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Pre-Sowing Seed Treatment with some Organic Compounds for Controlling Root Rot Disease and Improving Faba Bean Productivity under Salinity Affected Soil Conditions

Abd El-Hai, K.M. [1] and Aml E. A. El-Saidy [2]

[1]Department of Leguminous and Forage Crop Diseases Research, Plant Pathology Research Institute, ARC, Egypt

[2]Department of Seed Technology Research, Field Crop Research Institute, ARC, Egypt

ABSTRACT

Greenhouse experiment was conducted to evaluate the pathogenicity of root rot fungi on faba bean plant. Lab. experiment was also conducted to study the effect of organic compounds and fungicide Rizolex-T50 on the linear growth of the tested pathogenic fungi. Two field experiments were also carried out to study the effect of organic compounds with different concentrations and fungicide Rizolex-T50 on reduction of root rot and enhancing some morphological, physiological characteristics and productivity of faba bean under saline affected soil conditions during 2013/2014 and 2014/2015 seasons. The results under greenhouse conditions indicated that all tested pathogenic fungi caused emergence damping-off. *Rhizoctonia solani* was the most effective in pre-emergence damping-off while, *Fusarium solani* gave the highest percentage of post-emergence damping-off. The pathogenic fungi caused anatomically deformation in root structure. Infected root with *F. solani* caused complete destruction of epidermal cells followed by degradation of cell wall in the cells of cortex. *In vitro*, Rizolex-T50 completely inhibited the growth of the tested pathogenic fungi. On the other hand, chitosan at 8 g/L was the most effective in reducing fungal growth followed by proline at 200 ppm. *In vivo*, the application of organic compounds counteracted the harmful effects of salinity. Humic acid highly reduced pre- and post-emergence damping-off and subsequently increased field emergence %. The high level of proline (200 ppm) was more effective in increasing morphological measurements (plant height, No. of branches and leaves/plant) and photosynthetic pigments i.e. chlorophyll a, b and carotenoids. While, the high concentration of HA (2000 ppm) significantly increased No. of pods/plant, seed yield/plant and 100-seed weight. Chitosan gave the highest phenols content followed by proline treatment. The high increase in carbohydrate content occurred under low level of HA (1000 ppm), followed by high level of proline (200 ppm). The efficacy of the tested organic compounds in control of root rot disease was reflected on the plant causing increase in yield components. It is suggested that pre-sowing seed soaking with HA (2000 ppm) or chitosan (8 g/L) or proline (200 ppm) could be considered as alternative fungicide for controlling root rot disease and increasing productivity of faba bean under salinity and natural infection conditions in the field.

Keywords: Organic compounds, humic acid, chitosan, proline, faba bean, root rot, salt stress.

INTRODUCTION

Fabaceous crops are either sensitive or moderately tolerant to salinity. Faba bean is moderately tolerant to salinity (Al-Tahir and Al-Abdulsalam, 1997), support biological nitrogen fixation and sustainable source of nitrogen to cropping systems (Crews and Peoples, 2004). On the other hand, saline conditions lead to reduced water potential in the root zone causing water deficit, phytotoxicity of ions and nutrient imbalance (Munns, 2002). Furthermore, salinity conditions had adversely affect the cellular content of plant cells of different species, such as soluble carbohydrates, nucleic acids and proteins (Younis and Tourky, 2014).

In Egypt, most of the newly reclaimed lands depend on underground water of various degrees of salinity for irrigation. Also, progressive accumulation of salts became a serious problem in many cultivated areas of the Delta as a result of high ground water table, especially when accompanied by poor drainage (Bekhet *et al*., 2009). The plant diseases are a major limitation to improve production efficiency and crop quality. Root rot disease is a major limiting factor in faba bean production. It is commonly caused by one or more of *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Phythium* spp. or *Sclerotinia* spp. (Al-Kahal *et al*., 2009). They added that infection can occur early causing seed rot and seedling death before emergence or collapse of seedlings shortly after emergence. Seedlings stems may be characteristically constricted at or near the soil surface. The root system of infected plants shows a black rot which may extend into the base of the stem. The cortical tissue is invaded and a longitudinal section through the root and lower stem reveals that the vascular tissue is intact.

Seed treatment by chemical fungicide before sowing is a common practice to prevent seed and seedling rots, damping-off and other fungal diseases. Using of chemical fungicides lead to reduction in population of beneficial microorganisms, death of Rhizobia and its negative effects on public health and environmental balance (Al-Kahal *et al*., 2009). However, urgent need to use an interesting alternative to fungicide involves the use of some organic compounds. Humic acid overcoming the harmful effects of chocolate spot and rust diseases in faba bean plants (El-Ghamry *et al*., 2009). Some authors studied the effect of HA on plant structures under abiotic stresses especially salinity conditions (Hamideh *et al*., 2013). Humic acid also stimulate plant growth through the increase in cell division (Chen *et al*., 2004). Chitosan also used for controlling several plant diseases due to its fungicidal effects and elicitation of defense mechanisms in plant tissues (Prapagdee *et al*., 2007 and Ali *et al*., 2009). Also, proline induces the expression of salt-stress-responsive proteins and may improve the plant adaptation to salt stress (Khedr *et al*., 2003).

The present study aimed to measure the potential roles of humic acid, chitosan and proline on reduction of root rot disease and improving productivity of faba bean under saline affected soil conditions.

MATERIALS AND METHODS

Faba bean seeds cv. Giza 843 were obtained from Legume Crops Research Department, Field Crops Res. Instit., Agric. Res. Center, Giza, Egypt. Organic compounds i.e. humic acid (Actosol) containing 80% humic acid + 6% K_2O was obtained from Egyptian Fertilizer Development Center, El-Mansoura, Egypt, chitosan (Liponet) and L-proline were obtained from Sigma Chemical Company. Samples of faba bean plant showing root rot disease symptoms were collected from different locations of Dakahlia Governorate, Egypt during the winter season of 2012/2013. The infected roots were thoroughly washed in tap water and cut into small pieces, then surface sterilized with 2% sodium hypochlorite for 2 min. Sterilized pieces rewashed with sterilized water, dried between two sterilized filter paper, placed on potato dextrose agar (PDA) medium supplemented with streptomycin-sulfate (100 µg ml^{-1}) and incubated at 26º C. The growing fungi were purified according to Hawker (1960), then identified based on taxonomic criteria as described by Sneh *et al* (1992) for *R. solani* and Booth (1977) for *F. solani* and *F. oxysporum*. The fungal inoculum was prepared using sorghum, coarse sand and water (2:1:2 v/v) medium. The medium contents were mixed, bottled and autoclaved for 15 min. at 1.5 air pressure and 121^{o}C. The sterilized medium was inoculated using agar discs obtained from the peripheral of 7 days old colony of the isolated fungi. The inoculated media were incubated at 26º C for 15 days then used for soil infestation in greenhouse experiment.

Pot experiment was carried out in greenhouse for 40 days at the Department of Plant Pathology, Faculty of Agriculture, Mansoura University to evaluate the purified isolates of pathogenic fungi for their pathogenicity on faba bean under greenhouse conditions. Sterilized plastic pots (25 cm diam.) filled up with 4 kg loamy soil were artificially infected with the inoculum of the tested fungi at the rate of 0.4% (w/w). Four pots were used as replicate for each treatment as well as check (un-infected soil) using three replications. The infected pots were watered daily for one week to enhance growth distribution of fungal inoculum. Disinfected faba bean seeds (cv. Giza 843) were sown at the rate of 5 seeds/pot. During the growth period, disease development was observed. Pre-and post-emergence damping-off were recorded at 15 and 40 days after sowing, respectively. A completely randomized design with three replications was used in this experiment.

The anatomical changes in faba bean root due to the pathogenic fungi were determined after 30 days from sowing under greenhouse. Specimens of faba bean roots (5 mm in length) were killed and fixed in formalin: alcohol: acetic acid (1: 18: 1 v/v). The killing and fixing specimens washed and dehydrated in alcohol series and embedded in paraffin wax. Sections were prepared by a rotary microtome at 12-15 µ thick, stained in crystal violet and erythrosine, then cleared in xylol and mounted in Canada balsam (Gerlach, 1977). The sections were examined microscopically for determining the anatomical changes occurring due to pathogenic fungi.

The effect of organic compounds and fungicide Rizolex-T50 w.p. on the linear growth of the tested pathogenic fungi was *in vitro* evaluated. Humic acid at 1000 and 2000 ppm, chitosan at 6 and 8 g/L, proline at 100 and 200 ppm and Rizolex-T50 at 3 g/L were added to 10 ml of sterilized PDA before solidification and poured in sterilized perti-dishes. The plates were inoculated with fungal disc (5 mm) and incubated at 25 ºC. Three plates were prepared to serve as control for each fungus and three plates for each particular treatment for each fungus were used as replicates. Fungal linear growth was observed daily and diameter of fungal colonies were measured when the full growth of tested fungi was observed in the check treatment. A completely randomized design with three replications was used in this experiment.

Field experiments were carried out under natural infection during the two growing seasons of 2013/2014 and 2014/2015 at Tag El-Ezz Research Station, Dakahlia Governorate, Egypt. The soil of farm was divided into three blocks (1.5, 2.5 and 3.5 ds m^{-1}, approximately) by measuring the electrical conductivity (E. C.). Faba bean seeds of cv. Giza 843 were soaked for 10 h in each tested organic compounds while Rizolex was used as seed coating. Treated faba bean seeds were sown in 27th and 16th November in the first and second seasons, respectively. Treatments (Humic acid at 1000 and 2000 ppm, chitosan at 6 and 8 g/L, proline at 100 and 200 ppm and Rizolex-T50 at 3 g/L) also used as a foliar spraying at 35 and 50 days from sowing as active doses. Field emergence and pre-emergence damping-off were recorded at 15 days from sowing as well as post-emergence damping-off was recorded at 40 days from sowing. A split plot design with three replicates was used in field experiments. Main plots were occupied by salinity levels and sub-plots were occupied by organic compounds treatments. Each plot consisted of 5 ridges 3.5 m long and 60 cm width, occupying an area of 10.5 m^2 (1/400 fed.). Seeds were sown in hills spaced 20 cm apart with two seeds/hill. Phosphorus fertilizer was applied in the form of calcium superphosphate (15.5% P_2O_5) during land preparation at the rate of 100-150 kg/fed. Nitrogen fertilizer was applied in the form of ammonium nitrate (33.5 % N) at the rate of 15 kg/fed (after thinning to one plant/hill), whereas potassium sulphate (48% K_2O) was added at the rate of 50 kg K_2O/fed after 35 days from sowing. The normal cultural practices for growing faba bean were conducted as recommended by Ministry of Agricultural and land Reclamation.

After 75 days from sowing, ten plants were randomly selected from each plot to determine plant height (cm), number of branches / plant and number of leaves / plant. The blade of the 3rd leaf from plant tip (terminal leaflet) was taken to determine photosynthetic pigments (chl a, b and carotenoids) which extracted with methanol 90% after adding traces of calcium carbonate (Robinson and Britz, 2000) and determined according to Mackinney,1941. Total phenols (mg

catecole 100 g $^{-1}$ fresh weight) in fresh shoot were determined using the Folin - ciocalteau reagent (Singleton and Rossi, 1965). Proline content (µ moles/g dry weight) in dry shoot was estimated according to the method of Bates *et al* (1973) Total soluble carbohydrates (mg/g dry weight) in dry shoot was determined using the antherone method (Hedge and Hofrciter, 1962). At harvest time, ten plants were randomly taken from each plot to estimate number of pods plant $^{-1}$, seed yield/ plant (g) and weight of 100 seeds.

All data statistically analyzed according to the technique of analysis of variance (ANOVA) for completely randomized design (greenhouse and laboratory experiments) and split plot design for field experiments as published by Gomez and Gomez (1984) by means of MSTAT-C computer software package. Least significant of difference (LSD) method was used test the differences between treatment means at 5 % level of probability as described by Snedecor and Cochran (1982)

RESULTS

Three species of two fungal genera were isolated from samples of faba bean plants showing root rot symptoms. Three isolates of fungi i.e. *Rhizoctonia solani*, *Fusarium solani* and *Fusarium oxysporum* were identified and tested to determine their pathogenic capability of faba bean plants (c.v. Giza 843). Data in Table (1) show that all tested fungi were pathogenic and caused root rot symptoms under greenhouse conditions. *R. solani* was the most pathogenic fungi in pre-emergence damping-off (43.3%) followed by *F. solani* (36.6%). On the other side, *F.solani* gave highest percentage post-emergence damping-off (25.7%) followed by *R. solani* (22.0%). Meanwhile, *F. oxysporum* was the lowest pathogenic one which recorded 25.0% and 17.0% of pre and post-emergence damping-off, respectively.

Table 1. Pathogenicity test of the isolated fungi on faba bean damping-off (%) during 2013/2014 season

Characters / Treatments	Pre - emergence	Post - emergence	Survival
Check	3.7	0.0	96.3
Rhizoctonia solani	43.3	22.0	34.7
Fusarium solani	36.6	25.7	37.7
Fusarium oxysporum	25.0	17.0	58.0
F test	**	**	**
LSD at 5 %	2.9	2.0	6.0

**** indicate significant**

Fig. (1) illustrated that clear differences of root structure were found among cross sections of healthy and infected plants with pathogenic fungi. Remarkable differences occurred mainly in epidermis and cortex in the cross sections of infected root compared with control. The infected with fungi led to complete destruction of the epidermis that exhibited dark brown epidermal surface. The injuries changes followed by degradation of cell wall and hydrolysis of cell components in cortex and some area of vascular cylinder (pericycle, phloem and xylem). *R. solani* was the most aggressive followed by *F. solani*.

The inhibitory effect of tested organic compounds against linear growth of faba bean root rot fungi are presented in Table (2). Rizolex-T50 completely inhibited the linear growth of all tested pathogenic fungi. Moreover, complete inhibition in the growth of *R. solani* and *F. oxysporum* was recorded with chitosan at the concentration of 8 g/L. high reduction in fungal growth was observed with chitosan at 8 g/L and proline at 200 ppm however, HA came late. *R. solani* showed the most sensitive to organic compounds followed by *F. oxysporum*.

Table 2. The linear growth (cm) of faba bean root rot pathogens as affected by organic compounds treatment during 2013/2014 season

Characters / Treatments	*Rhizoctonia solani*	*Fusarium solani*	*Fusarium oxysporum*
Check	8.83	9.00	8.86
HA 1000 ppm	6.70	7.93	7.36
HA 2000 ppm	5.26	6.23	5.30
Chitosan 6g L^{-1}	2.60	3.06	3.33
Chitosan 8g L^{-1}	0.00	2.1	0.00
Proline 100 ppm	4.26	6.06	4.4
Proline 200 ppm	2.93	4.13	2.93
Rizolex - T 50	0.00	0.00	0.00
F test	**	**	**
LSD at 5 %	0.70	0.28	0.32

**** indicate high significant**

Data in Table (3) show that the field emergence percent of faba bean seeds decreased significantly with increasing salinity level. The highest reduction occurred under high salinity level. On the other hand, damping-off increased significantly under natural infection with increasing salinity level. It is worthy to mention that pre-emergence damping-off was more than post-emergence damping-off in both seasons.

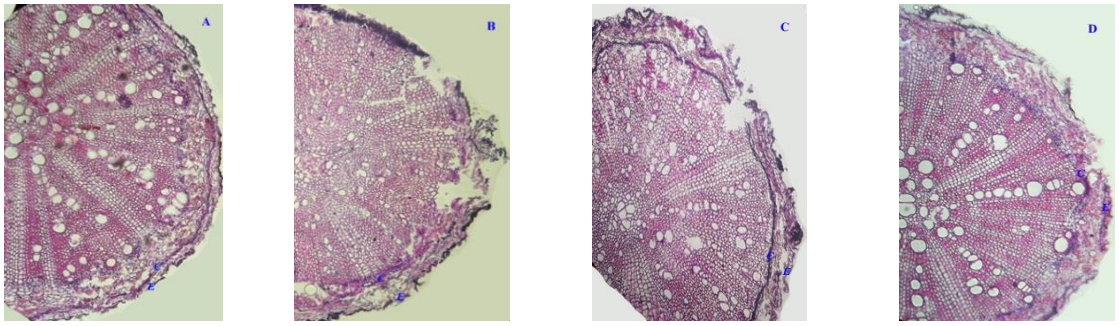
Table 3. Field emergence, pre and post emergence damping – off (%) as affected by salinity levels (S) and organic compounds treatment during 2013/2014 and 2014/2015 seasons

Characters / Treatments	Field emergence		Pre – emergence		Post – emergence	
	13/14	14/15	13/14	14/15	13/14	14/15
A- Salinity levels:						
1.5 dS m^{-1}	85.7	87.7	14.3	12.3	8.5	5.4
2.5 dS m^{-1}	68.5	74.2	31.5	25.8	12.8	9.2
3.5 dS m^{-1}	55.3	64.6	44.7	35.4	16.5	14.3
F test	**	**	**	**	**	**
LSD at 5 %	1.6	0.7	1.6	0.7	0.9	0.5
B- Organic compounds:						
Check	61.7	65.6	38.3	34.4	19.0	17.0
HA 1000 ppm	76.2	82.6	23.8	17.4	13.7	10.0
HA 2000 ppm	67.9	73.7	32.1	26.3	8.1	6.9
Chitosan 6g L^{-1}	65.1	69.2	34.9	30.8	16.0	11.6
Chitosan 8g L^{-1}	70.7	76.3	29.3	23.7	12.3	9.1
Proline 100 ppm	66.0	71.6	34.0	28.4	15.0	10.9
Proline 200 ppm	73.8	80.2	26.2	19.8	10.3	8.0
Rizolex - T 50	77.4	84.8	22.6	15.2	6.2	3.4
F test	**	**	**	**	**	**
LSD at 5 %	1.1	1.3	1.1	1.3	0.9	0.8
C- Interaction	**	**	**	**	**	**

*** indicate high significant**

Concerning the effect of organic compounds, data in also show that all tested organic compounds at both concentrations increased significantly field emergence percent and decreased significantly damping-off in both seasons compared with check. After Rizolex-T50, HA was more effect followed by proline then chitosan. The interaction between the two factors had significant effects on field emergence %, pre and post emergence damping – off in the two seasons (Table 3).

The response of faba bean plants growth to the application of organic compounds under salinity stress conditions was determined by means of measuring plant height , No. of branches and leaves/plant. Data in Table (4) indicate that salinity decreased significantly plant height, number of branches/plant and leaves/plant in both seasons. The highest reduction in these parameters occurred under high salinity level.



A: helthy plant **B: *Rhizoctonia solani*** **C: *Fusarium solani*** **D: *Fusarium oxysporum***

Fig 1: Root structure of faba bean as affected by pathogenic fungi

Table 4. Plant height, No. of branches and leaves / plant as affected by salinity levels (S) and organic compounds treatment during 2013/2014 and 2014/2015 seasons

Characters	Plant height (cm)		No. of branches / plant		No. of leaves / plant	
Treatments	**13/14**	**14/15**	**13/14**	**14/15**	**13/14**	**14/15**
A- Salinity levels:						
1.5 dS m^{-1}	109.7	105.9	7.0	8.2	48.9	51.1
2.5 dS m^{-1}	84.9	86.0	6.0	7.0	39.1	43.9
3.5 dS m^{-1}	62.0	65.0	5.0	5.4	28.6	33.6
F test	**	**	**	**	**	**
LSD at 5 %	2.1	2.1	0.3	0.4	1.9	1.8
B- Organic compounds:						
Check	76.5	72.8	4.6	5.4	32.6	36.5
HA 1000 ppm	86.6	88.5	6.1	6.8	37.8	41.6
HA 2000 ppm	90.5	92.0	6.4	7.3	39.7	43.8
Chitosan 6g L^{-1}	84.0	85.5	5.7	6.6	40.1	44.0
Chitosan 8g L^{-1}	80.1	81.1	6.4	7.2	42.1	45.5
Proline 100 ppm	95.5	95.5	6.7	7.7	41.6	46.3
Proline 200 ppm	101.3	100.2	7.4	8.4	44.8	48.5
Rizolex - T 50	69.7	69.4	4.6	5.5	32.3	36.7
F test	**	**	**	**	**	**
LSD at 5 %	1.7	1.9	0.5	0.5	1.5	1.6
C- Interaction	**	**	*	**	**	**

**** indicate high significant**

Regarding the effect of organic compounds and fungicide Rizolex-T50, data in Table (4) revealed that plant height and No. of branches and leaves/plant increased significantly under any dose of organic compounds. Proline at high level (200 ppm) was more effective. Meanwhile, Rizolex-T50 decreased plant height and has insignificant effect on number of branches and leaves.

The interaction between salinity levels and organic compounds treatment had significant effect on faba bean plant growth in both seasons (Table 4).

Data tabulated in Table (5) show that the highest values of plant height and number of leaves/plant were recorded from the application of proline followed by humic acid, the high concentration was more effective than the low concentration. It is worthy to mention that there is a negative correlation between salinity levels and number of branches under any dose of organic compounds used. While, Rizolex-T50 decreased plant height and had insignificant effect on number of branches and leaves/plant at the different levels of salinity.

Data in Table (6) show that chlorophyll a and b decreased significantly with increasing salinity levels. However, a significant increase in carotenoids content was noticed with increasing salinity level. The application of organic compounds at any concentration increased significantly all photosynthetic pigments. The highest increase in chlorophyll a and b was observed with proline at 200 ppm followed by HA at 2000 ppm. Moreover, the maximum increase in carotenoids content occurred under proline treatment followed by chitosan. The high concentration was more effective. However, chlorophylls and carotenoids were not affected significantly under Rizolex-T50 application.

All interaction between salinity level and organic compounds had significant effect on chlorophyll a and b. However, the interaction between salinity levels and organic compounds had no effect on carotenoids content (Table 6).

Data in Table (7) show that total phenols and proline content increased significantly with increasing salinity levels. While, carbohydrate content was decreased. All tested organic materials and Rizolex-T50 increased significantly the above parameters in both seasons. Chitosan gave the highest values of phenols followed by proline then HA, while Rizolex-T50 came late.

Table 5. Plant height, No. of branches and leaves / plant of faba bean as affected by the interaction between organic compounds treatment and salinity levels (S) during 2013/2014 and 2014/2015 seasons

Organic compounds \ Salinity	Plant height (cm)			No. of branches / plant			No. of leaves / plant		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
			2013/2014						
Check	98.3	78.3	53.0	5.7	4.7	3.7	40.0	35.0	23.0
HA 1000 ppm	111.3	86.7	62.0	7.3	6.3	4.7	44.3	39.3	30.0
HA 2000 ppm	115.7	90.7	65.3	7.7	6.7	5.0	48.3	40.3	30.7
Chitosan 6 gL^{-1}	108.3	83.0	60.7	6.3	5.3	5.7	57.7	37.3	25.3
Chitosan 8 gL^{-1}	102.0	80.3	58.0	7.0	6.0	6.3	59.3	39.0	28.0
Proline 100 ppm	123.0	92.0	71.7	8.0	7.0	5.3	50.0	41.7	33.3
Proline 200 ppm	127.0	101.3	75.7	8.7	7.7	6.0	52.7	46.3	35.7
Rizolex - T50	92.7	67.0	49.7	6.0	4.7	3.3	39.3	34.3	23.3
LSD at 5%		2.9			0.9			2.6	
			2014/2015						
Check	90.3	75.0	53.3	7.0	5.3	4.0	43.7	38.7	27.3
HA 1000 ppm	109.3	89.3	67.0	8.3	7.3	5.0	45.0	45.0	35.0
HA 2000 ppm	113.0	92.7	70.3	9.0	7.7	5.3	47.7	47.3	36.7
Chitosan 6 g L^{-1}	104.7	87.7	64.3	7.3	6.3	6.3	61.0	41.0	30.0
Chitosan 8 g L^{-1}	99.0	82.3	62.0	8.0	7.0	6.7	63.7	41.3	31.7
Proline 100 ppm	119.0	94.0	73.7	9.3	8.0	6.0	51.0	48.3	39.7
Proline 200 ppm	124.7	99.0	77.0	10.3	8.7	6.3	54.3	50.7	40.7
Rizolex - T 50	87.3	68.0	53.0	6.7	6.0	4.0	43.0	39.0	28.3
LSD at 5%		3.5			0.9			2.8	

S1=1.5 dSm^{-1} S2=2.5 dSm^{-1} S3 =3.5 dSm^{-1}

Table 6. Chlorophyll A, chlorophyll B and carotenoids of faba bean as affected by salinity levels (S) and organic compounds treatment during 2013 /2014 and 2014/2015 seasons

Characters	Chlorophyll A (mg/g fresh weight)		Chlorophyll B (mg/g fresh weight)		Carotenoids (mg/g fresh weight)	
Treatments	13/14	14/15	13/14	14/15	13/14	14/15
A- Salinity levels:						
1.5 dS m^{-1}	1.177	1.262	0.774	0.852	0.282	0.320
2.5 dS m^{-1}	0.994	1.061	0.607	0.650	0.335	0.362
3.5 dS m^{-1}	0.815	0.872	0.507	0.560	0.393	0.409
F test	**	**	**	**	**	**
LSD at 5 %	0.011	0.044	0.013	0.023	0.007	0.003
B- Organic compounds:						
Check	0.856	0.931	0.547	0.598	0.285	0.314
HA 1000 ppm	1.040	1.108	0.652	0.714	0.308	0.331
HA 2000 ppm	1.091	1.152	0.691	0.752	0.326	0.345
Chitosan 6g L^{-1}	0.930	0.978	0.585	0.635	0.338	0.362
Chitosan 8g L^{-1}	0.982	1.075	0.618	0.664	0.355	0.386
Proline 100 ppm	1.077	1.151	0.685	0.762	0.374	0.414
Proline 200 ppm	1.140	1.208	0.714	0.791	0.421	0.445
Rizolex - T 50	0.846	0.917	0.544	0.585	0.285	0.312
F test	**	**	**	**	**	**
LSD at 5 %	0.024	0.059	0.016	0.015	0.009	0.010
C- Interaction	**	*	**	**	NS	NS

*** and ** indicate significant and high significant , respectively NS= non-significant**

Low level of HA was more effective than high level. Moreover, proline led to maximum increase in proline content followed by chitosan. The highly increase in carbohydrate content occurred under low level of HA (1000 ppm) followed by high proline level. There is a positive significant effect for the interactions between salinity level and all tested materials on total phenols content in the second season only, prolin and carbohydrate content in both seasons.

The effect of soil salinity, tested materials and their interaction on average number of pods/plant, seed yield and weight of 100-seeds of faba bean plant during the two growing seasons are presented in Tables (8) and (9). It is clear from Table 8 that yield parameters were decreased significantly with increasing salinity levels. On contrast, the efficacy of all tested treatments in control of root rot disease was reflected on the plant stand causing increase in the faba bean yield. Humic acid at high concentration (2000 ppm) gave the highest values of the tested parameters followed by high proline concentration (200 ppm) then high concentration of chitosan (8 g/L).

The interaction between organic compounds treatment and salinity levels had significantly affected on No. of pods/plant, seed yield /plant and 100-seed weight in the first and second seasons, as shown in Table (9). The highest values of previous characters were produced from seed soaking with 2000 ppm HA followed by 200 ppm proline under any salinity level compared with other treatments. However, the lowest No. of pods and seed yield/plant results from untreated seed (check). It is worthy to mention that, the lowest 100-seed weight was recorded from Rizolex-T50 treatment at any salinity level in both seasons.

Table 7. Phenols, proline and carbohydrates content of faba bean as affected by salinity levels (S) and organic compounds treatments during 2013/2014 and 2014/2015 seasons

Characters / Treatments	Phenols content (mg/100 g fresh weight)		Proline content (µ moles/g dry weight)		Carbohydrates content (mg/g dry weight)	
	13/14	14/15	13/14	14/15	13/14	14/15
A- Salinity levels:						
1.5 dS m^{-1}	614.8	631.8	44.5	52.8	127.8	135.8
2.5 dS m^{-1}	645.8	656.2	52.2	61.2	105.2	120.9
3.5 dS m^{-1}	656.9	669.8	59.2	68.8	82.2	96.9
F test	**	**	**	**	**	**
LSD at 5 %	1.4	1.5	0.6	1.1	2.2	1.3
B- Organic compounds:						
Check	606.1	623.2	41.0	50.0	93.9	104.3
HA 1000 ppm	634.6	650.3	50.1	59.2	118.4	134.8
HA 2000 ppm	622.3	637.2	45.7	53.7	111.2	127.2
Chitosan 6g L^{-1}	664.2	673.6	53.8	61.7	105.4	117.2
Chitosan 8g L^{-1}	669.6	681.8	57.1	65.2	99.0	111.9
Proline 100 ppm	646.4	657.6	60.8	69.8	107.9	120.8
Proline 200 ppm	654.3	666.7	66.1	78.6	115.7	130.9
Rizolex - T 50	615.7	630.4	41.1	49.2	88.8	95.9
F test	**	**	**	**	**	**
LSD at 5 %	4.3	2.9	1.7	1.6	1.7	1.7
C- Interaction	NS	**	**	*	**	**

***and ** Significant and high significant, respectively NS= non-significant**

Table 8. No. of pods / plant, seed yield / plant and 100-Seed weight as affected by salinity levels (S) and organic compounds treatment during 2013/2014 and 2014/2015 seasons

Characters / Treatments	No. of pods / plant		Seed yield / plant (g)		100-Seed weight (g)	
	13/14	14/15	13/14	14/15	13/14	14/15
A- Salinity levels:						
1.5 dS m^{-1}	61.9	64.5	74.8	77.1	63.6	64.2
2.5 dS m^{-1}	42.8	45.5	57.2	60.4	61.3	60.7
3.5 dS m^{-1}	25.6	30.0	47.7	50.1	56.5	56.8
F test	**	**	**	**	**	**
LSD at 5 %	1.4	0.9	0.8	0.4	0.7	0.1
B- Organic compounds:						
Check	30.5	31.1	47.1	47.6	58.4	59.0
HA 1000 ppm	46.2	48.9	62.4	65.1	61.1	61.1
HA 2000 ppm	54.0	60.7	73.2	76.2	63.9	63.1
Chitosan 6g L^{-1}	40.8	43.0	53.6	57.0	59.9	60.1
Chitosan 8g L^{-1}	48.1	52.3	64.9	69.1	61.2	61.8
Proline 100 ppm	42.8	45.7	57.4	60.9	60.2	60.9
Proline 200 ppm	51.4	56.0	69.4	71.6	62.8	62.2
Rizolex - T 50	33.7	35.6	50.8	57.0	56.2	56.3
F test	**	**	**	**	**	**
LSD at 5 %	1.0	1.3	1.5	1.2	0.5	0.6
C- Interaction	**	**	**	**	**	**

*** and ** indicate significant and high significant, respectively NS= non-significant**

Table 9. No. of pods / plant, seed yield / plant and 100-Seed weight as affected by the interaction between organic compounds treatment and salinity levels (S) during 2013/2014 and 2014/2015 seasons

Salinity / Organic compounds	No. of pods / plant			Seed yield / plant (g)			100-Seed weight (g)		
	S1	S2	S3	S1	S2	S3	S1	S2	S3
				2013/2014					
Check	40.7	32.0	19.0	63.0	41.7	36.7	61.3	60.0	54.0
HA 1000 ppm	67.3	45.0	26.3	77.3	60.7	49.3	63.7	62.3	57.3
HA 2000 ppm	75.3	54.7	32.0	87.0	72.0	60.7	66.7	64.0	61.0
Chitosan 6 g L^{-1}	61.0	38.3	23.0	70.0	50.3	40.3	63.0	60.7	56.0
Chitosan 8 g L^{-1}	70.7	46.0	27.7	79.7	63.0	52.0	64.7	62.3	56.7
Proline 100 ppm	63.0	41.3	24.0	74.0	54.3	44.0	63.7	61.0	56.0
Proline 200 ppm	73.3	50.7	30.3	81.0	70.3	57.0	65.3	62.7	60.3
Rizolex - T50	44.0	34.7	22.3	66.0	45.0	41.3	60.7	57.7	50.3
LSD at 5%		1.7			2.5			0.9	
				2014/2015					
Check	43.0	31.0	19.3	64.3	41.7	36.7	62.7	59.7	54.7
HA 1000 ppm	68.3	46.7	31.7	80.0	64.7	50.7	65.3	60.7	57.3
HA 2000 ppm	81.0	60.7	40.3	89.3	75.7	63.7	66.0	62.7	60.7
Chitosan 6 g L^{-1}	62.0	40.7	26.3	74.0	53.7	43.3	63.3	60.7	56.3
Chitosan 8 g L^{-1}	72.7	50.7	33.7	82.0	70.7	54.7	65.3	61.7	58.3
Proline 100 ppm	66.0	42.3	28.7	76.7	57.7	48.3	64.7	60.7	57.3
Proline 200 ppm	76.7	55.7	35.7	83.0	72.7	59.0	65.3	62.0	59.3
Rizolex - T 50	46.0	36.3	24.3	67.3	46.7	44.0	60.7	57.7	50.7
LSD at 5%		2.3			2.0			1.1	

S1=1.5 dSm^{-1} S 2=2.5 dSm^{-1} S3 =3.5 dSm^{-1}

DISCUSSION

Infection with root rot fungi causes damping-off due to root rot and seedling death before emergence or collapse of seedlings shortly after emergence (Hussain *et al*., 1989). *Fusarium* spp. produces fusaric acid which inhibits seeds germination and cause rotten lesions on seed cotyledons and plumule soft rot (Gally *et al*., 1998). In this study, the tested pathogenic fungi causes destruction of root epidermis followed by degradation of cell wall and hydrolysis of cortex cells in turn breakdown the lateral roots and decrease the absorption surface of water and nutrients leading to damping-off.

The reverse effect of salinity might be due to hormone imbalance (El-Saht, 1994), decrease protein building (Parida and Das, 2005) and decrease in chlorophyll content and metabolic activities (Abd El-Hai *et al*., 2010 and Mohsen *et al*., 2013). Generally the harmful effects of salinity on plants may be due to deficit nutrients imbalance and phytotoxicity of ions (Abdelhamid *et al*., 2010). Thus, salinity leads to injuries effects in plant health combined with increase in disease infection.

The beneficial effects of humic acid on plant growth and development may be due to their effect on cell membranes which enhances minerals transport, promoted photosynthesis, plant hormone-like activity, modified enzyme activities, improved protein synthesis and reduction of active levels of toxic minerals (Hamideh *et al*., 2013). The activities of plant hormone-like (i.e. auxin) led to increase in total phenol, activity of catechol oxidase and increase in calcium content which protect plants against pathogens infection (Chowdhury, 2003). HA enhances chlorophyll synthesis and/or delay chlorophyll degradation (Nardi *et al*., 2002). The application of HA increased the uptake of P, K, Mg, Na, Cu and Zn (El-Galad *et al*., 2013). These nutrients have an essential role in plant growth and plant protect against disease infection, for example Zn is an essential constituent for three plant enzymes i.e. carbonic anhydrase, alcohol dehydrogenase and superoxide dismutase (SOD).

The data of this study show that chitosan at 8 g/L gave the highest reduction of fungal growth (Table 2). Chitosan also increased significantly field emergence %, morphological characters, photosynthetic pigments, phenols, proline, carbohydrates and yield characters. On the other hand, chitosan decreased significantly emergence damping-off. The antifungal activity of chitosan against many fungi was demonstrated by several studies (Ali *et al*., 2009). They found that chitosan could inhibit the growth of many fungi, including root rot pathogens. Chitosan enhance growth due to their effect on physiological processes (Farouk, 2005). In addition, chitosan increase phenolic compounds and synthesis of specific phytolaexins with antifungal activity (Ben-Shalom *et al*., 2003). Chitosan also has unique biological properties of various pathogenic fungi and ability to be a potent elicitor of plant defense reactions against soil-borne pathogens (Prapagdee *et al*., 2007).

Total free amino acids accumulation notably proline is one of the most frequently reported modifications induced by stress condition. So, proline is often considered to be involved in stress resistance mechanisms. Also, proline act as a storage compound for energy and reduced carbon and nitrogen needs. In addition, proline has a clear role on osmoticum and radical detoxification (Taie *et al*., 2013). Proline improves the plant adaptation to salt stress and induces the expression of salt-stress responsive proteins (Khedr *et al*., 2003). Moreover, proline protecting the photosynthetic apparatus and displaying an antioxidant activity (Ashraf *et al*., 2008). In this investigation the positive effect of organic compounds (humic acid, chitosan and proline) may be due to the increase in photosynthetic pigments, total phenols, proline and carbohydrate content which play an important role in plant defense, growth and productivity.

From the previous results and discussion, it may be suggested that using humic acid (2000 ppm) or chitosan (8 g/L) or proline (200 ppm) as seed soaking treatment and foliar spray after 35 and 50 days from sowing could be applied for reducing root rot disease in faba bean and improving yield under salinity affected soil conditions

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معاملة التقاوى قبل الزراعة ببعض المركبات العضوية لمقاومة مرض عفن الجذور وتحسين إنتاجية الفول البلدي تحت ظروف الأراضي المتأثرة بالملوحة

قمر محمد عبد الحى[١] و أمل الصعيدي عبد ربه الصعيدى[٢]

[١] قسم بحوث أمراض البقوليات والعلف - معهد بحوث أمراض النبات - مركز البحوث الزراعية - مصر

[٢] قسم بحوث تكنولوجيا البذور - معهد بحوث المحاصيل الحقلية - مركز البحوث الزراعية – مصر

أقيمت تجربة تحت ظروف الصوبة لتقييم القدرة المرضية لبعض مسببات أمراض عفن الجذور على نبات الفول البلدي. كما أقيمت تجربة معملية لدراسة تأثير بعض المركبات العضوية والريزوليكس تى ٥٠ كمبيد فطرى على نمو الفطريات المختبرة و تجربتين حقليتين لدراسة تأثير معاملة تقاوى الفول البلدى ببعض المركبات العضوية (حامض الهيوميك ، الشيتوزان والبرولين) بتركيزات مختلفة والريزوليكس تى ٥٠ كمبيد فطرى على مقاومة مرض عفن الجذور وتحسين بعض الصفات المورفولوجية والفسيولوجية وإنتاجية الفول البلدى تحت ظروف الأراضى المتأثرة بالملوحة خلال عامي ٢٠١٤/٢٠١٣ و ٢٠١٥/٢٠١٤ . أشارت النتائج أن العدوى بجميع المسببات المرضية المختبرة تحت ظروف الصوبة أدت إلى موت البادرات. وكان فطر الريزوكتونيا سولانى الأكثر فعالية فى موت البادرات قبل ظهورها فوق سطح التربة بينما أعطى الفيوزاريوم سولانى أعلى نسبة فى موت البادرات بعد ظهورها فوق سطح التربة. كما أدت الفطريات المسببة للأمراض إلى تغير فى التركيب التشريح للجذر. ونتج عن إصابة الجذور بفطر الفيوزاريوم سولانى تحطم كامل لخلايا البشرة متبوعة بتدهور جدر خلايا القشرة. وأظهرت النتائج أن معاملة الريزوليكس تى ٥٠ تحت ظروف المعمل أدت إلى تثبيط كامل لنمو الفطريات المختبرة . وعلى الجانب الآخر كانت المعاملة بالشيتوزان بتركيز ٨ جم/لتر هي الأكثر فعالية فى الحد من نمو الفطريات ويليها المعاملة بالبرولين بتركيز ٢٠٠ جزء فى المليون. كما أوضحت الدراسة أن المعاملة باستخدام المركبات العضوية تحت الدراسة أدت إلى مقاومة الآثار الضارة للملوحة تحت ظروف الحقل حيث أدت المعاملة باستخدام حامض الهيوميك إلى تقليل موت البادرات قبل وبعد ظهورها فوق سطح التربة وزيادة نسبة التكشف الحقلى. كما أشارت النتائج إلى أن المستوى العالي (٢٠٠ جزء فى المليون) من البرولين كان أكثر تأثيرا فى زيادة القياسات المورفولوجية (طول النبات وعدد الفروع والاوراق/نبات) وكذا صبغات التمثيل الضوئى (كلوروفيل ا ، ب والكاروتينويدات). بينما أدى التركيز العالى من حامض الهيوميك (٢٠٠٠ جزء فى المليون) إلى زيادة معنوية فى عدد القرون /نبات ، محصول البذور /نبات و وزن الـ ١٠٠ بذرة. وأعطت المعاملة بالشيتوزان أعلى محتوى من الفينول ويليها المعاملة بالبرولين. ونتجت أعلى زيادة فى محتوى الكربوهيدرات تحت المستوى المنخفض من حامض الهيوميك (١٠٠٠ جزء فى المليون) ويليه المستوى العالى من البرولين (٢٠٠ جزء فى المليون) وقد انعكست فعالية المركبات العضوية المختبرة فى مقاومة مرض عفن الجذور على النبات مما أدى زيادة مكونات المحصول. تقترح الدراسة نقع البذورلمدة ١٠ ساعات فى محلول حامض الهيوميك بتركيز 2000 جزء فى المليون أو ٨ جم / لتر شيتوزان أو ٢٠٠ جزء فى المليون برولين كبديل لإستخدام المبيدات الفطرية لمقاومة مرض عفن الجذور وزيادة إنتاجية الفول البلدى تحت ظروف الاراضى المتاثرة بالملوحة والإصابة الطبيعية في الحقل.