

## POSSIBILITY OF IMPROVING GROWTH, PHYSIOLOGICAL BEHAVIOUR AND YIELD OF CANOLA PLANTS CULTIVATED IN SALINE RECLAIMED SOILS WITH APPLICATION OF HUMIC AND ASCORBIC ACIDS

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**ABSTRACT:** *The possibility of enhancing growth physiological behaviour and yield of canola plants grown under reclaimed soil with salinity level of about 9000 ppm was investigated in the Experimental Farm, Faculty of Agriculture, Fayoum University, Fayoum, Egypt, during 2007/2008 and 2008/2009 seasons. For verifying this aim, the plants produced from humic acid treated- or untreated-seed beds were sprayed with ascorbic acid at the rates of 0 (control), 150, 300, 450 and 600 mgL<sup>-1</sup>. Significant increments in growth traits (plant height, number of leaves plant<sup>-1</sup>, total leaves area plant<sup>-1</sup> and dry weight of leaves plant<sup>-1</sup>), chemical constituents (photosynthetic pigments, some photosynthates, some macro-and micronutrients) and canola yields (seed yield plant<sup>-1</sup> and feddan<sup>-1</sup> as well as seed oil and protein yields) were obtained by application of humic acid alone or in combination with all ascorbic acid applications. As for ascorbic acid, plants sprayed with all studied rates revealed significant increases in all aforementioned parameters with the super results were obtained from the rate of 450 mgL<sup>-1</sup> as compared with untreated plants. Thereon, the study led us to be concluded that canola plants "cv. Serw 4", produced from humic acid fecundated-seed beds and sprayed with 450 mgL<sup>-1</sup> ascorbic acid solution tackled them to overcome the adverse conditions of reclaimed soils particularly, salinity up to 9000 ppm and consequently, and consequently produced economic seed and oil yields.*

**Key Word:** *Canola, Yield and Quality, Chemical Growth, Humic and Ascorbic Acids, Reclaimed Soils.*

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### INTRODUCTION

Egypt suffers from drastic shortage in the edible oils. The local oil production was about 250,000 t in 2009; encounter to about 1,700,000 t consumption (FAS, 2009). This wide gap between the production and consumption of vegetable oil reached to 85.3%. Thus, the need for importation became indispensable. So, it is important to introduce new crops able to partially overcome this gap especially, those which can be cultivated in newly reclaimed soils such as canola (*Brassica napus* L.).

Canola is an oil seed crop belongs to the family Brassicaceae and it is a specific type of rapeseed associated with high quality oil and meal. Canola

trademark is held by the Canadian Canola Association and it is permitted for use to describe rapeseed type with less than 2% of erucic acid in the oil and less than 30  $\mu\text{mol g}^{-1}$  of aliphatic glucosinolate in the oil free meal (Gunstone, 2004).

Canola now holds the third position among the oilseed crops in the world and its seeds contain about 40-44% oil and the meal contains about 30-35% protein after oil extraction. Canola oil contains less than half of saturated fats persist in any other vegetable oil (<60%) and it has a favorable mix of mono- and polyunsaturated fats as well as contains no cholesterol that benefits in different oil industries. Furthermore, oil free meal of canola had a high protein needed for livestock and poultry feed (Gunstone, 2004).

It is worth mentioning that much of the newly reclaimed areas in Egypt are salinity affected soils. Saline conditions disrupt several physiological processes in plants leading to a general reduction in growth and yield (El-Saidi, 1997 and Greenway and Munns, 1980). The injurious influence of salinity on plant growth and metabolism was attributed, principally, to the enhanced  $\text{Na}^+$  uptake which causes ion excess in plant tissues (Abbas *et al.*, 1991) beside, the inhibition of  $\text{K}^+$ ,  $\text{Ca}^{2+}$  and  $\text{NO}_3^-$  uptake by plant roots (Maas, 1986). In addition, it is well established that salinity stress damages plant cells through production of reactive oxygen species including superoxide, hydrogen peroxide, hydroxyl anions and singlet oxygen (Scandalios, 1997). Efforts have been paid to control salinity by technological means, viz reclamation, drainage, use of high leaching fractions and application of soil amendments (Abdel-Naby *et al.*, 2001). On the other hand, some trials have been made to alleviate the disturbances in plant metabolism excreted by salinity stress by using natural and safety substances among them ascorbic and humic acids which may help to overcome some of these inhibitory effects (Rady, 2006, Osman and Ewees, 2008 and Rady and El-Sawah, 2009).

Ascorbic acid is an important antioxidant defense in plant cells (Foyer and Halliwell, 1976) to protect them by scavenging the reactive oxygen species. It also regulates respiration, activates cell division and many enzymes activities (Rautenkranz *et al.*, 1994). It has synergistic effects on growth, yield and its components as well as chemical composition of several crops under favourable and unfavourable environmental conditions i.e. salinity (Ahmed *et al.*, 2003; Mostafa, 2004, Rady, 2006 and Rady and El-Sawah, 2009). It has been proved that, application of humic acid, as an organic soil amendment used either individually or in combination with others, resulted in significant increases in growth, chemical constituents and seed yield in the sandy and other soils through its effective role in improving hydrophysical properties, nutrient availability and seeds germination as well as helping the plants to resist drought (Salib, 2002, Salman *et al.*, 2005, Abou Zeid *et al.*, 2005, Sayed *et al.*, 2007 and Osman and Ewees, 2008).

The present work was planned and executed for studying the influence of fecundating the seed-beds with humic acid and foliar the produced plants

## Possibility of improving growth, physiological behaviour and .....

with ascorbic acid on plant growth and some chemical constituents as well as seed and oil yields of canola plants grown under saline reclaimed soil conditions.

### **MATERIALS AND METHODS**

During the two successive seasons of 2007/2008 and 2008/2009, a field trial was carried out at the Experimental Farm, Faculty of Agriculture, Fayoum University, Egypt, for realizing the aforementioned aim. Before sowing, soil samples to 30 cm depth from the experimental site were collected and analyzed by the standard procedures of Jackson (1967) and the obtained results are presented in Table (1).

Table (1): Physical and chemical properties of the experimental site soil in both 2007/2008 and 2008/2009 seasons.

Property	2007/2008	2008/2009
<b>Physical:</b>		
Clay%	17.50	18.25
Silt%	27.25	27.00
Sand%	55.25	54.75
Soil texture	Loamy sand	Loamy sand
<b>Chemical:</b>		
pH (1:2.5)	7.900	7.750
ECe (dS m <sup>-1</sup> )	14.150	13.950
Organic matter%	0.950	0.980
CaCO <sub>3</sub> %	8.150	8.250
Total N%	0.069	0.071
<b>Available nutrients (mg Kg<sup>-1</sup> soil):</b>		
K	65.20	70.10
P	17.15	16.75
Fe	08.15	07.20
Mn	06.10	05.80
Zn	01.75	01.95
Cu	01.50	01.35

Seeds of canola cv. "Serw 4" obtained from Agricultural Research Center, Giza, Egypt were sown on November 15, 2007 and 20, 2008 in the first and second season, respectively. The area of each experimental unit was 9 m<sup>2</sup> (3m length x 3m width) included 10 rows, 60 cm apart. Thinning was done 30 days after sowing leaving two plants hill<sup>-1</sup>. A seasonal total of 150, 200 and 50 kg feddan<sup>-1</sup> calcium superphosphate (15.5% P<sub>2</sub>O<sub>5</sub>), ammonium nitrate (33.5% N) and potassium sulphate (48% K<sub>2</sub>O), respectively were applied as recommended. Other recommended cultural practices for growing canola plants were applied. Treatments comprised 5 ascorbic acid rates; 0 (control), 150, 300, 450 and 600 mgL<sup>-1</sup>. These treatments were applied alone or in combination with humic acid seed-beds application.

#### **Method of seed-beds application with humic acid:**

Humic acid (at the rate of 6 kg feddan<sup>-1</sup>), produced by Alpha Chemika Co., Mumbai, India, was well mixed with a part of the tested soil and added at

standard amounts to the seed-beds directly before sowing. Results of analysis of humic acid are presented in Table (2).

**Table (2): Chemical constituents of humic acid (H.A.) used in this study.**

Component	H.A.	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	Na	Others
%	90.20	0.92	1.04	1.46	2.81	0.92	0.48	0.61	0.21	0.32	0.55	0.04	0.44

### **Method of ascorbic acid application:**

Ascorbic acid at the above mentioned concentrations was sprayed on shoots of plants to run off, two times; 5 and 8 weeks after sowing. Few drops of Tween-20 were added to the spraying solution as a wetting agent.

The experimental design used was a split-plot with four replicates. The main plot was devoted for humic acid treatment and ascorbic acid treatment was occupied the sub-main plot.

### **Recorded data:**

#### **Vegetative growth traits:**

Twelve weeks after sowing, four plants were randomly chosen from each experimental unit to determine the averages plant height (cm), number of leaves plant<sup>-1</sup>, total leaves area plant<sup>-1</sup> (dm<sup>2</sup>) and dry weight of leaves plant<sup>-1</sup> (g).

#### **Chemical constituents:**

Leaves of plants submitted to vegetative growth traits determinations, were also subjected to chemical determinations. Chlorophyll a, chlorophyll b, total chlorophyll and total carotenoids were extracted by acetone (80%) then, determined (mg g<sup>-1</sup> fresh leaves) using colorimetric method as described by Arnon (1949). Ascorbic acid was determined (mg g<sup>-1</sup> fresh leaves) using the dye 2,6-dichlorophenol indophenol method as outlined by A.O.A.C. (1995). Total soluble sugars were colorimetrically determined (mg g<sup>-1</sup> dry matter) using phosphomolybdic acid reagent according to Dubois *et al.* (1956). Free proline was extracted by 5-sulphosalicylic acid (3%) then, determined (mg g<sup>-1</sup> dry matter) colorimetrically using acid ninhydrin reagent as outlined by Bates *et al.* (1973). Total free amino acids were extracted using ethanol (80%) then, colorimetrically determined (mg g<sup>-1</sup> dry matter) using acid ninhydrin reagent method as outlined by Jayarman (1981). Nitrogen (%) was colorimetrically determined using the Orange G dye according to the method of Hafez and Mikkelsen (1981). For P, K, Na, Fe, Mn, Zn and Cu determinations, the wet digestion of 0.1 g of fine dry material of leaves of each treatment was done with sulphuric and perchloric acids mixture as mentioned by Piper (1947). Phosphorus (%) was colorimetrically estimated using chlorostannus molybdo-phosphoric blue color method in sulphuric acid system as described by Jackson (1967). Potassium and sodium (%) were determined using a Perkin-Elmer, Flame Photometer (Page *et al.*, 1982). Iron, manganese, zinc and copper (ppm) were determined using a Perkin-

Elmer, Model 3300, Atomic absorption Spectrophotometer (Chapman and Pratt, 1961).

**Seed yield and its components as well as seed oil and protein:**

At harvest time, ten plants were randomly taken from each experimental unit to determine the averages of number of fruiting branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup> and seed yield plant<sup>-1</sup> (g). Seed yield feddan<sup>-1</sup> (t) was determined on the whole plot basis. Seed oil and protein percentages were determined by using NMR apparatus (Grunland and Zimmerman, 1975).

**Statistical analysis:**

Due to similar variance trend of the data of the two seasons, combined analysis of variance and LSD at 0.05 to differentiate means were done according to Snedecor and Cochran (1980).

**RESULTS AND DISCUSSION**

**1. Vegetative growth traits:**

Plant height, number of leaves plant<sup>-1</sup>, total leaves area plant<sup>-1</sup> and leaves dry weight plant<sup>-1</sup> presented in Table (3) were significantly increased by 11.6%, 21.1%, 29.1 and 33.5%, respectively in plants produced from seed-beds treated with humic acid as compared with those of plants of humic acid untreated-seed-beds. These improving effects on growth traits may be due to plant hormone-like material contained in the humic substances (O'Donnell, 1973). In addition, Senn and Kingman (1973) showed that presence of auxin type reactions by humic substances resulted in increased growth. Recently, it was reported that humic acids contain cytokinins and their application led to increased endogenous cytokinin and auxin levels which possibly leading to improve growth under stress conditions (Zhang and Schmidt, 2000 and Zhang and Ervin, 2004). Furthermore, Senn and Kingman (1973) and Hsu (1978) stated that the presence of Fe in the humic acids (Table 2) had a positive effect on the growth of various groups of microorganisms which may excrete a range of vitamins, growth substances and antibiotics which all may be promoted plant growth. Sayed *et al.* (2007) added that, humic acid improved chemical properties of the soil because it increased soil microorganisms which enhanced nutrient cycling positively reflecting on photosynthesis and growth. Moreover, Raviv (1998) stated that humic acids may exert direct enzymatic or hormonal effects on plant growth. Regarding results illustrated herein, increasing macro-and microelements (N, P, K, Fe, Mn, Zn and Cu) as shown in Tables (6 & 7) and the decrease in Na<sup>+</sup> (Table 6) induced by soil treated with humic acid may be led to the significant increase in vegetative growth traits.

**Table (3): Influence of humic acid soil practices (B) and ascorbic acid foliar application (A) on some growth traits of canola plants grown under saline reclaimed soil conditions (data over the two seasons).**

Ascorbic acid (mgL <sup>-1</sup> )	0	+	Mean	0	+	Mean	0	+	Mean	0	+	Mean
	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B
	Plant height (cm)			Number of leaves plant <sup>-1</sup>			Total leaves area plant <sup>-1</sup> (dm <sup>2</sup> )			Dry weight of leaves plant <sup>-1</sup> (g)		
0	65.8	75.2	70.5	8.3	10.7	9.5	2.81	3.43	3.12	6.54	9.86	8.20
150	76.4	85.9	81.2	10.5	12.9	11.7	3.41	4.39	3.90	9.58	12.49	11.04
300	83.5	92.8	88.2	13.1	16.4	14.8	4.36	5.88	5.12	12.36	16.42	14.39
450	91.4	99.8	95.6	16.2	18.8	17.5	5.78	7.32	6.55	16.18	19.98	18.08
600	80.7	90.3	85.5	13.3	15.7	14.5	4.26	5.60	4.93	10.02	14.24	12.13
Mean A	79.6	88.8		12.3	14.9		4.12	5.32		10.94	14.60	
LSD <sub>0.05</sub> A	8.2			1.3			0.46			1.28		
LSD <sub>0.05</sub> B	4.7			0.7			0.25			0.70		
LSD <sub>0.05</sub> AxB	14.1			2.2			0.78			2.17		

0 H.A.= humic acid free seed-beds & + H.A.= humic acid fecundated-seed-beds

Regarding the effect of ascorbic acid, significant increases in all studied vegetative growth traits, viz. plant height, number of leaves plant<sup>-1</sup>, total leaves area plant<sup>-1</sup> and leaves dry weight plant<sup>-1</sup> were revealed with raising ascorbic acid rate up to 600 mgL<sup>-1</sup> (Table, 3). The maximum increases were recorded with ascorbic acid at the rate of 450 mgL<sup>-1</sup> which surpassed the results of water foliar spray by 35.6%, 84.2%, 109.9% and 120.5% for plant height, number of leaves plant<sup>-1</sup>, total leaves area plant<sup>-1</sup> and leaves dry weight plant<sup>-1</sup>, respectively. These results indicated that the most pronounced counteracted effects of studied soil salinity (Ca. 9000 ppm) on vegetative growth traits under study were existed by the exogenous application of ascorbic acid which led to the increase in endogenous level of this substance (Table 5) and consequently protect plant cells including protect the photosynthetic activities by scavenging reactive oxygen species (Zhang and Schmidt, 2000) thus, vigorous plant growth could be obtained under salinity stress. In this connection, Prusky (1988) and Elade (1992) reported a positive action for antioxidants, especially ascorbic acid on growth and attributed this finding to their effects on counteracting drought, salinity and diseases stresses and protecting plant cells against free radicals that responsible for plant senescence as well as to their auxinic action and consequently enhancing growth characters. Various authers,demonstrated the effect of ascorbic acid in regulating cell wall expansion, cell division and cell elongation through its action in cell vacuolarization (Arrigoni, 1994; Navas and Gomez-Diaz, 1995 and Cordoba-Pedregosa *et al.*, 1996), improving the nutritional status (Tables 6&7) and absorbing phenolic compounds which lead to save the growing tissues from toxic effects of the oxidized phenols (Gupta *et al.*, 1980) and/or enhancing the biosynthesis of soluble sugars (Table 5) and carbohydrates (Rady, 2006). The present findings are in coincidence with those obtained by Ahmed *et al.* (1998), Ragab (2002),

## **Possibility of improving growth, physiological behaviour and .....**

Ahmed *et al.* (2003), Mostafa (2004), Rady (2006), and Rady and El-Sawah (2009).

The interaction effects between humic acid and ascorbic acid treatments on all vegetable growth traits were significant. The highest values of plant height, number of leaves plant<sup>-1</sup>, total leaves area plant<sup>-1</sup> and leaves dry weight plant<sup>-1</sup> were recorded by the combined treatment of spraying the plants with 450 mgL<sup>-1</sup> ascorbic acid under fecundating the soil with humic acid which recorded 32.7%, 75.7%, 113.4% and 102.6% increases, respectively, compared to the combined treatment of zero mgL<sup>-1</sup> ascorbic acid foliar application under soil treatment with humic acid and recorded 51.7%, 126.5%, 160.5 and 205.5% increases, respectively above the combined treatment of tap water foliar treatment under humic acid free soil. The superiority of this treatment might come from improving the photosynthetic pigments (Table 4), nutritional status of plants (Tables 6&7) as well as the increase in total soluble sugars, total free amino acids and free proline (Table 5) saving more osmotic solutes which enabled plant cells to maintain more water against salinity.

### **2. Chemical constituents:**

#### **a. Leaf photosynthetic pigments and photosynthates under study:**

Data in Tables (4 and 5) reveal that, chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, total soluble sugars, total free amino acids, free proline and ascorbic acid in plant leaves were significantly increased in the order of 8.9%, 15.7%, 10.4%, 21.6%, 26.1%, 16.9%, 25.0% and 80.7%, respectively as a result of treating the seed-beds with humic acid, compared with humic acid untreated-seed-beds. These pronounced increments may be due to the increase in ascorbic acid in plant leaves (Table 5) which had an auxinic actions and synergistic effects on biosynthesis of sugars and carbohydrates (Al-Qubaie, 2002). Enhanced the chlorophyll content due to humic effect was inferred from its role in enhancing leaves nutritional status (Tables 6&7) especially, N as an important element of chlorophyll molecule. Moreover, as reported by Marschner (1995) humic acids lead to raise uptake K element leading to corresponding increase in the chlorophyll fluorescence which can serve as an indicator of stress induced accompanied with alteration of the endogenous hormones balance reflecting in more biosynthesis of sugars and other photosynthates. Also, BaoLin *et al.* (2000) noticed that, humic acids promoted growth, increased chlorophyll content, enhanced photosynthesis as well as accumulated carbohydrates and other photosynthates in leaves.

Regarding the influence of ascorbic acid on chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, total soluble sugars, total free amino acids, free proline and ascorbic acid concentrations in plant leaves, data

shown in Tables (4 and 5) exhibit significant increases in these parameters as a result of foliar application with ascorbic acid.

**Table (4): Influence of humic acid soil practices (B) and ascorbic acid foliar application (A) on leaf photosynthetic pigments of canola plants grown under saline reclaimed soil conditions (data over the two seasons).**

Ascorbic acid (mgL <sup>-1</sup> )	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B
	Chlorophyll a (mg g <sup>-1</sup> fresh weight)			Chlorophyll b (mg g <sup>-1</sup> fresh weight)			Total chlorophyll (mg g <sup>-1</sup> fresh weight)			Total carotenoids (mg g <sup>-1</sup> fresh weight)		
0	0.68	0.76	0.72	0.42	0.49	0.46	1.13	1.29	1.21	0.30	0.36	0.33
150	0.77	0.83	0.80	0.48	0.56	0.52	1.29	1.43	1.36	0.34	0.41	0.38
300	0.84	0.90	0.87	0.55	0.62	0.59	1.43	1.57	1.50	0.39	0.48	0.44
450	0.92	0.98	0.95	0.62	0.70	0.66	1.61	1.75	1.68	0.46	0.54	0.50
600	0.74	0.82	0.78	0.50	0.58	0.54	1.27	1.43	1.35	0.36	0.44	0.40
Mean A	0.79	0.86		0.51	0.59		1.35	1.49		0.37	0.45	
LSD <sub>0.05</sub> A	0.07			0.05			0.13			0.04		
LSD <sub>0.05</sub> B	0.04			0.03			0.08			0.03		
LSD <sub>0.05</sub> AxB	0.12			0.09			0.22			0.07		

0 H.A.= humic acid free seed-beds & + H.A.= humic acid fecundated-seed-beds

**Table (5): Influence of humic acid soil practices (B) and ascorbic acid foliar application (A) on some leaf photosynthates of canola plants grown under saline reclaimed soil conditions (data over the two seasons).**

Ascorbic acid (mgL <sup>-1</sup> )	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B
	Total soluble sugars (mg g <sup>-1</sup> dry weight)			Total free amino acids (mg g <sup>-1</sup> dry weight)			Free proline (mg g <sup>-1</sup> dry weight)			Ascorbic acid (mg g <sup>-1</sup> fresh weight)		
0	12.8	15.4	14.1	1.87	2.19	2.03	0.18	0.23	0.21	0.81	1.38	1.10
150	14.5	18.5	16.5	2.12	2.50	2.31	0.21	0.27	0.24	0.92	1.62	1.27
300	17.2	21.8	19.5	2.46	2.88	2.67	0.25	0.33	0.29	1.02	1.88	1.45
450	20.3	26.7	23.5	2.84	3.30	3.07	0.32	0.38	0.35	1.24	2.32	1.78
600	15.7	19.3	17.5	2.26	2.62	2.44	0.22	0.28	0.25	1.46	2.66	2.06
Mean A	16.1	20.3		2.31	2.70		0.24	0.30		1.09	1.97	
LSD <sub>0.05</sub> A	1.8			0.25			0.04			0.15		
LSD <sub>0.05</sub> B	1.1			0.14			0.03			0.08		
LSD <sub>0.05</sub> AxB	3.2			0.43			0.07			0.26		

0 H.A.= humic acid free seed-beds & + H.A.= humic acid fecundated-seed-beds

The rate of 450 mgL<sup>-1</sup> ascorbic acid gave the highest increases all above mentioned parameters except, ascorbic acid content which gave a maximum increase by the rate of 600 mgL<sup>-1</sup>. The proportions of 31.9%, 43.5%, 38.8%, 51.5%, 66.7%, 51.2%, 66.7% and 87.27% for the aforementioned traits, respectively, were recorded for the super treatment; i.e. spraying plant shoots with ascorbic acid at the rate of 450 mgL<sup>-1</sup>, compared with the treatment free from ascorbic acid (tap water). The promotive effect of ascorbic acid on chlorophylls and carotenoids and the other components under study might be attributed to the enhancing effects of this antioxidant on the nutritional status of canola plants detected herein (Tables 6&7) since, N is one of the essential chlorophyll components. Besides, Fe and Mn are



## **Possibility of improving growth, physiological behaviour and .....**

necessary for biosynthesis of chlorophyll and Zn is necessary for biosynthesis of tryptophan which is the precursor of auxin biosynthesis and consequently more biosynthesis of these components in the face of cells elongation. Furthermore, the role of ascorbic acid as an antioxidant, which directly involved in the regulation and protection of photosynthetic processes (Farago and Brunhold, 1994) might be led to the enhancing effect on pigments and the photosynthates under study. This treatment having the highest values of these constituents under study acquired the tested plants the ability to satisfactorily overcome soil salinity in respect to their containing sufficient amounts of soluble sugars, amino acids and proline (Table 5) which form sufficient cellular solutes served as sustenance for cell turgor and leading to maintenance of metabolic activities in plant cells and/or protect plants against adverse conditions i.e. salinity and drought of such soil under study. The enhancing effect of ascorbic acid on tested soluble sugars might be attributed to its promotive effect also on the studied pigments (Table 4) leading to the enhancement of photosynthesis and consequently, photosynthates increases. The positive effects of ascorbic acid on pigments and photosynthates obtained in this study are in agreement with those obtained by Tarraf *et al.* (1999), Ali (2002), Ahmed *et al.* (2003), Rady (2006), and Rady and El-Sawah (2009) who found that leaf photosynthetic pigments and some photosynthates especially, sugars and proline as osmotic solutes increased with foliar application of ascorbic acid under favorable and unfavorable conditions.

Results of combined treatments exhibited in Tables (4 and 5) reveal that, the combination between humic acid fecundated-soil and spraying plant shoot with ascorbic acid at 450 mgL<sup>-1</sup> was preferable to all other combinations since it granted the increases of 28.9%, 42.9%, 35.7%, 50.0%, 73.4%, 50.7%, 65.2% and 68.1% for chlorophyll a, chlorophyll b, total chlorophyll, total carotenoids, total soluble sugars, total free amino acids, free proline and ascorbic acid, respectively, compared to humic acid treated-soil interacted with foliar spray with tap water. When compared with the combined treatment of ascorbic acid-free water treatment under humic acid untreated-soil, it scored increments of 44.1%, 66.4%, 54.9%, 80.0%, 108.6%, 76.5%, 111.1% and 186.4% for the same studied components, respectively. These increments scored as a result of the application with humic and ascorbic acids may be attributed to that humic acid reduced the harmful effects of salinity by saving more mineral elements in the rhizosphere against Na<sup>+</sup> which positively reflected on the nutritional composition of plant leaves (Tables 6&7) and consequently enhanced the photosynthetic activities of plants and increased the osmotic solutes; sugars, amino acids and proline (Table 5) in plants against salinity. Ascorbic acid as one of antioxidants prevented enzyme inactivation, and the generation of more dangerous radicals and allow flexibility in the production of photosynthetic assimilatory power besides, electron transfer to O<sub>2</sub> prevented over reduction of electron

transports chain, which reduced the risk of harmful back reaction within the photosystem (Foyer *et al.*, 1990). In addition, Elade (1992) and Farag (1996) proved that, most antioxidants were responsible for accelerating the biosynthesis of various pigments and consequently more photosynthesis producing more quantities of photosynthates. Besides, Shahidi and Wanasundara (1992) stated that, phenolic antioxidants play important roles as free radical terminators and sometimes, as a metal chelators.

#### **b. Macronrients, micronutrients and Na:**

It could be stated from data in Tables (6 & 7) that N, P, K, Fe, Mn, Zn and Cu were significantly increased with the treatment of humic acid fecundated-seed-beds as compared with the treatment of humic acid free seed-beds whereas Na behaved reversely. These increases were of 16.0%, 33.3%, 18.7%, 12.5%, 11.1%, 10.9% and 13.2% for N, P, K, Fe, Mn, Zn and Cu, respectively. On the other side, Na was decreased by 32.5% in plant leaves. Existence of some minerals such as Fe, Mn, Zn and Cu in humic acids (Table 2) might be explained the results of this study. Lee and Bartlett (1976) reported that as the concentration of humic acids increased so did the percentage of P in the plant, revealing that humate plays an important role in the phosphate utilization of plants. The availability of P and Fe was increased due to humic application (Senn and Kingman, 1973). Abadia (1984) proved that, humic acids may increased plant growth parameters through their modifications in the soil root interface raising the nutrients availability to plants. Moreover, Jianguo *et al.* (1998) found that, humic acid application improved the nutritional regulation of plants as indicated by changes in various physiological and biochemical indexes. The activity of superoxide dismutase and various metabolic processes were increased as shown by synthesis and accumulation of nutrients. In addition, humic acid plays an important role for increasing the supplying power of soil capacity against nutrient loss and deficiency besides, the principal physiological function of humic acids may be reduce oxygen deficiency in plants, which results in better uptake of nutrients (Osman and Ewees, 2008).

Data presented in Tables (6&7) reveal that in the time in which Na significantly reduced, all tested nutrients exhibited a significant gradual increases with increasing ascorbic acid rate. The applied treatment of ascorbic acid at the rate of 450 mgL<sup>-1</sup> ranked as the best treatment and had, in general, the most pronounced counteracted effect on soil salinity under study. Such treatment surpassed that of zero mgL<sup>-1</sup> ascorbic acid (tap water) by 47.6%, 84.6%, 42.9%, 28.8%, 29.5%, 35.2% and 33.4% for N, P, K, Fe, Mn, Zn and Cu, respectively. On the other hand, Na reduced up to 52.3%. The beneficial effect of ascorbic acid, especially at the rate of 450 mgL<sup>-1</sup> on increasing tolerance of canola plants, especially at the rate of 450 mgL<sup>-1</sup> under the studied soil salinity was reflected on improving vegetative growth traits (Table 3), photosynthetic pigments (Table 4), photosynthates among

**Possibility of improving growth, physiological behaviour and .....**

them endogenous ascorbic acid (Table 5) and surely reflected also on stimulating the nutritional status of plants. These results supported those of Ahmed and Abd El-Hameed (2004), Rady (2006) and Rady and El-Sawah (2009) who reported that the effect of antioxidants, especially ascorbic acid, on producing healthy plants of great ability for more uptake of nutrients. Moreover, Gonzalez-Reyes *et al.* (1994) concluded that, ascorbate free radical caused hyperpolarization of plasma membranes, and this energization could then facilitate transport processes across such membranes. Most of the previous results are consistent with those of Ali (2000), Rady (2006) and Rady and El-Sawah (2009).

**Table (6): Influence of humic acid soil practice (B) and ascorbic acid foliar application (A) on some macronutrients and Na of leaves of canola plants grown under saline reclaimed soil conditions (data over the two seasons).**

Ascorbic acid (mgL <sup>-1</sup> )	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B	0 H.A.	+ H.A.	Mean B
	N (%)			P (%)			K (%)			Na (%)		
0	1.52	1.80	1.66	0.11	0.15	0.13	1.61	1.92	1.77	0.52	0.36	0.44
150	1.77	1.99	1.88	0.13	0.18	0.16	1.80	2.20	2.00	0.44	0.30	0.37
300	1.98	2.30	2.14	0.17	0.22	0.20	2.02	2.48	2.25	0.38	0.24	0.31
450	2.28	2.62	2.45	0.20	0.28	0.24	2.30	2.76	2.53	0.28	0.14	0.21
600	1.80	2.12	1.96	0.14	0.18	0.16	1.92	2.08	2.00	0.40	0.30	0.35
Mean A	1.87	2.17		0.15	0.20		1.93	2.29		0.40	0.27	
LSD <sub>0.05</sub> A	0.19			0.03			0.20			0.05		
LSD <sub>0.05</sub> B	0.11			0.02			0.12			0.03		
LSD <sub>0.05</sub> AxB	0.32			0.05			0.34			0.09		

0 H.A.= humic acid free seed-beds & + H.A.= humic acid fecundated-seed-beds

Data presented in Tables (6 & 7) show that, the best combined treatment promoted plant leaves to collect the highest amounts of nutrients, except with the reverse regarding Na, was fecundating the soil with humic acid interacted with spraying plant shoots with 450 mgL<sup>-1</sup> ascorbic acid solution. This treatment scored 45.6%, 86.7%, 43.8%, 30.2%, 30.8%, 36.5% and 30.6% for N, P, K, Fe, Mn, Zn and Cu, respectively, compared with the combined treatment of humic acid interacted with spraying the plant shoots with tap water while, Na decreased down to 61.1%. In addition, the same best combined treatment granted increases at 72.4%, 154.5%, 71.4%, 43.9%, 42.6%, 50.3% and 49.4% for the same nutrients, respectively, compared with the treatment of the interaction between humic acid untreated-soil and spraying the plant foliage with tap water, whereas Na reduced up to 73.1%. The increments obtained from the above mentioned best combined treatment, may be due to the acidity of humic acid which facilitate more solubility and absorption of nutrients (Osman and Ewees, 2008). Moreover, the increase in the nutrient elements of plants of the best combined treatment may be explained with the nutrients-containing humic acid under study (Table 2). As for ascorbic acid, Wise and Naylor (1987) indicated that, antioxidants such as ascorbate, glutathione and  $\alpha$ -tocopherol are directly

correlated with the ability to defend plant cells against oxidative damage resulting from salinity stress and consequently producing healthy plants having a great ability for nutrients uptake.

**Table (7): Influence of humic acid soil practice (B) and ascorbic acid foliar application (A) on some micronutrients of leaves of canola plants grown under saline reclaimed soil conditions (data over the two seasons).**

Ascorbic acid (mgL <sup>-1</sup> )	0	+	Mean	0	+	Mean	0	+	Mean	0	+	Mean
	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B
	Fe (ppm)			Mn (ppm)			Zn (ppm)			Cu (ppm)		
0	442.2	488.6	465.4	284.8	310.6	297.7	154.7	170.3	162.5	66.8	76.4	71.6
150	470.6	532.8	501.7	308.2	338.4	323.3	168.3	186.9	177.6	74.0	84.2	79.1
300	512.2	578.6	545.4	329.7	372.5	351.1	188.4	208.2	198.3	80.7	92.5	86.6
450	562.4	636.2	599.3	364.8	406.2	385.5	206.9	232.5	219.7	91.2	99.8	95.5
600	494.4	556.4	525.4	314.5	352.3	333.4	174.3	192.1	183.2	76.6	88.0	82.3
Mean A	496.4	558.5		320.4	356.0		178.5	198.0		77.9	88.2	
LSD <sub>0.05</sub> A	51.4			32.3			18..2			8.4		
LSD <sub>0.05</sub> B	29.2			18.3			10.3			4.8		
LSD <sub>0.05</sub> AxB	88.1			55.4			31.2			14.4		

0 H.A.= humic acid free seed-beds & + H.A.= humic acid fecundated-seed-beds

### 3. Seed yield and its components:

Data in Tables (8 & 9) indicate that number of fruiting branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, seed yield plant<sup>-1</sup>, seed yield feddan<sup>-1</sup>, seed oil%, oil yield feddan<sup>-1</sup>, seed protein% and protein yield feddan<sup>-1</sup> under study were significantly increased as a result of treating the seed-beds, directly before sowing, with humic acid. The increases were 31.2%, 28.2%, 30.1%, 27.8%, 9.0%, 40.5%, 18.5% and 52.4% for the above mentioned traits respectively, compared with humic acid free seed-beds. The improvement of canola seed yield and seed quality may be due to the positive effect of humic acid on plant growth traits (Table 3) and leaf photosynthetic pigments (Table 4) as well as macro- and microelements (Tables 6 & 7) and other components; total soluble sugars and free proline (Table 5) as osmotic substances. Humic acids are partially capable to retain water and nutrients (Osman and Ewees, 2008) in the rhizosphere lead to overcome the harmful effect of salinity. Raviv (1998) stated that humic acids may exert desirable direct enzymatic or hormonal effects on plant growth and yield. Moreover, Abou Zeid *et al.* (2005) added that, using humic acids improved the productivity of some crops grown on a sandy soil.

***Possibility of improving growth, physiological behaviour and .....***

**Table (8): Influence of humic acid soil practice (B) and ascorbic acid foliar application (A) on seed yield and its components of canola plants grown under saline reclaimed soil conditions (data over the two seasons).**

Ascorbic acid (mgL <sup>-1</sup> )	0	+	Mean	0	+	Mean	0	+	Mean	0	+	Mean
	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B
	Number of fruiting branches plant <sup>-1</sup>			Number of pods plant <sup>-1</sup>			Seed yield plant <sup>-1</sup> (g)			Seed yield feddan <sup>-1</sup> (ton)		
0	3.01	4.22	3.62	51.8	69.6	60.7	3.97	5.36	4.67	0.43	0.58	0.51
150	3.82	4.74	4.28	66.9	83.5	75.2	5.15	6.60	5.88	0.58	0.73	0.66
300	4.20	5.38	4.79	84.6	116.0	100.3	6.68	9.28	7.98	0.77	1.09	0.93
450	4.86	6.44	5.65	112.6	138.6	125.6	9.01	11.37	10.19	1.10	1.38	1.24
600	3.68	4.88	4.28	76.8	95.2	86.0	5.90	7.36	6.63	0.71	0.84	0.78
Mean A	3.91	5.13		78.5	100.6		6.14	7.99		0.72	0.92	
LSD <sub>0.05</sub> A	0.44			8.8			0.66			0.09		
LSD <sub>0.05</sub> B	0.25			5.0			0.38			0.05		
LSD <sub>0.05</sub> AxB	0.75			15.0			1.13			0.15		

0 H.A.= humic acid free seed-beds & + H.A.= humic acid fecundated-seed-beds

**Table (9): Influence of humic acid soil practice (B) and ascorbic acid foliar application (A) on seed oil and protein yields, and seed quality of canola plants grown under saline reclaimed soil conditions (data over the two seasons).**

Ascorbic acid (mgL <sup>-1</sup> )	0	+	Mean	0	+	Mean	0	+	Mean	0	+	Mean
	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B	H.A.	H.A.	B
	Oil (%)			Oil yield (kg feddan <sup>-1</sup> )			Protein (%)			Protein yield (kg feddan <sup>-1</sup> )		
0	40.8	44.6	42.7	175.5	258.7	217.1	24.7	29.2	27.0	106.2	169.4	137.8
150	41.2	44.5	42.9	239.0	324.9	282.0	24.5	29.6	27.1	142.1	216.1	179.1
300	41.1	44.8	43.0	316.5	488.3	402.4	25.0	29.5	27.3	192.5	321.6	257.1
450	41.4	45.6	43.5	455.4	629.3	542.4	25.2	29.9	27.6	277.2	412.6	344.9
600	41.0	44.6	42.8	291.1	374.6	332.9	25.0	29.2	27.1	177.5	245.3	211.4
Mean A	41.1	44.8		295.5	415.2		24.9	29.5		179.1	273.0	
LSD <sub>0.05</sub> A	3.6			35.9			3.3			21.8		
LSD <sub>0.05</sub> B	N.S			20.5			N.S			12.1		
LSD <sub>0.05</sub> AxB	N.S			61.5			N.S			37.1		

0 H.A.= humic acid free seed-beds & + H.A.= humic acid fecundated-seed-beds

The treatment of ascorbic acid foliar application at the rate of 450 mgL<sup>-1</sup> (Tables 8 & 9) gave the highest significant increases for canola seed yield and its components, whereas seed oil and protein percentages revealed insignificant differences, compared with other ascorbic acid rates. These increments scored with the treatment of 450 mgL<sup>-1</sup> ascorbic acid solution were 56.1%, 106.9%, 118.2%, 143.1%, 149.8% and 150.3% for number of fruiting branches plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, seed yield plant<sup>-1</sup>, seed yield feddan<sup>-1</sup>, oil yield feddan<sup>-1</sup> and protein yield feddan<sup>-1</sup>, respectively compared to the treatment of ascorbic acid-free treatment. The improving effect of ascorbic acid on seed yield and seed quality was mainly attributed to its positive action on enhancing growth traits (Table 3), photosynthetic pigments of plant leaves (Table 4), cellular solutes, viz. total soluble sugars,

total free amino acids and free proline (Table 5) for sustenance of cells turgor leading to maintenance of metabolic activities in plants and plant nutritional status (Tables 6&7). In this respect, Al-Qubaie (2002) found that ascorbic acid as an antioxidant compound had an auxinic action and also synergistic effect on the biosynthesis of carbohydrates and controlling the incidence of most fungi on plants makes them in vigour states and reflects on seed yield, seed oil and protein. Besides, the induced effect of ascorbic acid as one of vitamins on growth and yield may be due to that vitamins are recognized to be coenzymes involved in specific biochemical reactions in plants such as oxidative and non-oxidative decarboxylations (Robinson, 1973). These positive results of ascorbic acid on seed yield and its components are confirmed with those reported by Ahmed *et al.* (2003), Mostafa (2004), Rady (2006) and Rady and El-Sawah (2009).

For the interaction between treated or untreated soil with humic acid and ascorbic acid foliar application at various rates, data presented in Tables (8&9) show that the highest significant increase was obtained from the application of 450 mgL<sup>-1</sup> ascorbic acid combined with fecundating the soil with humic acid for the tested canola seed yield and seed quality. These increases obtained from this combined treatment scored 52.6%, 99.1%, 112.1%, 137.9%, 143.3 and 143.6% for number of fruiting plant<sup>-1</sup>, number of pods plant<sup>-1</sup>, seed yield plant<sup>-1</sup>, seed yield feddan<sup>-1</sup>, oil yield feddan<sup>-1</sup> and protein yield feddan<sup>-1</sup>, respectively. This favourable yield may be due to the positive combined effect of humic and ascorbic acids. The former had acidic effect in the rizhosphere and consequently more solubility and absorption of nutrients by plant roots reflecting on seed yield and its components as well as seed quality yields. The latter; ascorbic acid as an antioxidant compound has an auxinic action and also synergistic effect on the biosynthesis of carbohydrates (Al-Qubaie, 2002) reflected on seed yield and its components and consequently seed oil and protein yields. Besides, the induced effect of ascorbic acid as one of vitamins on growth and yield (Robinson, 1973).

## **Conclusion and recommendation**

It can be concluded that the work within hand gave an evidence to the role of humic acid in improving the soil fertility as a result of increasing useful soil microorganisms for plants as well as improving drainage and aeration, and increasing the availability of nutrient elements and consequently affected plant growth and yield. In addition, it increased the water holding capacity of soil therefore; it enhanced plant resistance to drought and salinity and stimulated seed germination. Furthermore, it activated the biochemical processes in plants (respiration and photosynthesis) and promoted the growth by its auxinic effect and its cytokinin component. Also, the role of ascorbic acid, as an antioxidant by which plant foliage applied, in inducing salinity tolerance of canola plants cultivated in salt-affected reclaimed soils containing salts concentration up to 9000 ppm leading to a

## Possibility of improving growth, physiological behaviour and .....

favourable growth and consequently produced economic yields of seeds, protein and oil under such conditions. This may be due to that ascorbic acid had an auxinic action and also synergistic effect on the biosynthesis of carbohydrates and controlling the incidence of most fungi on plants made them in vigour states and reflected on seed yield and its components and consequently seed oil and protein yields. Besides, the induced effect of ascorbic acid as a vitamin that recognized to be coenzymes involved in specific biochemical reactions in plants such as oxidative and non-oxidative decarboxylations. Thus, it has been recommended as the result of this study, that cultivation of canola crop could be successfully accomplished in saline reclaimed soils possess salinity level up to 9000 ppm by using some assistant factors; fecundating the seed-beds with humic acid at the rate of 6 kg feddan<sup>-1</sup> and spraying the produced plants with ascorbic acid at the rate of 450 mgL<sup>-1</sup> two times; 5 and 8 weeks after sowing.

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***Possibility of improving growth, physiological behaviour and .....***

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## إمكانية تحسين النمو والسلوك الفسيولوجي والمحصول لنباتات الكانولا النامية في أراضي مستصلحة ملحية باستخدام حامضي الهيوميك والأسكوربيك

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### الملخص العربي

أجرى هذا البحث في المزرعة التجريبية الخاصة بكلية الزراعة، جامعة الفيوم - مصر خلال موسمي ٢٠٠٧/٢٠٠٨ و ٢٠٠٨/٢٠٠٩ بهدف دراسة تأثير معاملة التربة (مراقد البذرة) بحامض الهيوميك، ثم رش النباتات الناتجة بحامض الأسكوربيك بتركيز صفر (كنترول)، ١٥٠، ٣٠٠، ٤٥٠، و ٦٠٠ ملجم/لتر على إمكانية تحسين النمو والسلوك الفسيولوجي والقدرة الإنتاجية (بذور وزيت البذرة) لنباتات الكانولا النامية تحت ظروف التربة المستصلحة المتأثرة بالأملاح (حوالي ٩٠٠٠ جزء في المليون).

أدت معاملة التربة (مراقد البذرة) بحامض الهيوميك قبل الزراعة بمفردها أو مع جميع معاملات حامض الأسكوربيك إلى زيادات معنوية لكل من صفات النمو (ارتفاع النبات، عدد الأوراق/نبات، المساحة الكلية للأوراق/نبات، والوزن الجاف للأوراق/نبات)، المكونات الكيماوية محل الدراسة (صبغات البناء الضوئي، بعض نواتج التمثيل الضوئي، وبعض المغذيات الكبرى والصغرى)، والمحاصيل المختلفة (محصول البذور/نبات & فدان، ومحصول الزيت والبروتين/فدان) وذلك بالمقارنة بالكنترول (المعاملة الخالية من حامضي الهيوميك والأسكوربيك).

بالنسبة للمعاملة بحامض الأسكوربيك، فقد وجد أن النباتات التي عوملت رشاً بهذه المادة بجميع التركيزات المستخدمة في الدراسة (١٥٠، ٣٠٠، ٤٥٠، و ٦٠٠ ملجم/لتر) أظهرت زيادات

### Possibility of improving growth, physiological behaviour and .....

مغوية في جميع القياسات تحت الدراسة (مثل صفات النمو، المكونات الكيماوية، ومحاصيل البذور والزيت والبروتين ) ، وكانت أفضل النتائج هي المتحصل عليها من رش النباتات بمحلول حامض الأسكوربيك بمعدل ٤٥٠ ملجم/لتر مقارنة بالنباتات التي عوملت رشاً بالماء. في ضوء تلك النتائج يمكن استنتاج أن، نباتات الكانولا (صنف سرو ٤) الناتجة من مراقذ البذرة التي عوملت قبل الزراعة بحامض الهيوميك عند رشها بمحلول من حامض الأسكوربيك بتركيز ٤٥٠ ملجم/لتر قد تمكن النباتات من التغلب على الظروف المعاكسة للأراضي المستصلحة حديثاً، خاصة تحت ظروف الملوحة حتى تركيز ٩٠٠٠ جزء في المليون، وبالتالي إمكانية الحصول على محاصيل اقتصادية من البذور والزيت والبروتين في ظل الظروف سالفه الذكر.