Treatments to Alleviate Adverse Effect of Saline Soil on Growth and Productivity of Balady Mandarin Trees (*Citrus reticulata* Blanco)

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ABSTRACT

To study the effect of soil amendments combined with foliar application of Salicylic acid on soil properties, growth, yield, fruit quality and leaf nutrient content of Balady mandarin trees, an experiment in split plot format based on complete randomized block design with three replicates in Sidy Ghazy, Kafr El-Sheikh governorate was conducted. The main plots of experiment were used for foliar application of Salicylic acid with three concentrations (tap water, 200 and 300 ppm) and sub plots were for soil amendments with Humic acid, Biotol, Humic acid + Biotol and Control. The obtained results showed that application of Humic acid + Biotol combined with Salicylic acid foliar spray at 300 ppm in mandarin orchard appeared to be superior in improving saline soil by enhancing soil chemical parameters in terms of EC, pH, CEC, SAR, nutrients and microbial population, in addition it induced a significant improvement of tree size and vegetative growth under saline soil conditions. Moreover, leaf analysis showed highly significant increase of N, P, K, Fe, Mn and Zn leaf contents with soil amendments and foliar spray of Salicylic acid at 300 ppm. The highest yield with good quality was obtained with the interaction between Humic acid plus Biotol and along with Salicylic acid foliar spray at 300 ppm. Thus, Humic acid, Biotol and foliar spray with Salicylic acid could contribute to the reduction of soil nutrient loss and mitigation of the negative effects of saline stresses on plant growth and productivity. Therefore, this study recommended the application of Humic acid + Biotol + Salicylic acid foliar application at 300 ppm, which gave the best growth, yield, fruit quality and nutritional status of Balady mandarin trees.

Key words: humic acid, salt stress, biofertilizers, *Citrus reticulata*, foliar application.

INTRODUCTION

Soil salinity is one of the most important environmental factors limiting citrus growth and productivity. The adverse effects of saline soil on citrus trees growth and yield, due to osmotic inhibition of water uptake by roots, ion toxicity, nutritional imbalance and accumulation of Na^+ and Cl(Moya *et al*., 2002; Al-Yassin, 2005). So, strategies to alleviating adverse effect of salt stress on citrus trees have been considered to be very important in order to sustain productivity from these soil and save it from degradation. Salicylic acid (H_4SiO_4) is a natural growth regulator in vascular plants. Salicylic acid stimulates many physiological processes like photosynthesis, transpiration and ion uptake(Vazirimehr and Rigi 2014). Salicylic acid induces plant resistance to stresses at low and high temperatures, heavy metals, drought and salinity (Khan *et al*., 2015). The ameliorative effects of salicylic acid have been well documented in inducing salt tolerance via accelerating their photosynthesis performance and carbohydrate metabolism, salicylic acid increased the protein contents inside the plant cells that enhance the ability to tolerant salt stress(Li *et al.*, 2014). A few studies were done on different fruit species using salicylic acid as a foliar spray, under salt stress conditions, concluded that salicylic acid reduces the adverse effects of salinity stress on growth and productivity of fruit trees(Aly *et al*. 2015) on Valencia orange.

Similarly, humic acid as a bio-stimulant has enormous beneficial effects on soil and plant attributes. Humic acid improve the physical, chemical and biological properties of soil(Khaled and Fawy, 2011). Enhancement of total chlorophyll contents, stomatal conductance, net photosynthesis rate and transpiration rate resulted in greater plant growth with humate application(Abbas *et al*., 2013). Increasing the permeability of plant membranes due to humate application resulted in improving growth of various groups of beneficial microorganisms(Nardi *et al*., 2002). Moreover, humic acid application markedly minimized the harmful effects of salinity and enhanced salt tolerance(Abd El-Hamied 2014b and Abobatta 2015) on Valencia orange.

Large number of bacterial species mostly those associated with the plant rhizosphere are able to exert a beneficial effect upon plant. They have been called plant growth promoting rhizobacteria(Hayat *et al*., 2010). Azotobacter *spp.* and Azospirillum spp. are nitrogen fixing and produce different GAs specially GA_1 and GA_3(Bottini *et al*., 2004). The use of those bacteria as bio-fertilizers which are the most important for plant production and soil as they play an important role in increasing vegetative growth, yield and fruit quality(El-Khayat and Abdel

Rehiem, 2013 on Mandarin and El-Khawaga and Maklad 2013 on Valencia orange). In pervious studies, using biofertilizers as soil application to alleviate salinity stress lowered soil pH, increased the uptake of water and nutrients and enhanced soil fertility. In addition, they enhanced leaf chlorophyll content, yield, fruit quality and leaf minerals content (Abdelaal *et al.*, 2013 on Valencia orange).

This investigation aims to study the effect of Humic acid and Biotol(biofertilizer) as soil amendments along with Salicylic acid as foliar spray on soil properties, growth, yield, fruit quality and nutritional status of Balady mandarin trees in salt affected soils.

MATERIALS AND METHODS

The present study was carried out during 2013, 2014 and 2015 seasons, the first year was considered as a preliminary trial to prepare trees to received treatments. Seventy two Balady mandarin(Citrus reticulata Blanco*)* budded on sour orange(Citrus aurantium L.) rootstock, planted at 4x4 meter in a private orchard situated at Sidy Ghazy, Kafr El-Sheikh governorate were chosen. Selected trees were uniform in vigor and productivity and have fifteen years old. The trees received the same cultural practices and the fertilization program applied by the farmer for each tree was: ½ kg/tree superphosphate(37%) applied at one dosage in December, 4.5kg/tree ammonium sulphate(20.5%) given at three equal applications on February, May and late of June and 1.25kg/tree potassium sulphate applied at two equal doses on March and late June. Mechanical and chemical analysis of the experimental soil are shown in Table (1).

This experiment was planned in completely randomized block design in split plot arrangement with three replicates for each treatment and six trees per each treatment.

Treatments:

Main plots(factor A): Salicylic acid as foliar application

a_1 tap water

a_2 200 ppm

a_3 300 ppm

Sub plots(factor B): Soil amendments

b_1 Humic acid

b_2 Biotol (biofertilizer)

b_3 Humic acid + Biotol (biofertilizer)

b_4 Control (untreated trees).

Salicylic acid(H_4SiO_4) was added at three concentrations i.e. control(tap water), 200 and 300 ppm three times, before flowering, after fruit setting and in August via a foliar application. Humic acid at 20 ml/tree(2.9 % humic acid) was added in December, March, June and August via soil application. The Biotol which produced from the Agricultural Research Center(ARC, Giza, Egypt) contains N_2-fixing free living bacterial cultures Azotobacter chroococcum and Azospirillium lipoferum and phosphate dissolving bacterial culture Bacillus megaterium was added in four equal doses at the rate of 4 ml/liter Biotol/tree via soil application in the first week of December, March, June and August.

The following data was recorded:

A. Soil characteristics:

1. Physical and chemical soil analyses:

Before applying the treatments and at the end of experiment, soil samples were taken from each treatment at major root zone(0 – 60 cm depth). Soil samples were prepared for chemical analysis according to(Jackson, 1967). These soil samples were dried, sieved through a 2 mm mesh and analyzed for texture, exchangeable cations Ca, Mg, K and Na, soluble cations and anions, soil pH and EC as well as available N, P, K, Fe, Mn and Zn were determined according to Page et al.(1982).

2. Soil microbial population:

Samples were collected at harvest time(last week of December) from the rhizosphere region to microbial analyses: Total bacterial count was done using dilution plate method and nutrient agar medium. Enumeration density of Azotobacter spp. was measured using MPN technique and medium of(Abd El-Malek and Ishak, 1968). Enumeration density of Azospirillum spp. was determined using MPN technique and semi-solid medium (Dobereiner, 1978).

B. Horticulture parameters:

1. Vegetative growth:

Shoot length(cm), leaves number per shoot and leaf area(cm^2) was measured by using a leaf area meter Model Li 3100 area - meter .

2. Leaf nutrient contents:

In August, fully mature leaves were separated from nonbearing shoots, washed, cleaned and oven dried to constant weight at 60-65C° and weighted. The dried leaf samples of each replicate were grounded and digested with H_2SO_4 and H_2O_2 according Evenhuis and DeWaard(1980).

Table 1: Mechanical and chemical analysis of experimental soil.

Mechanical			Chemical			Cations (meq/l)			Anions (meq/l)			
Sand %	Silt %	Clay %	pH	Ec dS/m^{-1}	o.m %	Na^+	Ca^{++}	Mg^{++}	CO_3^-	HCO_3^-	Cl^-	$SO4^{--}$
17.1	21.4	61.5	8.2	3.8	1.09	65.6	50.8	22.5	--	4.6	91.2	166.8

In digested solution samples N, P, K, Fe, Mn and Zn were determined as follows: nitrogen was determined by micro-Kjeldahl method (A.O.A.C. 1985), K by flame photometer, P by spectrophotometer, Fe, Mn and Zn were assayed with Atomic Absorption spectrophotometer (Unican SP 1900) according to Chapman and Pratt (1961).

3. Yield:

At harvest time (late December); the yield of each tree was determined as the number and weight (kg) of fruits per tree.

4. Fruit quality:

Fifteen fruits were taken at random from the yield of each tree to determine physical characteristics such as: fruit weight(g), fruit diameter(cm) and chemical properties; soluble solids content was determined by hand refractometer. Total acidity as citric acid and vitamin C as ascorbic acid (mg/100 ml juice) were determined according to(A.O.A.C., 1985). SSC/acid was calculated.

Statistical analysis:

Statistical analysis was done as analysis of variance according to Snedecor and Cochran (1967), and the least significant differences(L.S.D. at 5% level) was used to compare the means value.

RESULTS AND DISCUSSION

A. Soil characteristics:

1. Soil chemical properties:

Data presented in Table 2 clearly showed that, application of Humic acid, Biotol and Humic acid + Biotol on saline soil were effective in decreasing soil electric conductivity(EC), soil pH and sodium absorption ratio (SAR). Meanwhile cation exchange capacity(CEC) was also increased at the end of experiment when compared with the value before the experiment. In this respect, treatment of Humic acid + Biotol gave the lowest values of EC, pH and SAR and the highest value of CEC was observed by treatments of Biotol and Humic acid respectively. Similar results were reported by Mohamed *et al.*, (2013) on Valencia orange and Abed El-Hamied, (2014a) on Clementine mandarin. They concluded that, Humic acid and biofertilizers improving soil properties included hydraulic conductivity, bulk density, aggregation, pH and EC. Such conclusion confirmed by Ouni *et al.*,(2014) who showed that under saline conditions the humic substances distribution decreased soil Na, EC and pH values.

2. Available nutrients in the soil:

Data in Table 3 cleared that, the treatments of Humic acid, Biotol and Humic acid + Biotol caused an increase in soil nitrogen, phosphorus, potassium, iron, manganese and zinc contents at the end of the experiment compared to soil analysis before treatments. Moreover, treatment of Humic acid + Biotol exhibited the highest soil nutrients followed by Biotol and Humic acid treatments in comparison with soil without treatments. Similar results were reported by Mohamed et al.(2013) on Valencia orange. Moreover, Abed El-Hamied(2014a) reported that treated soil with biofertilizers(EM) plus organic manure caused an increase in N, P, K, Fe, Mn and Zn of Clementine orchard soil under saline water irrigation.

Table 2: Effect of Humic acid and Biotol on some soil properties.

Soil treatments	Before Experiment	after experiment	% of increase or reduction
		EC dS/m^{-1}	
Humic acid	3.75	3.15	- 16.00 %
Biotol	3.75	2.93	- 21.87 %
Humic acid + Biotol	3.75	2.64	- 29.60 %
L.S.D. at 5%	ns	0.16	
		pH	
Humic acid	8.20	7.90	- 4.66 %
Biotol	8.20	7.60	- 7.32 %
Humic acid + Biotol	8.20	7.30	- 10.98 %
L.S.D. at 5%	ns	0.22	
		CEC $cmol_c\ kg^{-1}$	
Humic acid	37.55	38.34	+ 2.10 %
Biotol	37.55	38.79	+ 3.30 %
Humic acid + Biotol	37.55	40.80	+ 4.65 %
L.S.D. at 5%	ns	0.80	
		SAR ratio	
Humic acid	10.38	9.89	- 4.70 %
Biotol	10.38	9.79	- 5.70 %
Humic acid + Biotol	10.38	9.63	- 7.20 %
L.S.D. at 5%	ns	0.24	

ns = non significant

Table 3: Effect of Humic acid and Biotol on soil macro and micro-nutrients.

Soil treatments	Before Experiment	after experiment	% of increase or reduction
		N (mg/kg/soil)	
Humic acid	82.85	194.13	+ 134.3 %
Biotol	82.85	217.40	+ 162.5 %
Humic acid + Biotol	82.85	301.28	+ 263.6 %
L.S.D. at 5%	ns	4.62	
		P (mg/kg/soil)	
Humic acid	4.74	8.66	+ 82.7 %
Biotol	4.74	9.87	+ 108.2 %
Humic acid + Biotol	4.74	17.24	+ 263.7 %
L.S.D. at 5%	ns	0.34	
		K (mg/kg/soil)	
Humic acid	67.59	120.15	+ 77.8 %
Biotol	67.59	137.43	+ 104.8 %
Humic acid + Biotol	67.59	202.34	+ 199.4 %
L.S.D. at 5%	ns	0.81	
		Fe (mg/kg/soil)	
Humic acid	28.63	48.70	+ 70.1 %
Biotol	28.63	47.20	+ 64.9 %
Humic acid + Biotol	28.63	52.52	+ 83.4 %
L.S.D. at 5%	ns	1.02	
		Mn (mg/kg/soil)	
Humic acid	9.50	19.84	+ 108.8 %
Biotol	9.50	16.99	+ 78.8 %
Humic acid + Biotol	9.50	21.64	+ 127.8 %
L.S.D. at 5%	ns	0.54	
		Zn (mg/kg/soil)	
Humic acid	3.99	7.81	+ 95.7 %
Biotol	3.99	7.64	+ 91.5 %
Humic acid + Biotol	3.99	8.73	+ 118.8 %
L.S.D. at 5%	ns	0.15	

ns = non significant

3. Soil microbial population:

Data in Table 4 showed the effect of Humic acid, Biotol, Humic acid + Biotol on soil microbial population, and pointed out that all treatments led to an increase in total bacteria, Azotobacter chroococcum, Azospirillum lipoferum and Bacillus megaterium in root zone of Balady mandarin trees. In this respect, data clearly showed that the largest values of total bacteria, Azotobacter chroococcum, Azospirillum lipoferum and Bacillus megaterium were obtained when inoculated trees by Biotol plus Humic acid followed by Biotol and Humic acid respectively. The obtained results were in harmony with those obtained by Abed El-Hamied (2014a) who indicated that, application of organic manure and EM greatly increased the number of total fungi, total bacteria and total actinomycetes in root rhizosphere of Clementine mandarin under saline water irrigation. In addition, Mohamed *et al*. (2013) working on Valencia orange trees growing on salt affected soil treated with biofertilizers(mixture of Cyanobacteria and Azolla), indicate that treatments enhanced biological activity in root rhizosphere of the soil, in terms of increasing the total bacterial counts, total cyanobacterial counts and CO_2 evolution as compared to control treatment.

These results are supported by the findings of Fahramand *et al*.,(2014) who concluded that Humic acid had an important role in improving soil aggregation and water movement which leaching the excessive soluble salts. In this respect, Khaled *et al.* (2012) found that physical properties such as hydraulic conductivity, bulk density and total porosity of salt affected soil greatly improved when compost is applied. Moreover, Mohamed *et al*., (2013) found that the electric conductivity (EC) of the soil treated with Humic acid was lower than the non-treated one. Moreover, in salt affected soil, the sodium percentage in water generally increases, in this situation humus complex was considered the effective amelioration methods to removl of exchange of soluble sodium and changing the ionic composition of soils.

Table 4: The accumulative effect of Humic and Biotol on the activity of three kind of bacteria.

Soil treatments	Before Experiment	after experiment	% of increase or reduction
	Total bacterial counts $\times 10^5$ cfu/g soil		
Humic acid	60.98	79.98	+ 31.1 %
Biotol	60.98	110.44	+ 81.1 %
Humic acid + Biotol	60.98	140.70	+ 130.8 %
L.S.D. at 5%	ns	0.86	
	Azotobacter counts $\times 10^5$ cfu/g soil		
Humic acid	16.83	20.62	+ 22.5 %
Biotol	16.83	30.10	+ 78.8 %
Humic acid + Biotol	16.83	43.05	+ 155.8 %
L.S.D. at 5%	ns	0.61	
	Azospirillum counts $\times 10^5$ cfu/g soil		
Humic acid	13.84	18.21	+ 31.6 %
Biotol	13.84	29.74	+ 114.9 %
Humic acid + Biotol	13.84	39.30	+ 183.9 %
L.S.D. at 5%	ns	0.88	
	Bacillus counts × 105 cfu/g soil		
Humic acid	30.28	40.81	+ 34.8 %
Biotol	30.28	80.39	+ 165.5 %
Humic acid + Biotol	30.28	91.37	+ 201.8 %
L.S.D. at 5%	ns	0.79	

ns = non significant

At the same time, leaching the sodium salts out of the soil profile was reported by (Ouni *et al*., 2014)**.** In addition, Andrade *et a*l. (1998) reported that soil aggregation is a dynamic process in which plants and the soil microorganisms play a major role. It has been supposed that vascular arbscular mycorrhizal fungi and N-fixing bacteria (*Azotobacter spp*. and *Rhizobium spp*.) could play several important roles including the binding of the soil particles into stable aggregates.

B. Horticulture parameters:

1. Vegetative growth:

Data in Table 5 showed that, soil applications of Humic acid and Biotol combined with Salicylic acid foliar spray had a significant effect on all vegetative growth parameters in both seasons, it was clear that shoot length, leaves number per shoot and leaf area were significantly increased as compared with control in both seasons.

As for the effect of foliar application of Salicylic acid, it was clear that foliar application of Salicylic acid concentrations had a positive effect on all growth parameters in this study compared to spray with tap water. The highest values of shoot length, leaves number per shoot and leaf area were recorded on trees sprayed with 300 ppm Salicylic acid. These results agreed with those of Al-Taey(2009) who stated that application of acetyl salicylic acid (1000, 2000 mg/l) significantly increased orange seedlings tolerance to salt water stress in term of increasing the average of leaf area , shoot length and fresh and dry weights of shoots. In addition, Abdulhussein and Awadh (2014) revealed that sour orange seedlings sprayed with 200 mg/l salicylic acid showed an increase in seedling height, leaf area, fresh and dry weight of vegetative and roots under saline irrigation water. In this respect, Khoshbakht and Asgharei (2015) indicated that exogenously applied salicylic acid resulted in a significant increase of Valencia orange growth (total leaf area, leaves number per plant and total dry mass) both under saline and non-saline conditions. Aly *et al.*, (2015) reported that magnetic irrigation water and sprayed with anti-salinity substances types; ascorbic, salicylic and proline acids enhanced growth parameters of Valencia orange trees, while salicylic acid had a marked superiority over other types of anti-salinity substances on vegetative growth attributes i.e. leaf area, shoot length, shoot number and shoot thickness.

Foliar application of Salicylic acid gave the best results concerning shoot length, leaves number per shoot and leaf area of Balady mandarin trees grown under saline conditions, this maybe due to Salicylic acid induced the nitrate reductase activation due to increased nitrate metabolism in the leaves and roots of plant (Hayat *et.al*.,2005), the increasing in pigments of photosynthesis will be reflected positively, to improve the vegetative growth of plants. The ability of Salicylic acid for plant tolerance may explain the formation of ROS (Reactive oxygen species) in the photosynthetic tissue of plants during salt stress and osmotic stress (Borsani *et al.*, 2001).

Table 5: Effect of soil amendments and salicylic acid foliar application on growth of Balady mandarin trees in 2014 and 2015 seasons.

Treatments		Shoot length Cm		Leaves number per shoot		Leaf area cm^2	
Salicylic acid ppm	**Soil amendments**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
Tap water	Humic acid	32.10	34.51	19.41	20.22	12.81	14.83
	Biotol	38.81	41.60	23.52	21.23	14.89	16.65
	Humic acid + Biotol	42.29	45.32	24.71	26.51	16.71	16.46
	Control	22.51	26.61	13.62	14.81	12.25	14.62
200 ppm	Humic acid	34.61	36.32	23.33	25.82	15.29	16.45
	Biotol	41.53	44.78	28.50	27.41	15.21	16.86
	Humic acid + Biotol	43.21	48.22	30.21	32.31	19.10	18.54
	Control	28.90	30.82	21.38	21.82	13.12	14.87
300 ppm	Humic acid	39.32	40.80	28.81	29.52	16.15	16.15
	Biotol	44.20	44.91	34.60	33.71	19.70	18.85
	Humic acid + Biotol	45.42	50.30	36.58	35.31	19.80	19.45
	Control	30.11	32.50	24.12	27.52	14.42	16.78
L.S.D. at 5%		1.95	1.60	2.22	3.14	1.61	2.10
Average of Main plots							
Tap water		33.93	37.01	20.32	20.69	14.16	15.64
200 ppm		37.06	40.03	25.86	26.84	15.68	16.68
300 ppm		39.76	42.13	31.03	31.51	17.51	17.80
L.S.D. at 5%		0.39	0.55	0.55	1.85	0.25	0.87
Average of Sub plots							
Humic acid		35.34	37.21	23.85	25.19	14.75	15.81
Biotol		41.51	43.76	28.87	27.45	16.60	17.45
Humic acid + Biotol		43.64	47.95	30.50	31.38	18.54	18.15
Control		27.17	29.97	19.71	21.38	13.26	15.42
L.S.D. at 5%		1.06	0.78	1.07	1.13	0.85	0.88

Salicylic acid pre-treatment also provided protection against salinity in plants, probably due to the increased activation of aldose reductase and APx enzymes and to the accumulation of osmolytes, such as sugars, sugar alcohol or proline (Tari *et al*., 2002 and Szepesi *et al*., 2005). Also, plants pre-treated with SA exhibited less Ca^{2+} and more K^+ accumulation and soluble sugars in roots at the expense of these contents in the plant.

Soil application of Humic acid, Biotol and Humic acid + Biotol had significantly positive effect on all vegetative growth parameters of Balady mandarin trees as compared with control trees in both seasons. The highest values of vegetative growth parameters were obtained with Humic acid + Biotol followed by Biotol then Humic acid respectively in both seasons. The lowest values of shoot length, leaf number per shoot and leaf area belonged to control treatment in both seasons.

The interaction between soil application of Humic acid, Biotol and Humic acid + Biotol combined with foliar application of salicylic acid had a significant effect on growth characters of Balady mandarin trees in both seasons. The highest values were belonged to treatment of(Humic acid + Biotol + salicylic acid spray at 300 ppm) followed by (Biotol + Salicylic acid spray at 300 ppm),(Humic acid + Biotol + Salicylic acid spray at 200 ppm) and (Humic acid + Biotol + tap water) respectively, while the least values were belonged to the control treatment. These results agreed with those of El-Khawaga and Maklad(2013) appling Azotobacter chroococcum, Bacillus megaterium and *Bacillus circulans* combined on Valencia orange trees, they showed that shoot length, leaves number per shoot and leaf area were significantly increased as compared to untreated trees. Mohamed *et al*., (2013) and Abobatta(2015) revealed that biofertilizer, diatoms as a source of Silicon and K-Humate improved canopy volume and leaf area of Valencia orange trees under salinity stress.

The obtained increase in vegetative growth, as a result of applied treatments may be due to Humic acid and Biotol treatments combined with foliar salicylic acid caused an improvement of nutrient availability and uptake, this is due to humus complex and microorganisms activity produced acids and CO_2 dissolved in soil solution resulting in a reduction of pH and thus increased available nutrients especially micronutrients. In addition, nitrogen-fixing bacteria such as Azotobacter chroococcum and Azospirillium lipoferum or phosphate dissolving bacterial culture Bacillus megaterium stimulate growth and increase yield by enhancing the nitrogen and phosphorus status of trees(Hayat *et al*., 2010). On the other hand, Azospirillum spp. and Azotobacte*r* spp. produced different GAs specially GA_1, GA_3, GA_9, GA_{19} and GA_{20} that are responsible for plant growth promotion that occurs upon inoculation onto plants (Bottini *et al*., 2004). In general, the Humic acid, Biotol(free living bacteria fixing nitrogen and dissolving phosphorus) and foliar spray with salicylic acid could contribute to reduction of the burden of soil nutrient loss and counteracting part of the negative effects of saline stresses on plant growth and productivity.

2. Nutritional status:

Data in Table 6 showed the effect of soil application of humic acid, Biotol and foliar concentrations of salicylic acid and their interaction on leaf N, P, K, Fe, Mn and Zn contents of Balady mandarin trees grown in salt affected soil.

Foliar application of salicylic acid had a significant effect on leaf nutrient contents as compared with control treatment. The highest values of leaf nutrient contents were recorded with high concentration(300 ppm) in both seasons. Karlidag *et al*.,(2009) concluded that, salicylic acid reduced Na uptake of Strawberry plants and/or increased the uptake of N, P, K, Ca, Mg and the other minerals as compared to control treatment under salt stress. Therefore, exogenous salicylic acid stimulated N, P, K, Fe, Mn and Zn uptake and enhanced nutritional status of trees, which was reflected on alleviating adverse effect of saline soil on the trees and encouraged their growth and productivity. In this line, Al-Absi(2009) concluded that Salicylic acid spray reduced Na^+ and Cl^- uptake and increased concentration of K^+ and Ca^{++} in plants under salt stress and could ameliorate the deleterious effects of salinity on growth and yield.

Soil application of Humic acid, Biotol and Humic acid + Biotol had a positive effect on leaf nutrient contents of Balady mandarin compared to control treatment. The highest values of leaf nutrient contents were recorded on trees treated with Humic acid + Biotol followed by Biotol, Humic acid and control in both seasons respectively. The differences were significant among treatments in both seasons. These findings are in line with those of Abd El-Hamied(2014b) who found that Humic acid and anti-salinity compounds appling in Valencia orange orchard alone caused an increase in leaf contents of N, P, K, Fe, Zn and Mn under saline irrigation water. Also,. Mohamed *et al*.,(2013) revealed that Fe, Zn and Mn in leaves of Valencia orange were increased when trees treated with biofertilizers and magnetite iron under salt affected soil, also biofertilizers plus 750g/tree magnetite iron reduced Na^+ uptake and increased K^+ in leaves which alleviating adverse effect of Na^+ toxicity. In addition, Abdelaal *et al.*, (2013) cleared that application of yeast, Minia Azotene, EM had an increase and significant promoting on N, P, K, Mg, Ca, Fe, Mn, Zn and Cu rather than non- application of Valencia orange trees grown in salt affected soil.

The interaction between soil amendments and foliar application of Salicylic acid had a significant effect on leaf nutrient contents of Balady mandarin trees in both seasons. The highest values were belonged to combined of (Humic acid + Biotol + foliar spray with Salicylic acid at 300 ppm) followed by (Biotol + foliar spray with Salicylic acid at 300 ppm), (Humic acid + Biotol + foliar spray with Salicylic acid at 200 ppm) and (Humic acid + foliar spray with Salicylic acid at 300 ppm) respectively, while the least values belonged to control trees.

3. Yield:

Data in Table 7 clear that, yield as weight (kg/tree) and number of fruits per tree was positively affected by soil amendments and their combination with Salicylic acid as compared with control trees in both seasons.

As for the effect of Salicylic acid concentrations, it is clear that all Salicylic acid concentrations cause a significant increase in yield as weight (kg/tree) or fruit number /tree compared to the control which produced the minimum yield. Also, the highest yield was obtained from trees sprayed by 300 ppm of Salicylic acid in both seasons. These results agreed with those of Samra *et al*.,(2012) on Balady mandarin and Aly *et al*.,(2015) on Valencia orange, they concluded that yield as weight(kg/tree) or fruit number per tree was improved when trees sprayed with Salicylic acid. The beneficial effect of Salicylic acid on improving yield of Balady mandarin under salt affected soil may be due to positive effect in increasing nutrients uptake(Table 4) which will reflected on improving most vegetative growth parameters as shown in Table 3.

The data in Table 7 also clarify that, soil amendments of Humic acid, Biotol, Humic acid + Biotol significantly increased yield as weight or fruit number per tree compared to control treatment in both seasons. The treatment of Humic acid + Biotol gave the highest yield as weight or number/tree of Balady mandarin followed by treatment of Biotol and treatment of Humic acid compared to control in both seasons, respectively. These results are similar to those obtained by El-Khayat and Abdel-Rehiem(2013) on mandarin. El-Mohamedy and Ahmed(2009) they concluded that Humic acid caused the highest yield as number of fruits/tree or weight(kg/tree) compared with untreated trees of mandarin. In this respect, Abbas *et al.*,(2013) showed that kinnow mandarin tree received Humic acid at 30 ml exhibited a significant enhancement in number of fruits per branch.

Table 6: Effect of soil amendment and salicylic acid foliar application on leaf nutrients of Balady mandarin trees in 2014 and 2015 seasons.

Treatments		N %	P %	K %	Fe ppm	Mn ppm	Zn ppm
Salicylic acid	**Soil amendments**			**2014 season**			
Tap water	Humic acid	2.120	0.120	1.150	70.52	27.92	29.74
	Biotol	2.270	0.123	1.220	84.31	31.58	34.24
	Humic acid + Biotol	2.330	0.130	1.250	93.41	31.62	38.19
	Control	1.900	0.090	0.990	51.44	21.85	19.88
200 ppm	Humic acid	2.220	0.129	1.250	87.33	31.11	35.26
	Biotol	2.320	0.130	1.270	90.94	35.24	36.11
	Humic acid + Biotol	2.470	0.135	1.280	108.85	36.32	42.07
	Control	1.980	0.105	1.120	81.75	28.30	25.13
300 ppm	Humic acid	2.500	0.135	1.330	100.55	34.09	39.13
	Biotol	2.500	0.140	1.360	103.15	38.06	45.20
	Humic acid + Biotol	2.530	0.150	1.390	125.16	40.17	46.10
	Control	2.150	0.110	1.280	84.97	31.74	29.14
L.S.D. at 5%		0.103	0.009	0.065	1.52	2.05	1.66
Average of Main plots							
Tap water		2.155	0.115	1.152	74.92	28.24	30.51
200 ppm		2.247	0.124	1.230	92.21	32.74	34.64
300 ppm		2.420	0.133	1.315	103.45	36.01	39.89
L.S.D. at 5%		0.041	0.002	0.028	0.62	1.42	0.64
Average of Sub plots							
Humic acid		2.280	0.128	1.243	86.13	31.04	34.71
Biotol		2.363	0.131	1.283	92.80	34.96	38.52
Humic acid + Biotol		2.443	0.138	1.306	109.14	36.04	42.12
Control		2.010	0.101	1.096	72.72	27.29	24.71
L.S.D. at 5%		0.046	0.003	0.026	0.96	0.78	0.86
Treatments				**2015 season**			
Tap water	Humic acid	2.166	0.127	1.180	75.48	26.32	30.06
	Biotol	2.313	0.132	1.320	90.51	28.62	36.72
	Humic acid + Biotol	2.390	0.135	1.240	103.49	30.50	40.04
	Control	1.916	0.098	1.060	59.77	23.70	21.71
200 ppm	Humic acid	2.233	0.138	1.270	90.64	29.44	37.10
	Biotol	2.530	0.140	1.280	106.06	33.80	37.42
	Humic acid + Biotol	2.640	0.140	1.350	115.06	35.30	40.08
	Control	2.050	0.112	1.133	85.18	26.60	29.09
300 ppm	Humic acid	2.620	0.149	1.350	108.08	32.88	41.30
	Biotol	2.630	0.150	1.370	119.29	34.30	45.05
	Humic acid + Biotol	2.680	0.160	1.380	129.12	36.22	47.06
	Control	2.100	0.120	1.160	87.20	28.43	31.79
L.S.D. at 5%		0.200	0.004	0.064	2.10	1.55	2.11
Average of Main plots							
Tap water		2.196	0.123	1.177	82.31	27.28	32.13
200 ppm		2.363	0.132	1.258	99.23	31.28	35.92
300 ppm		2.507	0.144	1.315	110.92	32.95	41.30
L.S.D. at 5%		0.065	0.001	0.006	0.42	0.38	1.41
Average of Sub plots							
Humic acid		2.340	0.138	1.266	91.40	29.55	36.15
Biotol		2.491	0.140	1.293	105.28	32.24	39.73
Humic acid + Biotol		2.570	0.145	1.323	115.89	34.00	42.39
Control		2.022	0.110	1.117	77.38	26.24	27.53
L.S.D. at 5%		0.090	0.002	0.003	1.29	0.75	0.99

The positive response of yield as a result of soil amendments of Humic acid and Biotol (Azotobacter and Azospirillum and Bacillus megaterium) may be due to high ability of these microbes in N^2-fixation and the secretion of several compounds that increase soil fertility. Moreover, Humic acid increased bacteria activity and number of this bacteria thus it can fix atmospheric nitrogen, increase phosphorus availability in soil and enhanced elements absorbed by Balady mandarin tree, that reflected to tree's ability to grow and increase productivity.

As for the combinations between soil amendments and foliar application of Salicylic acid concentrations, it is clear that yield as weight (kg/tree) or number of fruits/tree was significantly increased by all treatments compared to control in both seasons. The highest yield was harvested from trees treated by(Humic acid + Biotol + foliar spray with Salicylic acid at 300 ppm) followed by(Biotol + foliar spray with Salicylic acid at 300 ppm), (Humic acid + Biotol + foliar spray with Salicylic acid at 200 ppm) and(Humic acid + foliar spray with Salicylic acid at 300 ppm) respectively, while the lowest yield came from untreated trees. Abd El-Hamied(2014a) reported that yield and number of fruits/tree of Clementine mandarin was significantly improved by appling EM and organic manure in salt affected soil. These results could be due to that soil amendment as Humic acid and Biotol combined with Salicylic acid spray were very effective in raising the fertility of salt-affected soil which reflected in improving the yield. Biotol (biofertilizers) play an important role in enhancing crop productivity through increasing nitrogen fixation, phosphate solubilization, plant hormone and controlling salinity harmful effect. Also, Humic acid improved soil nutrients and soil properties by decreasing soil bulk density and enhancing soil pH, so, increasing crop yield (Canellas *et a*l., 2015).

4. Fruit quality:

Data in Tables(7 and 8) showed the effect of soil amendments and foliar concentrations of Salicylic acid and their interaction on fruit quality in terms of fruit weight(g), fruit diameter(cm), SSC %, acidity %, SSC/acid ratio and vitamin C of Balady mandarin trees.

Table 7: Effect of soil amendments and salicylic acid foliar application on yield, fruit weight and fruit diameter of Balady mandarin trees in 2014 and 2015 seasons.

Treatments		Yield				Fruit weight (g)		Fruit diameter (cm)	
		Number/tree		Kg/tree					
Salicylic acid ppm	Soil amendments	2014	2015	2014	2015	2014	2015	2014	2015
Tap water	Humic acid	357.08	389.25	40.40	41.32	102.16	106.41	5.38	5.53
	Biotol	400.07	411.23	44.05	44.18	110.20	108.20	5.82	5.63
	Humic acid + Biotol	505.21	433.48	56.60	48.62	112.13	112.42	5.91	5.85
	Control	314.09	317.92	30.71	31.27	97.41	98.51	5.10	5.12
200 ppm	Humic acid	463.19	474.18	51.52	51.25	111.45	108.11	5.86	5.62
	Biotol	502.06	564.78	56.42	63.53	112.55	112.71	5.92	5.86
	Humic acid + Biotol	545.07	590.20	61.32	68.65	112.66	115.40	5.93	6.10
	Control	333.48	425.88	33.70	35.58	100.32	102.50	5.28	5.33
300 ppm	Humic acid	583.31	522.33	65.60	58.56	112.72	112.21	5.95	5.83
	Biotol	585.04	545.82	67.40	63.39	115.33	116.20	6.06	6.14
	Humic acid + Biotol	588.19	575.12	68.20	68.28	116.18	118.70	6.11	6.17
	Control	401.07	438.20	39.32	42.37	102.80	105.80	5.40	5.50
L.S.D. at 5%		4.02	2.96	2.19	2.57	4.19	3.26	0.17	0.12
Average of Main plots									
Tap water		394.11	387.97	42.94	41.34	105.47	106.39	5.55	5.53
200 ppm		460.95	513.76	50.74	54.75	109.24	109.68	5.75	5.72
300 ppm		539.40	520.36	60.13	58.15	111.75	113.22	5.88	5.91
L.S.D. at 5%		1.91	1.94	0.29	0.22	1.88	1.18	0.03	0.03
Average of Sub plots									
Humic acid		467.86	461.92	52.51	50.37	108.78	108.91	5.73	5.66
Biotol		495.72	507.27	55.96	57.03	112.69	112.37	5.93	5.87
Humic acid + Biotol		546.16	532.93	62.04	61.85	113.66	115.50	5.98	6.04
Control		349.55	394.00	34.58	36.40	100.17	102.27	5.26	5.31
L.S.D. at 5%		2.47	1.67	1.35	1.58/	2.16	1.21	0.08	0.06

Table 8: Effect of soil amendments and salicylic acid foliar application on SSC %, acidity %, SSC/acid ratio and vitamin C of fruits of Balady mandarin tree in 2014 and 2015 seasons .

Treatments		SSC %		Acidity %		SSC/ acid Ratio		Vitamin C mg/100 ml juice	
Salicylic acid ppm	Soil amendments	2014	2015	2014	2015	2014	2015	2014	2015
Tap water	Humic acid	10.93	11.39	1.14	1.13	9.58	10.07	32.12	31.15
	Biotol	11.75	11.53	1.18	1.18	9.95	9.74	30.51	30.45
	Humic acid + Biotol	11.93	12.16	1.06	1.09	11.25	11.15	33.42	32.15
	Control	10.73	10.64	1.31	1.21	8.19	8.79	29.55	29.67
200 ppm	Humic acid	10.56	10.63	1.11	1.14	9.51	9.32	32.85	31.95
	Biotol	11.03	10.76	1.21	1.10	9.11	9.79	31.41	32.15
	Humic acid + Biotol	11.20	11.48	1.09	1.05	10.27	10.93	34.15	33.81
	Control	10.53	10.33	1.30	1.18	8.10	8.75	30.90	30.50
300 ppm	Humic acid	10.90	11.51	1.18	1.16	9.23	9.92	33.40	32.95
	Biotol	11.78	10.71	1.21	1.13	9.73	9.47	32.59	33.20
	Humic acid + Biotol	11.91	11.35	1.10	1.04	10.82	10.91	35.22	35.12
	Control	10.70	10.32	1.28	1.17	8.35	8.82	31.48	30.88
L.S.D. at 5%		0.11	0.33	ns	ns	0.06	0.05	0.74	2.56
Average of Main plots									
Tap water		11.33	11.43	1.17	1.15	9.74	9.93	31.40	30.85
200 ppm		10.83	10.80	1.17	1.11	9.24	9.69	32.32	32.10
300 ppm		11.32	10.97	1.19	1.12	9.53	9.78	33.17	33.03
L.S.D. at 5%		0.10	0.01	0.01	0.01	0.01	0.04	0.07	1.30
Average of Sub plots									
Humic acid		10.79	11.17	1.14	1.14	9.44	9.77	32.79	32.01
Biotol		11.52	11.00	1.20	1.13	9.59	9.66	31.50	31.93
Humic acid + Biotol		11.68	11.66	1.08	1.06	10.78	10.99	34.26	33.69
Control		10.65	10.43	1.29	1.18	8.21	8.78	30.64	30.35
L.S.D. at 5%		0.04	0.18	0.03	0.04	0.04	0.03	0.31	0.96

ns not significant

As for the effect of Salicylic acid spray, it is clear that the heaviest and largest fruits were harvest from trees sprayed with Salicylic acid at 300 ppm followed by 200 ppm as compared with those taken from control trees, the differences were significant among three concentrations of Salicylic acid for fruit weight and diameter in both seasons (Table 7). Also, fruits from tree sprayed with 300 ppm Salicylic acid had the highest values of vitamin C compared to control in both seasons. These results agreed with those of Ahmed *et al.*,(2014) and Ahmed *et al*.,(2015) on different fruit crops. On the other hand, the highest values of SSC/acid ratio were found in fruits harvested from control, but the lowest value of SSC/acid ratio came from tree sprayed with 200 ppm Salicylic acid in both seasons. The values of SSC% and acidity % were not affected by Salicylic acid spray and it was not taken any constant trend in both seasons. These results are in line with those of Vatanparast *et al.*, (2012) on pomegranate.

Also, data in Tables 7 and 8 showed that most fruit quality parameters were significantly affected by different soil amendments, it is clear that highest fruit weight, diameter, SSC%, SSC/acid ratio and vitamin C were recorded from fruits harvested from trees treated with Humic acid + Biotol followed by Biotol and Humic acid compared to control treatment in both seasons. All soil amendment slightly decreased fruit acidity as compared to control in both seasons. These results agreed with those of, Abobatta(2015) on Valencia orange.

With regard to, the effect of interaction between soil amendments and foliar concentrations of Salicylic acid on fruit quality, it is clear from Tables 7 and 8 that fruit quality attributes were significantly affected by all combinations in both seasons. The highest values of fruit weight, diameter, SSC%, SSC/acid ratio and vitamin C belonged to combined of (Humic acid + Biotol + foliar spray with Salicylic acid at 300 ppm) followed by(Biotol + foliar spray with Salicylic acid at 300 ppm), (Humic acid + Biotol + foliar spray with Salicylic acid at 200 ppm) and(Humic acid + Biotol + foliar spray with tap water) respectively, while the least values belonged to control treatment in both seasons. Concerning fruit acidity percentage, fruits harvested from untreated trees (control) achieved the highest values without significant differences among treatments in both seasons. These results agreed with those of

Hagagg *et al*.,(2013) and Abbas *et al.*,(2013). It is obvious from data in Tables(7 and 8) that, soil amendment combined with Salicylic acid foliar application significantly enhanced Balady mandarin yield and most of fruit quality attributes.

CONCLUSION

From the previously mentioned results, it was clear the great role of Humic acid, Biotol, Humic + Biotol as soil amendments combined with Salicylic acid foliar application for counteract part of the negative effects of saline stresses on Balady mandarin trees grown in a slightly saline soil, as it is indispensable for improvement of growth and the nutritional status of the Balady mandarin trees and production of maximum yield and quality. Thus, this study recommended the superiority of application of Humic acid + Biotol + Salicylic acid foliar application at 300 ppm, which gave the best growth, yield, fruit quality and nutritional status of Balady mandarin trees.

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الملخص العربى

تخفيف الآثار السلبية للتربة المالحة على نمو وانتاجية اشجار اليوسفى البلدى

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اجريت تجربة بحثية في منطقة سيدي غازي محافظة كفر الشيخ و ذلك لدراسة تأثير محسنات التربة و الرش بحمض الساليسيليك على النمو والمحصول وجودة الثمار ومحتوى الأوراق من العناصرالغذائية في أشجار اليوسفي البلدي، تم استخدم القطع المنشقة في تصميم قطاعات كاملة العشوائية مع ثلاثة مكررات، تضمنت القطع الرئيسية تركيزات حمض الساليسيليك وهي a_1:الماء العادي و a_2 : ٢٠٠ و a_3 : ٣٠٠ جزء في المليون، والقطع التجريبية المنشقة تضمنت محسنات التربة وهى b_1: حمض الهيوميك و b_2: سماد حيوي البيتول وb_3 : حمض الهيوميك +سماد حيوي البيتول و b_4 : الكنترول.

الساليسيلك بتركيز ٣٠٠ جزء في المليون في بستان اليوسفي اظهرت تفوق في تحسين الخصائص الكيميائية للتربة المالحة حيث انخفضت ملوحة التربة ورقم الحموضة ونسبة ادمصاص الصوديوم وزادت السعة التبادلية الكاتيونية والعناصر الميسرة والميكروبات، بالإضافة إلى إحداث تحسن ملحوظ في حجم الشجرة والنمو الخضري تحت ظروف الأرض الملحية.

وعلاوة على ذلك، أظهر تحليل الأوراق زيادة معنوية في محتواها من النيتوجين والفوسفور والبوتاسيوم والحديد والزنك والمنجنيزعند المعاملة بمحسنات التربة والرش بحمض الساليسيليك بتركيز ٣٠٠ جزء في المليون. تم الحصول على أعلى محصول وجودة الثمار بشكل كبير مع التفاعل بين حمض الهيومك + السماد الحيوي البيتول والرش بحمض الساليسيليك بتركيز ٣٠٠ جزء في المليون.

لذا، حمض الهيومك مع السماد الحيوي البيتول و الرش الورقي بحمض الساليسيليك اظهر استجابة في تخفيف العبء عن فقدان المغذيات من التربة ومواجهة جزء من الآثار السلبية للإجهاد الملحي على نمو النبات والإنتاجية. ولذا، توصى الدراسة بإضافة حمض الهيومك + السماد الحيوي البيتول + رش الأوراق بحمض الساليسيلك بتركيز ٣٠٠ جزء في المليون، وذلك لأنها أعطت أفضل نمو للآشجاروالمحصول وجودة الثمار والحالة الغذائية لأشجار اليوسفي البلدي تحت ظروف الأرض الملحية.