assay mixture was composed of the following components, i.e., 10ml phosphate buffer pH 5, 4ml ascorbic acid solution 0.44%, 1ml orthotalidine alcohol solution 0.5%, 4ml hydrogen peroxide solution 30%. The mixture was up to 50ml with distilled water. Incubation was performed at 25°C for 10min, after which 10ml of each sample was pipette on to 2.5ml 2N H_2SO_4 in a conical flask .

One drop of starch indicator, the incubated extract was titrated against appropriate blanks with N/250 iodine solution until blue color was formed and persisted for 30sec.

The activity was calculated as ml N/250 iodine consumed by gram dray matter of each sample.

Nucleic acids estimation

Bluck RNA (mainly cytoplasmic RNA) contents were estimated in fresh leaves of sudan grass plants.

The extraction procedure was essentially that of Nitsan & Long (1966) which was modified by Sharaky (1982) operations up to ethanol/each was carried out at 4°C. Half gram of leaf tissue was homogenized with 4ml of 0.15m NaCl 0.1µ EDTA (Ethylendiamine tetra acetic acid) buffer solution pH 8.0 by a mortar and pestle. An equal volume of 4% (w/v) trichloroacetic acid was added immediately to tissue homogenate. After standing the mixture for 3h at -5^{o}C, the precipitate was extracted successively with methanol (twice), ethanol (twice), boiling ethanol/either (3:1) for 3 min at 60-70^{o}C (twice) and either (once). The air dried residue was extracted with 3ml of 0.N KOH at 30^{o}C/20h (alkaline extraction of nucleic acid).

Nucleic acid (RNA and DNA) were recovered by centrifugation after extraction (5000rpm/min) about half hour. The supernatant which contains nucleic acid was collected. The precipitate was once more rinsed with 3ml of 0.3N KOH then centrifuged and supernatant was collected. The two supernatant were combined. Mixed and acidified with 10% perchloric acid to pH 1-2. After standing the acidified nucleic acid mixture for 6h/-10^{o}C, it was centrifuged (5000rpm/min) about half hour to precipitate DNA protein complex. The supernatant which contains RNA as free polyriboncleotides was collected while the DNA protein complex precipitate was once again washed with prechloric acid 5% and centrifuged. The two supernatant were mixed and made up to acertain volume by prechloric acid 5%. The quantity (mg/g fresh weight) of RNA and DNA were estimated according to the equations of Nieman & Poulesn (1967**)**.

Carbohydrate farctions

Total carbohydrate and total sugar were determined in the dried samples of shoots photometrically according to Bernfeld (1955) and Miller (1959) methods with some modifications.

Color reagent preparation

One gram of 3, 5 dinitro salicylic acid was dissolved in 20ml of 2N NaOH , then 50ml distilled water and 30gm of Rochelle salt were added and the mixture was shaken well until dissolving the salt, then the volume was made up to 100ml with distilled water.

Total carbohydrate

One tenth gram of dry shoot and grains of Sudan grass powder with 20ml of 6N HCl were taken in a carbohydrate tube, then the samples were heated for 6h in a boiling water bath then filtered using whatman paper No.1.

Twenty ml of 6N NaOH were added to the filtrate for neutralization, then made up to 100ml with distilled water. Five ml from the filtrate were added to 2ml of color reagent in a test tube, shaked well and heated exactly for 10min in a boiling water bath then cooled under running tap water. The color intensities were measured colorimetrically at 550nm using spectronic-20 spectrophotometer.

Total sugars

From the filtrate of the above mentioned reducing sugars, 10ml were taken in a carbohydrate tube, 5ml 6N HCl were added and incubated for 2h in water bath. After incubation, 5ml of 6N NaOH were added for neutralization and 2ml of the color reagent were mixed as previously mentioned in case of total carbohydrate.

Statistical analysis

Data of the current study were subjected to an analysis of variance for a split-split plot design, after testing for the homogeneity of error variances. Statistically 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).

Results and Discussion

Plant vegetative characters

Data present in Table 3 showed the effect of humus component, foliar application of MLE, soil salinity and their interaction on plant height, fresh weight (FW) and dry weight (DW) of Sudan grass plants. Data revealed that plant height, FW and DW of Sudan grass plants were decreased significantly with increasing soil salinity levels. This trend was found true under different humus component with Moringa leaf extract at all three cuts. In control treatment, the plants in thethird cut were due to high salinity level (12.33dSm^{-1}). Soil salinity reduced plant growth, i.e., plant height may be due to reduction in cell division or cell elongation in both case and/or reduction could raise from toxic effect of ions (Na and Cl) on metabolism or from adverse water relation (Hawker & Walker, 1978 and Zhaoliang et al., 1995**)**. Similar results were found by Elgharably (2008**)** who found that total dry mater of wheat plants was significantly decreased by salinity levels.

TABLE 3. Integrative effect of humus substance and Moringa extract on plant height (cm), fresh and dry weight (g pot^{-1}) of sorghum plants grown under salt stress conditions (Average of two growing seasons 2014 and 2015).

Salinity level	Humus materials	Moringa extract	Plant height			Fresh weight			Dry weight		
			1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
S1	Without	Without	141	124	72	584	527	401	97	92	67
		With	150	133	73	585	551	422	98	95	70
	HA	Without	154	151	75	603	580	426	101	95	71
		With	168	158	89	610	582	439	102	97	73
	FA	Without	152	137	72	591	566	415	99	94	69
		With	168	141	79	601	572	429	100	97	71
	HA+FA	Without	160	155	87	607	581	433	101	97	72
		With	171	161	93	621	599	450	103	100	75
	Mean		158	145	80	600	570	427	100	96	71
S2	Without	Without	103	86	44	552	510	335	92	85	56
		With	110	92	52	563	516	361	94	86	60
	HA	Without	130	100	57	574	533	362	94	89	60
		With	136	116	66	582	543	383	96	91	64
	FA	Without	120	90	54	558	527	353	93	88	59
		With	124	96	56	568	530	370	95	88	62
	HA+FA	Without	129	101	58	578	542	377	96	90	63
		With	140	123	72	656	550	390	97	92	66
	Mean		124	100	57	579	531	366	95	89	61
S3	Without	Without	69	44	0	522	470	0	87	78	0
		With	78	51	0	531	490	0	89	79	0
	HA	Without	92	55	36	534	482	234	89	82	39
		With	98	71	39	542	502	250	90	84	42
	FA	Without	80	50	31	526	474	225	88	80	38
		With	84	60	32	533	494	229	89	82	40
	HA+FA	Without	96	76	39	539	494	241	90	82	41
		With	100	79	42	549	509	259	92	85	43
	Mean		87	61	27	534	489	180	89	82	30
Mean of hums materials	Without	Without	104	85	39	553	503	245	92	85	41
		With	113	92	42	560	519	261	93	87	44
	HA	Without	125	102	56	570	532	340	95	89	57
		With	134	115	65	578	543	357	96	90	60
	FA	Without	117	92	52	558	522	331	93	88	55
		With	125	99	56	568	532	343	94	89	58
	HA+FA	Without	128	111	61	574	539	350	96	90	59
		With	137	121	69	609	553	366	97	92	62
	Grand mean		123	102	55	571	530	324	95	89	54
LSD 0.05%	A		1.5	1.2	0.7	2.3	2.0	2.1	0.4	0.3	0.4
	B		1.7	1.4	0.8	2.7	2.3	2.4	0.4	0.4	0.4
	AB		3.0	2.4	1.4	NS	4.1	4.2	NS	0.7	0.7
	C		1.2	1.0	0.6	1.9	1.7	1.7	0.3	0.3	0.3
	AC		NS	1.7	1.0	3.3	2.9	3.0	0.5	0.5	0.5
	BC		2.5	1.9	1.1	NS	NS	NS	NS	NS	NS
	ABC		4.3	3.3	1.9	NS	NS	5.9	NS	NS	1.0

HA: Humic acid, FA: Fulvic acid, A: Salinity effects, B: Humus effects, C: Moringa effects.

Individual application of humic acid or in combination with fulvic acid gave the best results of plant height, FW and DW of Sudan grass plants at three cuts under all salinity levels than single fulvic acid application in presence of Moringa extract. Humic acid ameliorate vegetative growth by increasing the availability of nutrients, decreased the harmful effect of salinity, advanced root growth and increased P, K of soil **(**Tattini et al., 1991 and Ulukan, 2008 a**)**. The application of humus materials improved chemical, hydropheysical and biological trait of saline soil. This finding remains in well concurrence with those of Basyouny et al. (2003).

From the obtained results, spraying Moringa extract increased significantly morphological characters as compared to the untreated plants. The highest values of plant height, FW and DW of Sudan grass plants at the three cuts under S1, S2 and S3 were observed with application of humic acid + fulvic acid under foliar spray of Moringa extract followed by humic acid alone. The lowest values were recorded under control plants in the absence of Moringa extract.

Moringa oleifera is an exceptionally nutritive multipurpose plant developed for new vegetable, domesticated animals grub, green fertilizer, biogas, medication, bio-pesticide and production of seed (Fuglie, 2000). Plant growth enhanced by MLE that is may be due to that Moringa contains many mineral elements (i.e., N, P, K and Ca) and rich by amino acid and zeatien (Makkar & Becker, 1996 and Basra et al., 2009). Such plant growth promoters impact plant development in a few different ways and furthermore support defense mechanisms of plant against stresses by increasing endogenous concentration of plant growth regulator's (PGR). Also, PGR can used as a foliar spray or seed priming agent. Use of MLE as plant growth increased crop production by 20-35%. Spray influences the harvests by longer life expectancy, heavier roots, stems and leaves, producing large and more fruits and increasing yield production by 20-35% (Foidle et al., 2001), accelerating growth of young plants and proved to be an ideal plant growth in many experiments (Makkar & Becker, 1996; Nouman et al., 2013 and Basra et al., 2005).

Physiological and biochemical properties

Photosynthetic pigments and photochemical activity

The obtained results in Tables 4 and 5 illustrated significant variation among salinity stress levels of chlorophyll *a*, *b* and carotenoids and photochemical activity of Sudan grass leaves. The results showed that photosynthetic pigments and photochemical activity were decreased by increasing soil salinity levels. This trend was found true under different humus component with or without Moringa leaf extract, at all three cuts. As mention before control plants with the untreated soil due to extremely high salinity ($12.33 dSm^{-1}$) at third cut was died.

The obtained results from the effect of salinity stress on the concentration of photosynthetic pigments and photochemical activity in plant leaves may be substantiated by finding of Horváth et al. (2015) in tomato and Porcela et al. (2015) in rice, indicated that photosynthetic pigments were decreased significantly under high salinity levels. Salinity stress decreased net photosynthetic rate by conductance of stomatal and transpiration (Chen et al., 2014). Decreasing chlorophyll content by salinity stress may be ascribed to increase activity of chlorophyll and degradation chloroplast enzymes (Reddy & Varo, 1986). The reductory effect of salinity on chlorophyll content might be attributed to its negative action on increase chlorophyllase activity which degradated chlorophyll (Sivtrev et al., 1973).

In this regard Yeo & Flovers (1983) and Malibari et al. (1993) reported that the reduction of chlorophyll content under salinity stress may be due to the inhibition effect of the accumulation ions of various salts on biosynthesis of different pigment fraction.

Data indicated that application humus component, i.e., humic acid and fulvic acid under salinity stress condition (S1, S2 and S3) showed significant differences in chlorophyll *a, b* and carotenoids as well as photochemical activity. Generally humic acid was more effective than fulvic acid in increasing photosynthetic pigments and photochemical activity. Similarly, (Hanafy et al., 2010) indicated that addition of humic acid significantly increased photosynthetic pigments concentration under calcareous soils conditions. Also, Fu et al. (1994) revealed that application of FA to rape plants increased chlorophyll content and intensity of photosynthesis. Increasing leaves chlorophyll content by application of HA might be attributed to accumulation of nutrient which enhancing photosynthesis.

Foliar application of MLE caused positive effect on photosynthetic pigments and photochemical activity of plant under salinity levels. The highest values of photosynthetic pigments and photochemical activity were obtained by mixed application of HA + FA under spray Moringa leaf extract, while, the lowest one was obtained in plants not receiving humus component as well as Moringa extract. Moringa leaves have been accounted for to be a rich wellspring of an important component (i.e., amino acid, zeatien, β-carotene, N, P, K and Ca) and act as a good source of some antioxidants (i.e., flavonoid, phenolic components and ascorbic acid) (Dillard & German, 2000 and Siddhuraju & Becker, 2003). So, the exogenous applications of Moringa leaf extract (MLE) improve the antioxidants which ameliorate the bad affect of salinity and increase photosynthetic pigments and photochemical activity as compared to the untreated one (control).

TABLE 4. Integrative effect of humus substance and Moringa extract on leaf photosynthetic pigments (mg/g FW) of sorghum plants grown under salt stress conditions (Average of two growing seasons 2014 and 2015).

Salinity level	Humus materials	Moringa extract	Chlorophyll *a*			Chlorophyll *b*			Carotoniods		
			1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
S1	Without	Without	1.19	1.02	0.76	0.46	0.35	0.26	1.06	1.10	0.51
		With	1.19	1.07	0.79	0.52	0.38	0.29	1.10	1.11	0.55
	HA	Without	1.24	1.19	0.84	0.57	0.42	0.36	1.14	1.12	0.61
		With	1.38	1.22	0.89	0.63	0.48	0.39	1.19	1.14	0.63
	FA	Without	1.20	1.17	0.80	0.55	0.36	0.30	1.10	1.11	0.58
		With	1.23	1.18	0.83	0.56	0.38	0.31	1.15	1.13	0.61
	HA+FA	Without	1.30	1.19	0.87	0.58	0.44	0.37	1.15	1.12	0.62
		With	1.42	1.31	0.91	0.75	0.49	0.40	1.27	1.22	0.66
	Mean		1.27	1.17	0.84	0.58	0.41	0.34	1.15	1.13	0.60
S2	Without	Without	0.99	0.98	0.69	0.35	0.26	0.14	1.00	0.99	0.46
		With	1.00	1.00	0.72	0.39	0.29	0.22	1.06	1.01	0.56
	HA	Without	1.15	1.06	0.74	0.53	0.30	0.19	1.04	1.01	0.49
		With	1.18	1.17	0.77	0.55	0.34	0.25	1.08	1.04	0.60
	FA	Without	1.11	1.00	0.71	0.41	0.29	0.15	1.02	1.00	0.47
		With	1.14	1.04	0.75	0.44	0.30	0.25	1.07	1.04	0.56
	HA+FA	Without	1.16	1.10	0.77	0.54	0.31	0.21	1.05	1.03	0.50
		With	1.21	1.18	0.79	0.55	0.36	0.29	1.09	1.05	0.60
	Mean		1.11	1.07	0.74	0.47	0.31	0.21	1.05	1.02	0.53
S3	Without	Without	0.98	0.84	0	0.29	0.11	0	0.84	0.71	0
		With	0.99	0.89	0	0.30	0.13	0	0.91	0.74	0
	HA	Without	1.06	0.96	0.67	0.45	0.19	0.11	0.91	0.86	0.42
		With	1.07	0.99	0.69	0.48	0.23	0.20	0.99	0.90	0.54
	FA	Without	1.04	0.89	0.62	0.32	0.17	0.11	0.98	0.79	0.41
		With	1.06	0.94	0.64	0.35	0.18	0.18	0.98	0.81	0.51
	HA+FA	Without	1.07	0.96	0.67	0.45	0.21	0.14	0.99	0.86	0.46
		With	1.10	1.03	0.70	0.51	0.26	0.20	0.99	0.99	0.56
	Mean		1.05	0.94	0.50	0.39	0.19	0.12	0.95	0.83	0.36
Mean of hums materials	Without	Without	1.05	0.95	0.48	0.37	0.24	0.13	0.97	0.93	0.32
		With	1.06	0.99	0.50	0.40	0.27	0.17	1.02	0.95	0.37
	HA	Without	1.15	1.07	0.75	0.52	0.30	0.22	1.03	1.00	0.51
		With	1.21	1.12	0.78	0.55	0.35	0.27	1.08	1.02	0.59
	FA	Without	1.12	1.02	0.71	0.43	0.27	0.19	1.03	0.97	0.49
		With	1.14	1.05	0.74	0.45	0.29	0.25	1.07	0.99	0.56
	HA+FA	Without	1.18	1.09	0.77	0.52	0.32	0.25	1.07	1.01	0.53
		With	1.24	1.17	0.80	0.60	0.37	0.30	1.12	1.09	0.61
	Grand Mean		1.14	1.06	0.69	0.48	0.30	0.22	1.05	1.00	0.50
LSD 0.05%	A		0.08	0.06	0.06	0.01	0.05	0.04	0.05	0.04	0.02
	B		NS	0.07	0.07	0.01	0.06	0.05	NS	0.04	0.03
	AB		NS	NS	0.11	0.01	NS	NS	NS	0.07	0.05
	C		NS	0.05	NS	0.01	NS	0.03	NS	NS	0.02
	AC		NS	NS	NS	0.01	NS	NS	NS	0.03	NS
	BC		0.13	NS	NS	0.01	0.08	NS	NS	NS	NS
	ABC		NS	NS	0.16	0.01	0.14	NS	NS	NS	NS

HA: Humic acid, FA: Fulvic acid, A: Salinity effects, B: Humus effects, C: Moringa effects.

TABLE 5. Integrative effect of humus substance and Moringa extract on photochemical activity (µmol/mg Chl./min), peroxidase (POX) (µmol H_2O_2/mg FW/min) and catalase (CAT) (µmol H_2O_2/mg FW/min) activities in sorghum plants grown under salt stress conditions.

Salinity level	Humus materials	Moringa extract	Photochemical activity			POX activity			CAT activity		
			1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
S1	Without	Without	48.4	36.2	34.2	3.41	2.76	2.61	310	249	143
		With	50.1	37.1	36.2	3.68	2.96	3.01	329	263	171
	HA	Without	55.3	40.3	39.7	3.84	3.03	3.01	331	255	151
		With	56.4	42.3	40.2	4.11	3.51	3.76	356	282	191
	FA	Without	49.0	39.8	38.7	3.68	2.81	2.96	326	251	146
		With	52.1	39.9	38.8	3.91	3.06	3.19	331	277	182
	HA+FA	Without	50.2	40.7	40.0	3.96	3.12	3.19	338	263	159
		With	58.9	44.7	44.1	4.16	3.69	3.96	362	291	198
	Mean		52.5	40.1	39.0	3.84	3.12	3.21	335	266	168
S2	Without	Without	39.1	29.6	26.3	3.62	3.48	3.31	463	341	255
		With	40.4	30.0	28.0	3.92	3.81	3.62	488	366	269
	HA	Without	41.1	32.8	30.4	3.82	3.96	3.81	471	368	270
		With	45.2	35.0	34.3	4.30	4.10	4.15	501	388	280
	FA	Without	39.8	31.3	29.8	3.77	3.76	3.59	467	352	261
		With	40.3	31.3	30.2	3.96	3.98	3.89	492	375	275
	HA+FA	Without	42.7	33.1	31.1	3.91	4.01	4.09	489	373	273
		With	46.2	37.0	35.7	4.38	4.19	4.69	503	392	281
	Mean		41.8	32.5	30.7	3.96	3.91	3.89	484	369	271
S3	Without	Without	31.3	20.2	0	4.81	4.02	0	569	418	0
		With	31.9	21.2	0	4.99	4.56	0	591	439	0
	HA	Without	36.8	23.9	20.4	5.03	4.76	4.46	582	440	242
		With	37.3	25.1	21.9	5.62	4.98	4.72	611	461	263
	FA	Without	33.2	21.9	19.0	4.93	4.19	3.96	576	421	331
		With	35.3	22.3	19.9	5.11	4.79	4.13	581	448	348
	HA+FA	Without	36.8	24.1	21.2	5.15	4.82	4.69	593	446	353
		With	40.0	27.0	23.9	5.83	5.19	4.81	623	468	375
	Mean		35.3	23.2	20.3	5.18	4.66	3.35	591	443	239
Mean of hums materials	Without	Without	39.6	28.7	20.2	3.95	3.42	1.97	447	336	133
		With	40.8	29.4	21.4	4.20	3.78	2.21	469	356	147
	HA	Without	44.4	32.3	30.1	4.23	3.92	3.76	461	354	221
		With	47.3	34.1	32.1	4.68	4.20	4.21	489	377	245
	FA	Without	40.7	31.0	29.2	4.13	3.59	3.50	456	341	246
		With	42.6	31.2	29.6	4.33	3.94	3.74	468	367	268
	HA+FA	Without	43.2	32.6	30.8	4.34	3.98	3.99	473	361	262
		With	48.4	36.3	34.6	4.79	4.36	4.49	496	384	285
	Grand mean		41.3	31.9	28.5	4.33	3.90	3.48	470	359	226
LSD 0.05%	A		0.27	0.29	0.21	0.27	0.23	0.24	3.3	14.0	16.5
	B		0.32	0.33	0.24	0.31	0.27	0.28	3.9	NS	19.0
	AB		0.55	0.57	0.42	NS	NS	0.49	NS	NS	32.9
	C		0.22	0.23	0.17	0.22	0.19	0.20	2.7	11.4	13.4
	AC		0.39	0.40	0.30	NS	NS	NS	NS	NS	23.3
	BC		0.45	0.47	0.34	NS	0.38	NS	5.5	NS	26.9
	ABC		0.77	0.81	0.59	NS	NS	NS	9.4	NS	46.5

HA: Humic acid, FA: Fulvic acid, A: Salinity effects, B: Humus effects, C: Moringa effects.

Peroxidase (POX) and catalase (CAT)activity

From the data in Table 5, it could be clearly noticed that increase soil salinity levels significantly increased activities of POX and CAT of Sudan grass homogenate in the three cuts, the high level salinity stress (S3) was the most effective one increased POX and CAT activity. These results were sustained by Lechno et al. (1997) who found that, NaCl treatment increased the activities of antioxidants enzymes. Salinity stress cause over production of reactive oxygen species (ROS) in plant tissue (Rady et al., 2015). The antioxidant defense system is essential for reduction oxidative destruction in plants under stress by releasing excessive reactive oxygen species (ROS) (Baloğlu et al., 2012). Increased antioxidative enzyme activities induced by salinity stress protected cell proteins, cell membrane and metabolic mechanism, which keep subcellular structure from damage by salinity stress (Sekmen et al., 2012).

CAT is a main enzyme that eliminates H_2O_2 in cell mitochondrion and microbody (Shigeoka et al., 2002). CAT enzyme is an important antioxidant system that catabolizes hydrogen peroxide, a precursor of reactive oxidants and reacts with H_2O_2 directly to form water and oxygen (Smironoff, 1993).

Regarding to the effect of application various humus component as well as foliar spray with Moringa leaf extract resulted in a positive significant increase of POX and CAT activities in plant under different levels of salinity stress during three successive cuts. The highest values of POX and CAT were obtained by mixed application of HA + FA with Moringa leaf extract spray under S3 (12.33dSm^{-1}) salinity level.

However, untreated plants under S1 (3.01dSm^{-1}) salinity level showed the lowest POX and CAT activities. Exogenous application of HA increased significantly POX and CAT activities with increasing HA levels (Zhang et al., 2013). Humic acid isable to control hormone levels, enhance plant growth and ameliorate stress tolerance (Saruhan et al., 2011). Humic acid regulation mechanism of antioxidative enzyme which mitigating the negative effect of stress (Zhang et al., 2013). Also, FA application substantially ameliorated the adversities of stress by increasing levels of antioxidant enzyme (superoxide dismutase (SOD), POD and CAT activities (Anjum et al., 2011). While, Moringa leaf extract contains antioxidant including proline and phytohormone such as indol acetic acid (IAA), gibberlline and cytokinin which improve plant growth, metabolism and antioxidant enzymes (Rehman et al., 2014).

Nucleic acids

Data present in Table 6 showed that the three salinity levels S1, S2 and S3 significantly decreased total DNA and RNA of sudan grass leaves in the three cuts, increasing salinity levels up to (12.33dSm^{-1}) was the most effective. Salinity had been showed to reduce the synthesis of total DNA, RNA and protein in many plants (Levitt, 1980). The decrease of RNA due to salinization may be explained by either a suppression in nutrient absorption as a result of NaCl or $CaCl_2$ uptake in combination with nutrient ions, or by excess accumulation of certain ion mainly Cl^- in the sweet pepper cells (Al-Bahrany, 1994). Increasing NaCl from 0.1 to 1.5mM decreased DND, RNA binding level in cowpea plants (Lee & Hacker, 2001). These reductions may be due to triggering increase in ribonuclease and protease activities (Galston, 1983).

Data also indicated that, application of different humus component as well as foliar spray with Moringa leaf extract caused positive effect on nucleic acid (RNA & DNA) of sudan grass plants grown under different levels of soil salinity. The highest values of RNA and DNA under S1, S2 and S3 level revealed 243, 192, 172mg/g FW, respectively for RNA and 18.81, 13.61, 9.11mg/g FW, respectively for DNA were obtained by mixed application with HA+FA under spray with Moringa leaf extract, while, the lowest one was obtained in plants not receiving humus materials as well as Moringa extract.

In this respect humic acid improve plant growth by enhancing nutrient uptake, photosynthesis, including synthesis total RNA & DNA and proteins as well as plasma membrane stabilization (Cimrin et al., 2010 and Saruhan et al., 2011).

Carbohydrate fractions

Data in Table 7 revealed that total carbohydrate ant total sugar of Sudan grass shoot were significantly and gradually decreased with increasing salinity levels especially under high salinity level (S3). In this concern, Farouk et al. (2001) stated that artificial salinity stress significantly decreased total carbohydrate content of shoots. While, Hajar et al. (1996) on *Nigella sativa* found that total sugar and starch decreased with increasing NaCl salinity. The synthesis of carbohydrate was depressed by water stress due to soil salinity **(**Kilany et al., 2006**)**. Abiotic stress represses cell expansion more than cell division, its diminished plant development by influencing different physiological and biochemical process, for example, ion uptake, nutrient metabolism and carbohydrate **(**Farooq et al., 2009**)**.

TABLE 6. Integrative effect of humus substance and Moringa extract on the contents of RNA and DNA (µg/g FW) of sorghum plants grown under salt stress conditions (Average of two growing seasons 2014 and 2015).

Salinity level	Humus materials	Moringa extract	RNA 1st cut	RNA 2nd cut	RNA 3rd cut	DNA 1st cut	DNA 2nd cut	DNA 3rd cut
S1	Without	Without	220	181	180	10.36	9.31	7.63
		With	224	185	181	14.92	9.32	8.68
	HA	Without	229	197	193	14.31	10.21	9.68
		With	236	203	200	16.62	13.11	12.01
	FA	Without	225	194	188	12.68	11.36	8.89
		With	229	195	191	13.93	12.10	9.12
	HA+FA	Without	231	199	196	15.63	11.65	10.16
		With	243	211	201	18.81	15.12	12.13
	Mean		230	196	191	14.66	11.52	9.79
S2	Without	Without	165	153	141	7.88	7.73	6.45
		With	171	156	146	8.31	7.91	6.69
	HA	Without	176	170	156	11.19	9.69	7.81
		With	186	185	166	13.03	10.88	7.93
	FA	Without	173	163	155	9.36	8.89	7.02
		With	178	168	155	9.36	9.03	7.06
	HA+FA	Without	179	174	161	10.01	9.12	7.91
		With	192	193	168	13.61	11.06	8.36
	Mean		178	170	156	10.34	9.29	7.40
S3	Without	Without	154	134	0	6.31	5.11	0
		With	155	146	0	7.92	6.23	0
	HA	Without	163	152	133	7.02	7.71	5.88
		With	170	161	140	7.61	8.73	6.13
	FA	Without	161	149	125	6.51	7.11	5.69
		With	165	151	129	8.02	7.64	5.88
	HA+FA	Without	168	155	138	8.61	7.99	6.12
		With	172	163	145	9.11	8.89	6.41
	Mean		164	151	101	7.64	7.43	4.51
Mean of hums materials	Without	Without	180	156	107	8.18	7.38	4.69
		With	183	162	109	10.38	7.82	5.12
	HA	Without	189	173	161	10.84	9.20	7.79
		With	197	183	169	12.09	10.42	8.69
	FA	Without	186	163	156	9.52	9.12	7.20
		With	191	171	158	10.44	9.59	7.35
	HA+FA	Without	193	176	165	11.75	10.07	8.06
		With	202	189	171	13.84	11.69	8.97
	Grand Mean		190	172	154	10.88	9.41	7.23
LSD 0.05%	A		2.8	1.64	2.04	0.76	0.68	0.69
	B		3.2	1.89	2.35	0.88	0.78	0.80
	AB		NS	3.28	4.07	1.53	NS	1.38
	C		2.3	1.34	1.66	0.62	0.55	0.57
	AC		NS	2.32	NS	NS	NS	0.98
	BC		NS	NS	NS	NS	1.10	NS
	ABC		NS	NS	NS	NS	NS	NS

HA: Humic acid, FA: Fulvic acid, A: Salinity effects, B: Humus effects, C: Moringa effects.

TABLE 7. Integrative effect of humus substance and Moringa extract on the contents of total carbohydrate and total sugar (%) of sorghum plants grown under salt stress conditions.

Salinity level	Humus materials	Moringa extract	Total carbohydrate			Total sugar		
			1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
S1	Without	Without	30.2	28.4	25.7	7.37	6.34	6.03
		With	32.9	30.1	26.3	7.99	6.52	6.52
	HA	Without	34.8	32.7	29.4	8.78	7.52	7.11
		With	36.2	33.4	31.2	9.11	8.13	7.77
	FA	Without	34.1	34.1	27.7	8.36	7.18	6.88
		With	34.7	31.2	28.2	8.76	7.21	7.23
	HA+FA	Without	35.0	31.3	30.0	8.93	7.98	7.76
		With	37.0	34.6	31.6	9.69	8.86	8.19
	Mean		34.4	32.0	28.8	8.62	7.47	7.19
S2	Without	Without	25.3	24.6	21.3	6.81	5.13	4.83
		With	27.6	25.0	21.9	6.98	5.42	4.92
	HA	Without	29.9	27.8	24.8	7.21	5.99	5.32
		With	32.1	29.9	25.4	7.82	6.38	5.93
	FA	Without	28.7	27.4	23.2	7.08	5.83	5.13
		With	28.9	27.7	23.9	7.19	5.93	5.19
	HA+FA	Without	29.8	28.2	23.9	7.53	6.11	5.87
		With	32.8	30.0	26.3	8.13	6.92	6.06
	Mean		29.4	27.6	23.9	7.34	5.96	5.41
S3	Without	Without	21.4	19.9	0	4.26	3.98	0
		With	22.4	20.1	0	4.99	4.03	0
	HA	Without	24.2	22.9	18.4	5.99	4.79	3.92
		With	25.4	24.9	19.7	6.89	4.93	4.63
	FA	Without	23.2	22.1	17.1	5.21	4.11	3.71
		With	24.0	22.9	19.8	5.23	4.52	4.11
	HA+FA	Without	24.8	24.2	20.7	6.63	4.88	4.63
		With	26.7	25.6	21.3	7.78	5.26	4.98
	Mean		24.0	22.8	14.6	5.87	4.56	3.25
Mean of hums materials	Without	Without	25.6	24.3	15.7	6.15	5.15	3.62
		With	27.6	25.1	16.1	6.65	5.32	3.81
	HA	Without	29.6	27.8	24.2	7.33	6.10	5.45
		With	31.3	28.6	25.4	7.94	6.48	6.11
	FA	Without	28.7	27.9	22.7	6.88	5.71	5.24
		With	29.2	27.3	24.0	7.06	5.89	5.51
	HA+FA	Without	29.9	28.7	24.9	7.70	6.32	6.09
		With	32.2	30.1	26.4	8.53	7.01	6.41
	Grand Mean		29.3	27.5	22.4	7.28	6.00	5.28
LSD 0.05%	A		0.82	0.71	0.56	0.75	0.63	0.51
	B		0.95	0.82	0.64	NS	0.72	0.59
	AB		NS	NS	1.12	NS	NS	1.02
	C		0.67	0.58	0.46	NS	0.51	0.42
	AC		NS	NS	0.79	NS	NS	NS
	BC		NS	NS	NS	NS	1.02	NS
	ABC		NS	NS	NS	NS	1.77	NS

HA: Humic acid, FA: Fulvic acid, A: Salinity effects, B: Humus effects, C: Moringa effects.

Its interest to mention that application of humus materials (HA & FA) exhibited the highest values of carbohydrate fraction as compared to control. In this respect HA was the more effective than FA. These results hold true at the three cuts. In all cases, the increase in carbohydrate fraction was over highly significant in comparison with untreated one. These results in agree with Ali et al. (2013) who reported that application of humic acid increased leaves total carbohydrate content under salinity stress. Also, humic acid ameliorate physiological process like osmotic adjustment and composition of carbohydrate (Jianguo et al., 1998).

Furthermore, spraying Moringa leaf extract in combination with humus materials were more effective than increasing carbohydrate fraction of Sudan grass plants under other treatments during three cuts under three levels of soil salinity. Increasing carbohydrate fraction reached its maximum values at HA + FA under spray Moringa leaf extract.

In this condition, foliar spray of MLE increased total soluble sugar and total carbohydrate (Abdalla, 2013 and Rady et al., 2015). Application of Moringa leaf extract gave chance to great translocation of the nutrient assimilated into healthy cells to be utilized in various metabolic process such as carbohydrate synthesis under saline soil (Semida et al., 2014).

The interaction between humus materials, Moringa leaf extract and soil salinity levels were insignificant variations for carbohydrate fractions.

Conclusion

Results of this study show that the integrative application of HM (HA+FA) and MLE was effective in alleviating the physiological response of salt stress damages. The beneficial effects of the integrative HM+MLE application were more pronounced under moderate soil salinity (6.12dS m^{-1}). The integrative HM+MLE-treated sorghum plants under salt stress maintained higher osmoprotectants, phytohormones, nucleic acids and antioxidants, concluding that this integrative treatment plays a very important role in plant growth, development and metabolism and responses to salt stress. It could also be concluded that HM and MLE can be used as plant bio-stimulants/nutritive means in integration under normal or abnormal conditions as an economic and natural source of mineral nutrients, phytohormones, amino acids, osmoprotectants, and antioxidants. Further studies are required to assess the accurate functions of HM and MLE in signaling pathways and physiological responses to abiotic stresses.

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استخدام المواد الدبالية ومستخلص أوراق المورنجا لتحسين الأثار الضارة لملوحة التربة على نباتات حشيشة السودان

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اجريت تجربة اصص خلال موسمى 2017، 2018 بصوبة كلية الزراعه جامعه الزقازيق بمحافظة الشرقية لدراسة تاثير اضافة المواد الدبالية (حامض الهيوميك، حامض الفولفيك) مع الرش الورقى بمستخلص أوراق المورنجا على الصفات المورفولوجية والفسيولوجية والبوكيمائية لنبات حشيشة السودان النامي تحت ثلاثة مستويات من ملوحة التربة.

أوضحت النتائج أن الملوحة ادت إلى حدوث انخفاض معنوى فى ارتفاع النبات والوزن الجاف والرطب للمجموع الخضرى وكذلك صبغات التمثيل الضوئى (كلورفيل أ، ب، الكاروتنويدات) والنشاط الكيموضوئي والأحماض النووية DAN & RNA والكربوهيدرات والسكريات الكلية بينما ادت إلى زيادة نشاط انزيمات البيروكسيديز الكتاليز. بينما ادى استخدام المواد الدبالية مع الرش الورقى بمستخلص أوراق المورنجا إلى تحسن تلك الصفات تحت ظروف الأجهاد الملحي.

كانت افضل النتائج المتحصل عليها لجيع الصفات السابقة عند استخدام خليط من حامض الهيوميك ، حامض الفولفيك متداخلا مع الرش الورقى بمستخلص أوراق المورنجا بينما كانت اقل النتائج فى النباتات الغير معاملة.