The Journal of Animal & Plant Sciences, 26(2): 2016, Page: 487-493
ISSN: 1018-7081

HUMIC ACID APPLICATION IMPROVES FIELD PERFORMANCE OF COTTON (*Gossypium barbadense* L.) UNDER SALINE CONDITIONS

M. M. Rady[1*], T. A. Abd El-Mageed[2], H. A. Abdurrahman[2] and A. H. Mahdi[3]

[1]Botany Department, [2]Soil and Water Department and [3]Agronomy Department, Faculty of Agriculture, Fayoum University, 63514 Fayoum, Egypt
[*]Corresponding author E-mail: mmr02@fayoum.edu.eg; mrady2050@gmail.com

ABSTRACT

Humic acid (HA) application may enhance plant stress-defence responses. This study was investigated the mitigation effect of HA on growth, photosynthesis, water use efficiency (WUE), nutritional status and yields of salt-stressed cotton plants grown for two seasons at two different sites with ECe of 3.46 and 12.86 dS m^{-1}. Each site was applied with HA at the level of 15 kg ha^{-1}. The improving effect of 15 kg HA ha^{-1} was better in the site with ECe of 3.46 dS m^{-1}. HA-treated plants showed improved photosynthetic efficiency, WUE, nutritional status, seed and lint yields and fiber quality compared to untreated plants. In contrast, the soil application of HA led to significant reductions in the leaf concentrations of Na, total soluble sugars and free proline. It has been concluded that HA has a pronounced positive effect on the growth, yields, fiber quality and WUE of salt-stressed cotton plants. Humic acid therefore has the potential to be used as a soil amendment for cotton plants to overcome the adverse effects of soil salinity.

Keywords: Cotton growth, Fiber quality, Humic substances, Soil salinity, Water use efficiency, Yield.

INTRODUCTION

Egyptian cotton (*Gossypium barbadense* L) is a major agricultural commodity that plays a prominent role in supporting the economy of country. It is grown as a textile fiber, nutritive and animal feeding crop. Cotton is classified as a salt tolerant crop, however, often adversely affected by soil salinity especially during emergence and seedling growth (Ashraf, 2002). Salinity is a major abiotic stress, reducing the growth and yield of a wide range of crops worldwide (Ashraf *et al.*, 2008; Hakim *et al.*, 2014; Seday *et al.*, 2014). Salt stress decreases the osmotic potential of soil water, reducing its availability for plants (Asik *et al.*, 2009). It also reduces the availability of essential nutrients for plants due to a poor structure of saline soils (Osman and Rady, 2012).

Efforts have been made to control salinity and others stresses by various technological means, including the application of soil amendments (Rady, 2011; Osman and Rady, 2012, Sadiq *et al.*, 2014; Semida *et al.*, 2015). Much attention has been paid to develop sustainable agriculture through the soil application of organic matters, including humic substances (HS) to alleviate the inhibitory effects of soil salinity by improving the physical and chemical properties of soils, increasing soil water retention and providing the nutrients during plant growth. Humic substances are well known as stimulators of plant growth (Vaughan, 1985) and might show anti-stress effects under salinity. These are found to increase membrane permeability and facilitate transport of essential elements within plant roots (Osman and Rady, 2012). Enhancing the uptake of nutrients and reducing the uptake of some toxic elements may be one of the mechanisms of humic acid (HA) to promote plant growth. The application of HA as soil amendment resulted in significant increases in plant growth and crop yields in reclaimed saline soils probably due to improvement of hydro-physical properties and nutrient availability of these soils (Osman and Rady, 2012).

The main purpose of this work was to evaluate the possible use of HA as a soil application to alleviate the harmful effects of salinity stress on cotton plants, explaining the role of HA in improving growth and yield of salt-stressed cotton plants and maximizing water use efficiency for optimal crop production.

MATERIALS AND METHODS

Two field experiments were conducted during summer of 2013 and 2014 at two different sites, selected in Sinnuris District, Fayoum (90 kilometers southwest Cairo, between latitudes 29° 29´ 41.28̀ N and longitudes 30° 52´ 30̀). The climatic data of studied area indicated that the total rainfalls does not exceed 7.5 mm per year and the mean minimum and maximum annual temperatures are 14.5 °C (in January) and 31.0 °C (in June), respectively. The evaporation rates coincide with temperatures where the lowest evaporation rate (1.9 mm per day) was recorded in January while the highest value (7.3 mm per day) was recorded in June (CLAC, 2010).

Salinity levels of the selected two sites were measured as the electrical conductivity (ECe) of a saturated-paste extract of the soil of each site. The main chemical characteristics of the irrigation water were: EC

3.22 dS m^{-1}; pH 7.46; sodium adsorption ratio 5.38; K^+ 1.32 meq L^{-1}; Mg^{2+} 8.00 meq L^{-1}; Ca^{2+} 8.00 meq L^{-1}; Na^+ 15.23 meq L^{-1}; $SO4^{--}$ 7.50 meq L^{-1}; Cl^- 19.50 meq L^{-1}; and HCO_3^- 5.55 meq L^{-1}. The main initial physical characteristics of the soil of both sites with loamy sand texture were: ECe 3.46 and 12.86 dS m^{-1}; sand 79.25 and 78.14%; silt 10.00 and 9.21%; clay 10.75 and 12.65%; bulk density 1.67 and 1.51 g cm^{-3}; Hydraulic conductivity 2.87 and 1.83 cm h^{-1}; field capacity 25.33 and 23.31%; welting point 9.73 and 12.48%; and available water 15.60 and 10.83%, respectively. In addition, the main chemical characteristics of the soil of both sites (Page *et al.*, 1982; Klute, 1986) are given in Table 1.

The main characteristics of the humic acid (HA) were: net HA content, 90.29% (w/w) on a dry weight (DW) basis; total N, 0.95% (w/w); total P, 1.04% (w/w); total K, 1.46% (w/w); total Ca, 2.81% (w/w); total Mg, 0.92% (w/w); total S, 0.48% (w/w); Fe, 615 mg kg^{-1} DW; Mn, 348 mg kg^{-1} DW; Zn, 301 mg kg^{-1} DW; Cu, 112 mg kg^{-1} DW; and Na, 210 mg kg^{-1} DW. Treatments on each of the both sites (3.46 and 12.86 dS m^{-1}) were soil application of two levels of HA (0 and 15 kg ha^{-1}). The selection of HA level of 15 kg ha^{-1} was based on a preliminary pot study (data not shown). Humic acid at rate of 15 kg ha^{-1} was mixed well with 150 kg fine sand, spread on the soil surface, and then mixed into the soil-surface layer. Treatments were replicated three times in a randomized complete blocks design. At each experimental site, seeds of cotton (*Gossypium barbadense* L., Giza 90 variety) were planted manually on 7 and 10 March, 2013 and 2014 seasons, respectively on hills of 20 cm apart and thinned for 2 plants per hill with the first irrigation. Calcium superphosphate (15.5% P_2O_5) at rate of 375 kg ha^{-1} was added during soil preparation. Ammonium nitrate (33.5% N) at rate of 175 kg ha^{-1} was applied in two equal splits with the 1st and 2nd irrigation. The cotton plants were irrigated ten days intervals using the amounts of applied water as 100% and the irrigation method was surface irrigation. The daily ETo was calculated from weather data according to the equation of FAO-PM (Allen *et al.*, 1998) using the average daily ETo in Fayoum region.

At seventy five days after sowing, leaf samples were taken from each experimental treatment to determine the concentrations of mineral nutrients, sodium ions, total soluble sugars, free proline and anthocyanin. Chlorophyll fluorescence was also measured.

The leaf nitrogen (N) concentration (mg g^{-1} DW) was determined according to Hafez and Mikkelsen (1981). Leaf phosphorus (P), potassium (K^+), calcium (Ca^{2+}), and sodium ions (Na^+) and leaf micro-elements (Fe, Mn, Zn and Cu) concentrations (mg g^{-1} DW) were assessed using a Perkin-Elmer Atomic Absorption and Flame Photometer (Page *et al.*, 1982). Leaf total soluble sugar concentrations were assessed according to Irigoyen *et al.* (1992). Leaf free proline contents were estimated using the rapid colourimetric method, as described by Bates *et al.* (1973). Leaf anthocyanin concentration was analyzed using the method described by Mancinelli (1990).

Chlorophyll fluorescence was measured on two different sunny days using a portable fluorometer (Handy PEA, Hansatech Instruments Ltd, Kings Lynn, UK). Fluorescence measurements included: Maximum quantum yield of PS II Fv/Fm calculated as; $Fv/Fm = (Fm - F0)/Fm$ (Maxwell and Johnson, 2000). Performance index of photosynthesis based on the equal absorption (PI_{ABS}) was calculated (Clark *et al.*, 2000).

At harvest time, the yield components [i.e. plant height (cm), total bolls per plant, seed cotton yield (t ha^{-1}), 100-seed weight (g) and total cotton yield (seed + lint) in t ha^{-1}] of each treatment were recorded from a randomly chosen ten plants of each treatment. Water use efficiency (WUE) values, as kg of yield per m^3 of applied water, were calculated for different treatments after harvest (Jensen, 1983).

Seed cotton was manually picked two times in each season (on August 18th and 23th, and on October 5th and 25th in both 2013 and 2014) from central rows of the plots to determine seed and lint yields. The measurements of fiber properties were determined at Cotton Technology Research Division, Cotton Research Institute, Giza, Egypt.

Data for two seasons were collected and subjected to a combined analysis using ANOVA procedures in GenStat statistical package (version 11) (VSN International Ltd, Oxford, UK). Difference between means was compared using LSD test at 5% level.

RESULTS

Cotton plants grown at high salinity level of 12.86 dS m^{-1} exhibited significant reduction in the concentrations of N, P, K, Ca, Fe, Mn, Zn and Cu by 41.5%, 26.1%, 62.8%, 35.2%, 14.2%, 22.3%, 35.2% and 17.4%, respectively, compared to plants grown under 3.46 dS m^{-1} (Tables 2 and 3). In contrast, Na^+ concentration was significantly increased by 19.6%. Humic acid (HA) application was observed to alleviate these adverse effects of soil salinity and significantly reduced Na^+ concentration in cotton plants under both 3.46 and 12.86 dS m^{-1} by 16.0% and 15.4%, respectively, compared to no HA application. In addition, the concentrations of N, P, K, Ca, Fe, Mn, Zn and Cu were significantly increased in cotton plants with HA application compared to control without any HA. In the saline soil with 12.86 dS m^{-1}, HA application increased the concentrations of N, P, K, Ca, Fe, Mn, Zn and Cu in cotton plants by 40.7%, 48.5%, 104.9%, 50.0%, 64.6%, 40.4%, 47.7% and 25.9%, respectively, while decreased the concentration of Na in cotton tissues by 20.5%.

The concentrations of total soluble sugars and free proline in cotton plants grown under salinity at the level of 12.86 dS m^{-1} significantly increased by 14.9% and 55.3%, respectively, compared to those in plants grown under 3.46 dS m^{-1} (Table 4). In contrast, anthocyanin concentration and chlorophyll fluorescence (Fv/Fm and PI_{ABS}) of cotton plants were significantly reduced by 9.1% and (48.7% and 71.6%), respectively. However, HA application significantly decreased the concentrations of total soluble sugars and free proline, while significantly increased anthocyanin concentration and chlorophyll fluorescence in cotton plants compared to plants grown in HA-free soil. In the saline soil at 12.86 dS m^{-1}, HA application increased anthocyanin concentration, Fv/Fm and PI_{ABS} of cotton plants by 24.0%, 105.1% and 170.0%, respectively, while decreased the concentrations of total soluble sugars and free proline in cotton plants by 14.5% and 6.4%, respectively.

The cotton plants grown under salinity at 12.86 dS m^{-1} level revealed significant reductions in plant height, number of bolls, seed yield, lint yield, total yield, fiber strength and water use efficiency (WUE) by 30.8%, 51.0%, 42.9%, 46.8%, 44.6%, 9.3% and 45.0%, respectively, compared to plants grown under 3.46 dS m^{-1} salinity level (Tables 5 and 6). In contrast, 100-seed weight, fiber length and micronaire value were not affected. HA application to saline soil was observed to overcome these adverse effects of soil salinity and significantly increased the above mentioned characteristics compared to plants grown in saline soil without HA application. However, in the saline soil at 12.86 dS m^{-1}, HA application increased plant height, number of bolls, seed yield, lint yield, total yield and WUE by 9.7%, 25.4%, 15.7%, 13.6%, 14.2% and 18.2%, respectively.

Table 1. Some initial chemical properties of the experimental soil

Properties	**Site 1**	**Site 2**
pH [at a soil: water(w/v) ratio of 1:2.5]	7.84	7.66
Ece (dS.m^{-1}; soil – paste extract)	3.46	12.86
CEC ($cmol_e$ kg^{-1})	10.11	9.91
$CaCO_3$ %	5.03	3.30
Organic matter %	1.30	1.14
ESP (exchangeable sodium percentage)	13. 54	15.94
Soluble ions (meq L^{-1}):		
Ca^{++}	12.0	19.0
Mg^{++}	8.50	12.5
Na^{++}	16.32	115.84
K^{+}	0.75	2.53
HCO_3^{-}	2.25	4.24
Cl^{-}	20.59	80.11
$SO4^{--}$	14.73	66.47
Exchangeable cations (meq $100g^{-1}$ soil):		
Ca^{++}	5.37	6.25
Mg^{++}	2.78	0.92
Na^{++}	1.36	1.58
K^{+}	0.42	1.09
Available nutrients (mg kg^{-1} soil):		
N	50.00	40.00
P	520.93	592.49
K	55.36	44.11
Fe	2.82	2.97
Mn	6.6	6.52
Zn	0.85	0.82
Cu	0.35	0.33

Table 2. Concentrations of N, P, K, Ca and Na in cotton leaves as influenced by humic acid (HA) application of saline soil

Treatments		N	P	K	Ca	Na
Soil salinity (dS m^{-1})	HA (kg ha^{-1})	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)	(mg g^{-1} DW)
3.46	0	27.7 ± 5.3b*	0.92 ± 0.02c	21.8 ± 1.2b	6.17 ± 0.60b	16.3 ± 0.5b
	15	35.9 ±2.4a	1.15 ± 0.05a	26.4 ± 0.7a	7.67 ± 0.15a	13.7 ± 1.9c
12.86	0	16.2 ± 1.4d	0.68 ± 0.03d	8.1 ± 0.9d	4.00 ± 0.5c	19.5 ± 0.8a
	15	22.8 ± 2.3c	1.01 ± 0.05b	16.6 ± 0.4c	6.00 ± 0.58b	15.5 ± 0.3b

*Values are means ± SE (n = 3). Mean values in each column followed by a different lower-case-letter are significantly different (LSD) at $P \leq 0.05$.

Table 3. Concentrations of Fe, Mn, Zn and Cu in cotton leaves as influenced by humic acid (HA) application of saline soil

Treatments		Fe	Mn	Zn	Cu
Soil salinity (dS m^{-1})	HA (kg ha^{-1})	(mg g^{-1} DW)	(mg kg^{-1} DW)	(mg kg^{-1} DW)	(mg kg^{-1} DW)
3.46	0	2.04 ± 0.25c*	49.4 ± 0.3c	165.4 ± 15.2b	30.4 ± 0.4b
	15	2.67 ± 0.04b	60.9 ± 0.6a	307.4 ± 10.7a	34.0 ± 0.9a
12.86	0	1.75 ± 0.30d	38.4 ± 0.7d	107.2 ± 6.6c	25.1 ± 0.9c
	15	2.88 ± 0.48a	53.9 ± 0.9b	158.3 ± 10.5b	31.6 ± 0.9b

*Values are means ± SE (n = 3). Mean values in each column followed by a different lower-case-letter are significantly different (LSD) at $P \leq 0.05$.

Table 4. Effect of humic acid (HA) application on leaf concentrations of total soluble sugars, free proline and anthocyanin, and leaf chlorophyll fluorescence ratio (Fv/Fm) and performance index (on absorption basis; PI_{ABS}) of cotton plants grown on saline soil

Treatments		Soluble sugars	Free proline	Anthocyanin	Fv/Fm	PI_{ABS}
Soil salinity (dS m^{-1})	HA (kg ha^{-1})	(mg g^{-1} DW)	(μg g^{-1} DW)	(mg g^{-1} DW)		
3.46	0	4.95 ± 0.26b*	1.52 ± 0.11c	0.55 ± 0.01b	0.76 ± 0.04b	3.63 ± 0.89c
	15	3.23 ± 0.68c	1.02 ± 0.04d	0.64 ± 0.01a	0.81 ± 0.03a	4.72 ± 1.01b
12.86	0	5.69 ± 0.39a	2.36 ± 0.16a	0.50 ± 0.02c	0.39 ± 0.03c	2.03 ± 0.02d
	15	4.86 ± 0.32b	2.21 ± 0.05b	0.62 ± 0.02a	0.80 ± 0.05ab	5.48 ± 1.34a

*Values are means ± SE (n = 3). Mean values in each column followed by a different lower-case-letter are significantly different (LSD) at $P \leq 0.05$.

Table 5. Effect of humic acid (HA) application on plant growth, and seed and lint yields of cotton plants grown on saline soil

Treatments		Plant height	Number	100-seed	Seed yield	Lint yield
Soil salinity (dS m^{-1})	HA (kg ha^{-1})	(cm)	of bolls per plant	weight (g)	(t ha^{-1})	(t ha^{-1})
3.46	0	83.7 ± 1.4b*	14.5 ± 0.3a	7.90 ± 0.10b	1.56 ± 0.07b	1.11 ± 0.06b
	15	99.9 ± 3.6a	15.4 ± 0.5a	8.67 ± 0.12a	1.81 ± 0.06a	1.30 ± 0.06a
12.86	0	57.9 ± 1.9d	7.1 ± 0.9c	7.90 ± 0.19b	0.89 ± 0.03d	0.59 ± 0.03d
	15	63.5 ± 0.8c	8.9 ± 0.6b	7.82 ± 0.17b	1.03 ± 0.03c	0.67 ± 0.04c

*Values are means ± SE (n = 10). Mean values in each column followed by a different lower-case-letter are significantly different (LSD) at $P \leq 0.05$.

Table 6. Effect of humic acid (HA) application on total yield (seed and lint), water use efficiency (WUE %) and fiber quality characteristics of cotton plants grown on saline soil

Treatments		Total yield (seed + lint) (t ha^{-1})	WUE (%)	Fiber length U.H.M. (mm)	Micronaire value	Fiber strength (tppsi)
Soil salinity (dS m^{-1})	HA (kg ha^{-1})					
3.46	0	2.67 ± 0.05b*	0.40 ± 0.01b	30.4 ± 0.6	4.37 ± 0.07a	107.0 ± 1.6a
	15	3.10 ± 0.08a	0.46 ± 0.03a	30.7 ± 0.2	3.93 ± 0.03b	110.0 ± 1.5a
12.86	0	1.48 ± 0.07d	0.22 ± 0.01d	30.0 ± 0.5	4.30 ± 0.06a	97.0 ± 1.7b
	15	1.69 ± 0.09c	0.26 ± 0.01c	30.7 ± 0.2	4.07 ± 0.07b	104.3 ± 1.7ab

*Values are means ± SE (n = 10). Mean values in each column followed by a different lower-case-letter are significantly different (LSD) at $P \leq 0.05$.

DISCUSSION

Due to salinity, agricultural productivity faces a great problem worldwide, particularly in the arid and semiarid regions. In these regions, salinity caused by many factors such as low rainfall, high evaporation rate, poor irrigation water and its management and accumulation of salts in the top layer of the soil due to over-irrigation (Rady *et al.*, 2013). In this study, the reduction in plant growth in saline soil could be attributed to the osmotic effect as a result of salt stress that caused increase in growth inhibitors (i.e., abscisic acid) and decreased growth promoters (i.e., indole-3-acetic acid and gibberellins), hence disturbances in the water balance of the stressed plants, leading to stomatal closure, ionic imbalance, reduction in photosynthesis, accumulation of toxic ions and consequently inhibition of growth (Rady *et al.*, 2013; Semida and Rady, 2014).

Humic acid (HA) application improved the chemical properties of soils because it increases the number of soil microorganisms, which enhance nutrient cycling, and reduce soil pH (Osman and Rady, 2012), thus leading to increase in the availability of mineral nutrients to plant roots in present study (Tables 2 and 3). HA also promotes plant growth through its effect on ion transfer at the root level by activating the oxidation-reduction state of the medium that leads to increase in the absorption of nutrients by preventing their precipitation in the nutrient solution of the growing medium (Osman and Rady, 2012). The improved leaf nutrient composition in cotton plants by HA application could be explained by the improved availability of essential nutrients in the root zone, resulting from their solubilization caused by the release of organic acids. HA can also reduce the surface tensión of water and increase the effectiveness of nutrients or chemicals. In addition, using the full irrigation requirements (100% of ET_o) with the application of HA significantly increased the leaf concentrations of N, P, K, Ca, Mn, Fe, Cu and Zn consequently increased their uptake by plant roots under soil salinity level of 12.86 dS m^{-1} (Table 2 and 3). This agreed with the earlier work reported by Rady (2012). He reported that humic acid (a component of the organo-mineral fertilizer) improves chemical properties of the soil by increasing the soil microorganisms, which enhance nutrient status of plants by increased absorption of nutrients by preventing precipitation in the nutrient solution. Humic acid led to higher concentrations of nutrients, including the elemental K, leading to a corresponding increase in chlorophyll fluorescence (Table 4), which can serve as an indicator of the stress induced by alterations in the balance of endogenous hormones (Marschner, 1995). HA also improves the cell permeability, making for a more rapid entry of nutrients into root cells result in higher uptake of plant nutrients (Rady, 2011).

Soluble sugars were increased in plants subjected to the adverse conditions of untreated saline soil (Table 4), but reduced when soil applied with HA. They contribute to osmotic adjustment (Hayashi *et al.*, 1997) and can directly or indirectly modulate the expression of genes included in metabolic processes and storage functions (Hebers and Sonnewald, 1998). In addition, the level of free proline increased under the adverse conditions of the saline soil without HA application. The acceleration of increased pool of proline results in an increase in the capacity of plant tolerance to the adverse conditions of the saline soil under study. The application of HA to saline soil reduced the concentrations of total soluble sugars and free proline in cotton plants, which may be attributed to the crucial role of HA in mitigating the deleterious effects of soil salinity. In addition, anthocyanin is utilized as a precursor with cytoprotective function in the secondary metabolism. Thus, the increased level of anthocyanin in cotton plants grown in saline soil treated with HA indicates an index for a good mechanism of plant tolerance towards the changes in the environmental conditions (Semida *et al.*, 2014).

Cotton plants grown in saline soil applied with HA had Fv/Fm values above 0.70, showing no stress effects, while the control plants had lower Fv/Fm values, representing stress effects. Significant increases were also observed in PI_{ABS} of the HA-treated plants compared to the control plants (Table 4). Mechanisms suggested to the stimulatory effect of HA hypothesize a ‘direct’ action on the plants, which is hormonal in nature, together with a positive ‘indirect action’ on the metabolism of soil microorganisms, the dynamics of uptake of soil nutrients, and soil physical condition (Semida *et al.*, 2014). Soil application with HA enhanced seed and lint cotton yields, water use effiency and fiber quality under soil salinity (Tables 5 and 6). This may be attributed to HA improved the soil structure and soil physical properties, increasing the uptake of essential nutrients, contributing to large improvement in these traits.

Conclusion: Application of HA to saline soils (ECe = 12.86 dS m^{-1}) improved plant stress-defence responses resulting better plant performance under stress in direct and indirect manners. Thus, the application of HA may provide a useful amendment to reduce the adverse effects of salinity stress on cotton plants grown in saline soils.

REFERENCES

Allen, R.G., L.S. Pereira, D. Raes, and M. Smith (1998). Crop Evapotranspiration Guidelines for Computing Crop Water Requirements. FAO Irrig. and Drain. Paper 56. United Nations, Rome, Italy, pages 30-42.

Ashraf, M. (2002). Salt tolerance of cotton: some new advances. Crit. Rev. Plant Sci. 21: 1–30.

Ashraf, M., H.R. Athar, P.J. Harris, and T.R. Kwon (2008). Some prospective strategies for improving crop salt tolerance. Adv. Agron. 97: 45–110.

Asik, B.B., M.A. Turan, H. Celik, and A.V. Katkat (2009). Effects of humic substances on plant growth and mineral nutrients uptake of wheat (*Triticum durum* cv. Salihli) under conditions of salinity. Asian J. Crop Sci. 1: 87–95.

Bates, L.S., R.P. Waldren, and I.D. Teare (1973). Rapid determination of free proline for water-stress studies. Plant Soil. 39: 205–207.

CLAC (2010). The Climatic Normal - El Fayoum Station. Annual report, Central Laboratory for Agricultural Climate (CLAC), Cairo, Egypt.

Clark, A.J., W. Landolt, J.B. Bucher, and R.J. Strasser (2000). Beech (*Fagus sylvatica*) response to ozone exposure assessed with a chlorophyll a fluorescence performance index. Environ. Poll. 109: 501–507.

Hafez, A.R., and D.S. Mikkelsen (1981). Colorimetric determination of nitrogen for evaluating the nutritional status of rice. Commun. Soil Sci. Plant Anal. 12: 61–69.

Hakim, M.A., A.S. Juraimi, M.M. Hanafi, M.R. Ismail, M.Y. Rafii, M.M. Islam, and A. Selamat (2014). The effect of salinity on growth, ion accumulation and yield of rice varieties. J. Anim. Plant Sci. 24(3): 874–885.

Hayashi, H., L.M. Alia, P. Deshnium, M. Ida, and N. Murata (1997). Murata transformation of *Arabidopsis thaliana* with the coda gene for choline oxidasa: accumulation of glycine betaine and enhanced tolerance to salt and cold stress. Plant J. 12: 133–142.

Hebers, K., and V. Sonnewald (1998). Altered gene expression: brought about by interand pathogen interactions. J. Plant Res. 111: 323–328.

Irigoyen, J.J., D.W. Emerich, and M. Sanchez-Diaz (1992). Water stress induced changesin the concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. Plant Physiol. 8: 455–460.

Jensen, M.E. (1983). Design and operation of farm irrigation systems. ASAE, Michigan, USA, p. 827.

Klute, A. (1986). Methods of Soil Analysis. Part-I: Physical and Mineralogical Methods (2nd Ed). American Society of Agronomy, Madison, Wisconsin, USA.

Mancinelli, A.L. (1990). Interaction between light quality and light quantity in the photoregulation of anthocyanin production. Plant Physiol. 92: 1191–1195.

Marschner, H. (1995). Mineral Nutrition of Higher Plants. 2nd Ed. Acad. Press Publ, NY, USA, pp. 559–579.

Maxwell, K., and G.N. Johnson (2000). Chlorophyll fluorescence - a practical guide. J. Exp. Bot. 51: 659–668.

Osman, A.SH., and M.M. Rady (2012). Ameliorative effects of sulphur and humic acid on the growth, antioxidant levels, and yields of pea (*Pisum sativum* L.) plants grown in reclaimed saline soil. J. Hortic. Sci. Biotechnol. 87: 626–632.

Page, A.I., R.H. Miller, and D.R. Keeny (1982). Methods of Soil Analysis. Part II. Chemical and Microbiological Methods. 2nd Ed. Amer. Soc. Agron, Madison, WI, USA, pp. 225–246.

Rady, M. M. (2011). Effects on growth, yield, and fruit quality in tomato (*Lycopersicon esculentum* Mill.) using a mixture of potassium humate and farmyard manure as an alternative to mineral-N fertilizer. J. Hortic. Sci. Biotechnol. 86: 249–254.

Rady, M. M. (2012). A novel organo-mineral fertilizer can mitigate salinity stress effects for tomato production on reclaimed saline soil. S. Afr. J. Bot. 81: 8–14.

Rady, M. M., B.C. Varma, and S. M. Howladar (2013). Common bean (*Phaseolus vulgaris* L.) seedlings overcome NaCl stress as a result of presoaking in *Moringa oleifera* leaf extract. Sci. Hortic. 162: 63–70.

Sadiq, S.A., D.M. Baloch, N. Ahmed, and Hidayatullah (2014). Role of coal-derived humic acid in the availability of nutrients and growth of sunflower under calcareous soil. J. Anim. Plant Sci. 24(5): 1737–1742.

Seday, U., O. Gulsen, A. Uzun, and G. Toprak (2014). Response of citrus rootstocks to different salinity levels for morphological and antioxidative enzyme activities. J. Anim. Plant Sci. 24(2): 512–520.

Semida, W.M., T.A. Abd El-Mageed, and S.M. Howladar (2014). A novel organo-mineral fertilizer can alleviate negative effects of salinity stress for eggplant production on reclaimed saline calcareous soil. Acta Hortic. 1034: 493–499.

Semida, W.M., T.A. Abd El-Mageed, S.M. Howladar, G.F. Mohamed, and M.M. Rady (2015). Response of *Solanum melongena* L. seedlings grown under saline calcareous soil conditions to a new organo-mineral fertilizer. J. Anim. Plant Sci. 25(2): 485–493.

Semida, W.M., and M.M. Rady (2014). Presoaking application of propolis and maize grain extracts alleviates salinity stress in common bean (*Phaseolus vulgaris* L.). Sci. Hortic. 168: 210–217.

Vaughan, D., and R.E. Malcolm (1985) Influence of humic substances on growth and physiological process. In: “Soil Organic Matter and Biological Activity” (Vaughan, D., and R.E. Malcolm, Eds.). Kluwer Acad. Publ., Dordrecht, Netherlands, pp. 37–75.