J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 7 (9): 241 - 248, 2016

Use of Endophytic Bacteria to Suppress Damping-Off of Cotton Seedlings Caused By *Fusarium oxysporum*

Nafisa M. Gomaa[1]; Shereen E. M. El-Nahas[2]; Eman A. M. Osman [2] and Maggie E. M. Hassan [2]

[1] Department of Botany, Faculty of Science, Fayoum University

[2] Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.

Corresponding author

E. Mail address: Maggie E. M. Hassan maggie2000_eg@yahoo.com

ABSTRACT

When two strains of bacteria (*Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*) were tested on seedlings of cotton cultivar Giza 86, they did not show any significant effects on plant height and dry weight. The two bacterial strains were also nonpathogenic (0.0 % infection). The *Pseudomonas aeruginosa* strain was more effective in reducing damping-off symptoms caused by *Fusarium oxysporum* isolates no_s 1 and 2 whereas, *S. maltophilia* was effective against *F. oxysporum* isolate no. 4. Although the two bacterial strains reduced damping-off on cotton seedlings caused by *Fusarium* fungal isolate no. 3, *P. aeruginosa* was more effective than *S. maltophilia*. *Fusarium oxysporum* isolates no_s. 1 and 2 significantly reduced plant height than isolates no_s. 3 and 4. Strain of *S. maltophilia* significantly increased dry weight of cotton seedlings when soil was infested with isolates no_s 3 or 4 of *F. oxysporum*. *In vitro* antagonism tests showed that *P. aeruginosa* inhibited growth of *F. oxysporum* isolates. The inhibition ranged from 19.33 to 31.0 %. On the other hand, *S. maltophilia* did not show any inhibition of *F. oxysporum* isolates growth. The two bacterial strains did not show any significant effects on the activities of peroxidase and polyphenol oxidase, while the ß- 1, 3 glucanase activity was significantly increased. The total and reduced sugar contents were significantly increased due to the application of the two bacterial strains but *P. aeruginosa* was more effective. *Pseudomonas aeruginosa* was the only strain which significantly increased free phenols.

Keyword: Endophytic bacteria, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, Cotton seedling damping-off, and *Fusarium oxysporum*.

INTRODUCTION

Cotton seedling damping-off is caused by a complex of seed-borne and soil inhabiting organisms. *Fusarium oxysporum, F. moniliforme*, and *F. solani* are the three pathogenic species involved in the etiology of cotton damping-off in Egypt (Abd-El Salam *et al*., 2006).

Fusarium spp. produce all the characteristic effects of the seedlings disease syndrome. Root and stem tissues become discolored, turn brown internally, and then rot. After emergence, necrotic lesions usually appear on the hypocotyls near the soil surface. The lesions are brown to reddish brown and girdle the stem (Watkins, 1981).

The widespread use of seed-dressing fungicides for controlling the disease has become indispensable under Egyptian conditions. However, it is becoming increasingly evident that fungicides widespread use is associated with some problems, such as the potential harmful effect on non-target organisms, the development of resistant races of pathogens, and possible carcinogenicity (Omar *et al*., 2009).

Biological control of plant pathogens, especially soil borne plant pathogens, by microorganisms has been considered a more natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Panlitz, 1996). Endophytic bacteria are plant-associated bacteria that live inside plant tissues without causing damage. They have received considerable attention due to their bioactivities including antibiotics production, biological control of plant diseases, plant growth stimulation and nitrogen fixation (Cui *et al*., 2002; Qiao *et al*., 2006; He *et al*., 2010).

Advantages of using biocontrol agent are that they live in the same ecological niche where plant pathogen live, thus providing competition reliable for inhibition of many plant diseases and that they do not cause environmental contamination (Misaghi and Donndelinger, 1990). Mechanisms responsible for antagonistic activity include (i) inhibition of pathogen by antibiotics, toxins and antibiosis, (ii) competition for colonization sites and nutrients, (iii) competition for minerals, (iv) degradation of pathogenicity factors of the pathogen such as toxins, (v) parasitism that may involve production of extracellular cell wall degrading enzymes such as chitinases and ß-1,3 glucanase and (vi) inducing host resistant (Bloemberg and Lugtenberg, 2001; Whipps, 2001).

Pseudomonas aeruginosa demonstrated a strong antagonistic activity against *Rhizoctonia* sp. and *Fusarium* sp where it produces a bioactive compound called pyrrolnitrin which showed high activity against the two fungi (Shtark *et al*., 2003; Reddy *et al*., 2009). Moreover, different antibiotics were isolated from this bacterium (Kumar *et al*., 2005).

Berg *et al*. (1996) reported that *Stenotrophomonas maltophilia* inhibited the growth of *R. solani* possibly as a result of antibiosis and production of some lytic enzymes. Jakcobi *et al*. (1996) reported that *S. maltophilia* produces an antibiotic called maltophilin that inhibits the growth of several phytopathogenic fungi.

Selim *et al*. (2014) reported that *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* have the capability to release extracellular bioactive compounds that play a role in the *in vitro* suppression of some plant pathogens.

2. Material and methods

Isolation and identification of endophytic bacteria

The two bacterial strains "*Pseudomonas aeruginosa* (B1) and *Stenotrophomonas maltophilia* (B2)" were isolated from peas (*Pisum sativum* L.) roots and cabbage (*Brassica oleracea* var *capitata* L.) stem,

respectively (Selim *et al*., 2014). Bacterial strains were identified morphologically, biochemically and by 16s-DNA sequencing as described previously (Selim *et al*., 2014).

Source of *F. oxysporum* isolates

Fusarium oxysporum isolates used in this study were obtained from the fungal collection of cotton and fiber crops Dis. Res Sec., Plant Pathology Res. Inst. Agric. Res. Center. All isolates were originally isolated from cotton seedling infected with damping-off.

Effect of bacteria on growth of cotton seedlings (*Gossypium barbadense* L.)

Autoclaved clay-loam soil was dispensed in sterilized 15-cm clay pots and sowed with 20 seed of cotton cultivar Giza 86 (the most predominant commercial cultivar in Egypt). Shaked cultures of the two bacterial strains (B1 and B2) were grown in 250 ml nutrient broth liquid medium with constant shaking (100 rpm) at 28ºC for 48 hrs. Bacterial suspensions were applied immediately after sowing, as soil drench at a rate of 10 ml/ pot [30 X 10^6cfu]. Pots were randomly distributed on a greenhouse bench where the temperature was [26ºC ±5]. Three replicates were used for each treatment. Infection, plant height and dry weight were recorded 40 days after sowing. Random sample of cotton seedlings were used for further chemical studies.

Biological control of cotton (*Gossypium barbadense* L.) seedling damping-off caused by *F. oxysporum* by two bacterial strains

Inocula of *Fusarium* isolates were prepared by growing each isolate in sterilized 500-ml glass bottles containing 50g of sorghum grains and 40 ml of water. The sterilized bottles were inculcated with fungal growth taken from one-week culture grown on PDA plates. The inoculated bottles were incubated for 3 weeks at 26ºC±5. During that period the inculcated bottles were shacked for 5 min every three days to ensure uniform distribution of the fungal growth. The growing cultures on sorghum were air-dried under greenhouse conditions. The air dried cultures were triturated to a powder by a blender (Aly, 1988). The powdered inoculum of each isolate was stored in plastic bags at 5ºc until use. Autoclaved clay-loam soil was infested with each of the four isolates of *F. oxysporum* separately [1, 2, 3, and 4]. The inoculum concentrations used was 50g/ kg soil. Soil infested with each fusarium was divided into three batches. The first batch was used as control treatment where bacterial growth medium was applied to soil. The suspensions of the two bacterial strains B1 and B2 were applied individually to the other two batches of soil as previously mentioned. The infested soil was dispensed in sterilized 15-cm clay pots and planted with 10 cotton seeds/ pot, (Cultivar Giza 86), with three replicates for each treatment (*Fusarium* isolate x bacterial strain) . Infection, plant height and dry weight were recorded 40 days after sowing.

Antagonistic activity of bacteria

The paired cultures of *Fusarium oxysporum* and bacteria were placed simultaneously on opposite sides of 9-cm diameter petri dishes containing 15 ml of PDA. Paired cultures were incubated at 26 ± 3 ºC for 7 days, then the area of *F. oxysporum* isolates growth on medium surface were determined by the following method: A transparency was divided into equal squares (0.5 X 0.5 cm), the number of squares determined and divided by 4. The obtained value was considered as the area of *F. oxysporum* isolates growth in cm^2 (Ali *et al*., 2001). Three replications (petri dishes) for each treatment.

Percentage of inhibition was calculated according the following formula: $\frac{A}{B}$

Inhibition % = $100-\left(\frac{A}{B} \times 100\right)$

Where A = area (cm^2) of fungal growth under the effect of bacterial strain and B= area (cm^2) of fungal growth in the control.

Preparation of enzyme extracts and the assay methods

Crude enzyme extracts for the assays were prepared according to (Aluko and Ogbadu, 1986). One gram of the homogenized cotton plant was extracted with 3 ml of 0.1 M phosphate buffer (pH 7). The homogenate was filtered and then centrifuged at 3000 rpm for 15 min. at 4ºC. The supernatant filtered and collected as an enzyme extract. Enzyme extract was stored at 2-5 ºC and aliquots of these assayed for enzyme activity in (spectronic 106) spectrophotometer.

Peroxidase enzyme assays

Peroxidase activity was determined according to (Worthington, 1972) as follows: 0.5ml of extracted enzyme sample and 1.5 ml of phosphate buffer pyrogallol, pH 6.0, 1.1ml H_2O_2 30%. The increase in absorbance was determined spectrophotometer (spectronic 601) at 430 nm every 30 second for 10 reads**.** Peroxidase activity was calculated as mg/g fwt/ min.

Polyphenol oxidase enzyme assays

Polyphenol oxidase activity was measured following the method described by (Esterbaner *et al*., 1977). 0.5 ml of the enzyme extract and 2.2 ml of 0.1 M phosphate buffer were mixed together in a cuvette and the sample was adjusted to zero absorbance in a Spectrophotometer at 495 nm. 0.5 ml of 0.01 M catechol in 0.1 M phosphate buffer was added to the above mixture and the reactants were quickly mixed. The enzyme activity was measured as the change in absorbance per minute at 495 nm immediately after the addition of catechol solution, which initiated the reaction.

glucanase enzyme assays

ß-1,3 glucanase activity was assayed using a reaction system containing 250 µL of a laminarin solution (1%) dissolved in sodium phosphate (pH 7) containing the substrate laminarin. buffer and 125 µL of enzyme solution. Reaction was allowed to proceed for 30 min at 37ºC and stopped by addition of 1.5 mL of 3, 5-dinitrosalicylic acid reagent. The reducing sugar formed was then determined spectrophotometrically at 550 nm according to (Miller, 1959). The enzyme activity was expressed as µg glucose released min-1 mg-1 of sample.

Preparation of samples for chemical studies of sugars and phenols

Fresh plant sample (10 g) from each replicate of each treatment was cut into small pieces and immediately macerated into 95% boiling ethanol for 10 min. The macerated were transferred into soxhlet unites containing 75% ethanol as an extraction solvent. The extract process resumed for 12 hrs. Ethanol extracts were filtrated and evaporated until the complete removal of ethanol. The dried residue was dissolved in 5ml isopropanol 50% and kept in freezer till analysis. The extracts were used, later for analysis of sugars and phenols.

Sugar contents

Total soluble sugars, reducing and non-reducing sugars were spectrophotometeric determined at 540 nm using the picric acid technique as described by (Thomas and Dutcher, 1924) as follows.

A volume of 0.5ml of each extract was placed in test tubes; containing 5ml of distilled water and 4ml picric solution were added. The mixture was boiled for 10 min. After cooling, 1ml sodium carbonate solution 20% was added and the mixture was boiled again for 15 min. After it was cooled, the tubes were completed to 10 ml with distilled water. Thereafter, the density of developed color was determined at 540 nm using spectrophotometer (spectronic 106) in presence of blank and using glucose as a standard.

The same described procedure for total sugars was used except that the picric- and sodium carbonate-solutions were added together at the same time. The same spectrophotometer and wavelength were used.

The content of non-reducing sugars was calculated as the difference between the total sugars and reducing sugars.

Phenol compounds

Total phenols determination was carried out as described by (Simons and Ross, 1971). Concentrate hydrochloric acid (0.25 ml) was added to 0.2 ml of the sample extract in test tube and mixed. The mixture was then boiled for about 10min. After cooling, 1ml Folin reagent and 5ml sodium carbonate solution (20%) were added and diluted to 10 ml using distilled water. After 30 min the density of the developed blue color was determined at 520 nm using chatichole as standard.

Free phenols determination was carried out using the same described method with some exception, since, 1ml Folin reagent and 3ml sodium carbonate solution (20%) were added to 0.2 ml of the sample extract, diluted with distilled water to 10 ml. After 30 min, the density of the developed blue color was determined at same wavelength.

Statistical analysis

Data collected were analyzed by MSTAT-C program. The means differences were compared by the least significant difference test (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

When two strains of bacteria [*Pseudomonas aeruginosa* (B1) and *Stenotrophomonas maltophilia* (B2)] were tested on seedlings of cotton cultivar Giza 86, they did not show any significant effects on plant height and dry weight (Tables 1 and 2, and Fig. 1). It worth noting that the two strains were also nonpathogenic (0.0% infection) on seedlings of Giza 86 (Data not shown). Although Schulz and Boyle (2006) reported that in most cases bioagents relationship with host plant is symbiotic or mutualistic with no visible damage or morphological changes in the host, in contrast others found that among members of *Pseudomonas* there are numerous species that are pathogenic to plants. For examples, *Pseudomonas* spp. was associated with pink eye disease of potato tubers (Flosom and Friedman, 1959) and wheat (Cherrington and Elliott, 1987).

Figure 1. Effect of two bacterial strains on growth of cotton seedlings of cultivars Giza 86

C = control (soil free of bacteria),
B1 = soil infested with *Pseudomonas aeruginosa*,
B2= soil infested with *Stenotrophomonas maltophilia*

Table 1. Analysis of variance of effects of two bacteria strains *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* on growth of cotton seedlings of caultivar Giza 86.

Variable	Source of variation	d.f	M.S	F. value	P> F.
1.Plant height	Replicates	2	3.538	8.747	0.035
	Treatments	2	2.254	5.574	0.07
	Error	4	0.404	-	-
2. dry weight	Replicates	2	0.016	0.217	0.814
	Treatment	2	0.141	1.933	0.259
	Error	4	0.073	-	-

Table 2. Effect of two bacterial strains on growth of cotton seedlings of cultivars Giza 86

Treatment	Variable	
	Plant height cm/plant	Dry weight g/ plant
Control[a]	11.767 [b]	6.610
B1	12.667	7.027
B2	13.500	6.717
L.S.D. (≤0.05)	NS	NS

[a] soil was free from bacterial strains.
[b] mean of 3 replicates (pots).

Heydari and Naraghi (2014) reported that it may be possible to promote and increase cotton seedlings growth by the use of some bacteria antagonists. *Fusarium* isolate, bacteria strain, and their interaction

on damping-off of Giza 86 were evaluated (Table 3 and Fig. 2). *Fusarium* isolates were a significant source of variation in plant height. Bacterial strains were significant source of variation in infection and dry weight. *Fusarium* isolate X bacterial strain interaction was significant source of variation in infection and dry weight. Data in Table 4 showed that *Pseudomonas aeruginosa* was effective as biocontrol agent against *F. oxysporum* isolates no. 1 and no.2. Pathogenicity of *F. oxysporum* isolate no.3 was significantly reduced by each of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* strains. However, *Pseudomonas* strain was more efficient as it reduced infection by 32.0 % compared with *Stenotrophomonas* strain, which reduced infection only by 28.0 %. The superiority of *Stenotrophomonas* strain in controlling damping-off was evident against *F. oxysporum* isolate no. 4. *Fusarium* isolates could be divided into two distinct groups regarding their effects on plant height. *Fusarium* isolates no_s. 1 and 2 were the more pathogenic, while isolates no_s 3 and 4 were the less pathogenic. Within each group the difference was non significant. Both strains of bacteria did not show any significant effect on dry weight of seedlings of cotton when the soil was infected with isolates no. 1 or no. 2. On the other hand strain of *Stenotrophmonas* significantly increased dry weight of seedling when the soil was infested with *F. oxysporum* isolates no. 3 or no. 4. *Pseudomonas* are characteristically fast growing, easy to culture and manipulate genetically in the laboratory, and are able to utilize a range of easily metabolized organic compounds, making them amenable to experimentation (De Weger *et al*., 1995). *Pseudomonas* spp. has shown activity in suppressing fungal infection (Chen *et al*., 2000). In addition these bacteria are known to promote plant growth and yield (Krebs *et al*., 1998 and Ryder *et al*., 1999). They activate systemically the plant's latent defense mechanisms called induced systemic resistance against pathogens (Ardakani *et al*., 2010). This mechanism operates through the activation of multiple defense compounds at sites distant from the point of pathogen's attack (Bharathi *et al*., 2004).

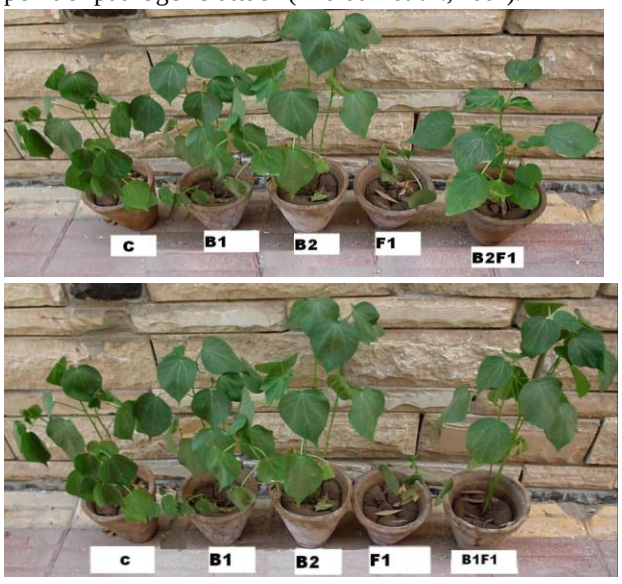

Fig. 2 a

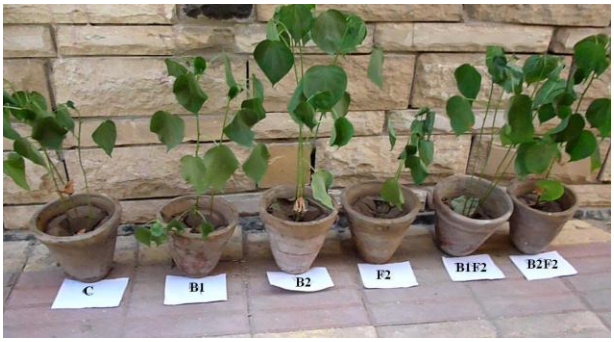

Fig. 2 b

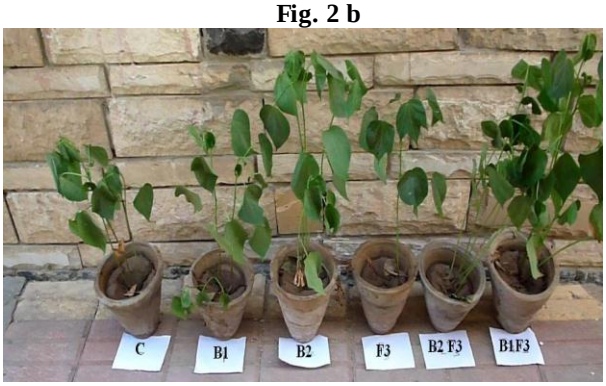

Fig. 2 c

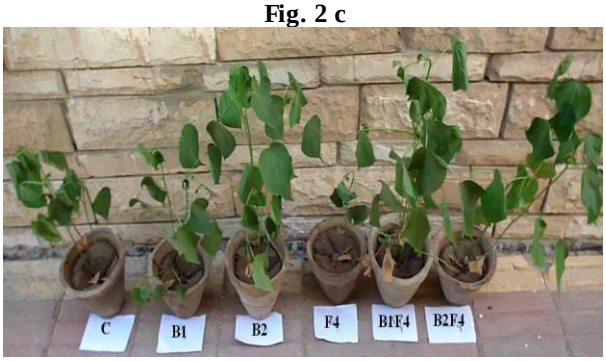

Fig. 2 d

Figure 2 Effects of *Fusarium* isolates F1 (fig.2 a), F2 (fig.2 b), F3 (fig.2 c), and F4 (fig.2 d); bacterial strains *Pseudomonas aeruginosa* (B1) and *Stenotrophomonas maltophilia* (B2); and their interaction on growth variables of cotton seedlings (cv Giza 86).

Table 3: Analysis of variance of effects of *Fusarium* isolates (F), bacteria strains (B), and their interaction on growth variables of cotton seedlings.

Variable	Source of variation	d.f	M.S	F. value	P> F.
Infection	Replicates	2	36.11	0.373	0.693
	F	3	166.67	1.723	0.191
	B	2	1102.778	11.402	0.00
	F x B	6	297.222	2.556	0.05
	Error	22	96.717		
Plant height	Replicates	2	14.818	0.23	0.797
	F	3	221.023	3.431	0.035
	B	3	49.725	0.772	0.474
	F x B	6	65.947	1.024	0.436
	Error	22	64.412		
Dry weight	Replicates	2	2.019	0.79	0.466
	F	3	6.453	2.523	0.084
	B	2	10.361	4.051	0.032
	F x B	6	8.545	3.341	0.017
	Error	22	2.557		

Table 4: Effect of *Fusarium* isolates (F), bacteria strains (B) and their interaction on damping-off of cotton seedlings cultivar Giza 86

Fusarium isolates	Infection (%)				Plant height (cm/plant)				Dry weight (g/ plant)			
	control	Bacteria		Mean	Control	Bacteria		Mean	control	Bacteria		Mean
		B1	B2			B1	B2			B1	B2	
F1	83.33	70.00	76.67	76.67	17.33	29.03	23.67	23.34	5.63	8.17	6.84	6.88
F2	76.67	50.00	83.33	70.00	25.50	27.21	21.06	24.59	6.58	8.98	4.74	6.77
F3	83.33	56.67	60.0	66.67	30.33	28.28	36.18	31.59	6.99	7.36	10.52	8.29
F4	76.67	66.67	63.33	68.89	30.55	30.61	38.58	33.24	6.78	8.41	9.68	8.29
Mean	80.0	60.84	70.83		25.92	28.78	29.87		6.50	8.23	7.95	
L.S.D. (≤0.05)	F		--			7.57				--		
	B		--			--				--		
	F x B		10.06			--				2.61		

When the antagonism of the two bacterial stains was evaluated against *Fusarium oxysporum* isolates on PDA medium, *Pseudomonas aeruginosa* strain inhibited fungal growth, the inhibition ranged from 19.33 to 31.0 % (Table 5 and Fig 3). This antagonism is in agreement with Rahman *et al*., (2007) who reported that *P. aeruginosa* inhibited spore germination of *Colletotrichum* by 68.45% during *in vitro* screening on PDA. Khanuchiya *et al*., (2012) found that *in vitro* antagonistic effect against *F. oxysporum* was maximum with *Pseudomonas aeruginosa*. It is worth noting that *Stenotrophomonas maltophilia* strain was *in vitro* ineffective in suppression the fungal growth while it showed efficiency (28 %) in reducing damping-off.

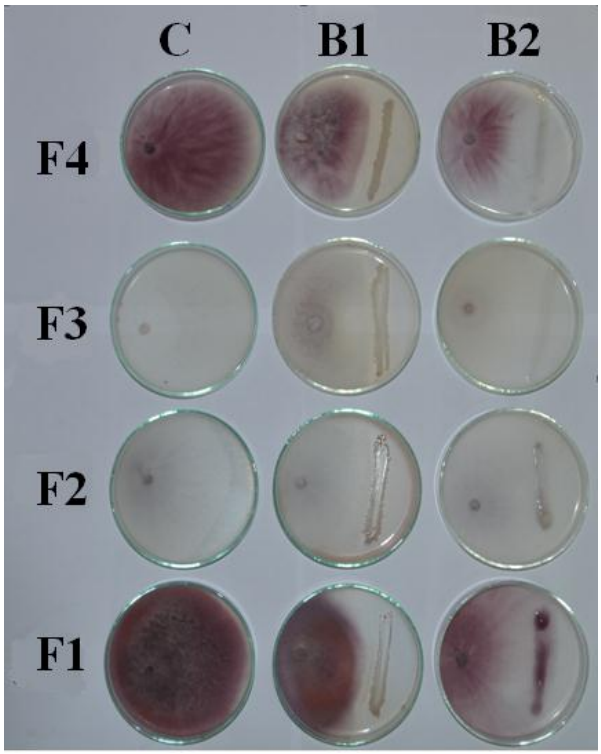

Figure 3. Antagonism on PDA medium between *Fusarium oxysporum* isolates (F1, F2, F3 and F4) and bacterial strains B1 (*Pseudomonas aeruginosa*) and B2 (*Stenotrophomonas maltophilia)*. C= control

Therefore, inducing resistance is a possible mode of action by which *S. maltophilia* strain suppress damping-off of cotton seedlings. This phenomenon help plant to utilize own defense mechanism to increase the level of resistance without changing plant genome (Van Loon, 1997). Selim *et al*., (2014) concluded that *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* have the capability to release extracellar bioactive compound that play a role in the *in vitro* suppression of *F. oxysporum* and *R. solani*. However, Ngoma *et al*., (2013) reported that the good results obtained *in vitro* cannot always be dependedably reproduced under field conditions.

Table 5: *In vitro* antagonism of two bacterial strains against four *Fusarium oxysporum*

Fungal isolate	Inhibition % of Bacterial strains		
	Pseudomonas aeruginosa	*Stenotrophomona maltophilia*	Control [b]
F1	30.67[a]	0	0
F2	31	0	0
F3	19.33	0	0
F4	27.77	0	0

[a] **Mean of 3 replicates (plates)**
[b] **The control was free of any bacterial strains**
Inhibition % = 100-(x100)
Where A = area (cm^2) of fungal growth under the effect of bacterial strain and B= area (cm^2) of fungal growth in the control.

In the present study, the two strains did not show any significant effects on the activities of peroxidase and polyphenol oxidase while they significantly increased the activity of ß- 1, 3 glucanase in cotton seedlings. *Pseudomonas aeruginosa* strain was more effective than *Stenotrophomona maltophilia* strain in increasing the activity of ß- 1, 3 glucanase (Tables 6 and 7). Production of lytic enzymes is among mechanisms which have been implicates in the supperession of fungal root disease by biocontrol *Pseudomonas* (Whipps, 2001; Raaijmakers *et al*., 2002; Haas and Defago 2005). Berg *et al*., (1996) reported that *Stenotrophomona maltophilia* inhibite the growth of *Rhizoctonia solani*, possibly as a result of antibiosis and production of some lytic enzymes. Some novel soluble proteins, which determined as pathogens related (PR) proteins, were effectively induced in response to different stresses such as infection with various pathogens, chemical treatment and/or physiological stresses. These proteins were identified as chitinases and ß - 1, 3 glucanases in several plant species (Van Loon, 1990). Boller and Metraux, (1988) and Fink, *et al*., (1988) indicated that induced of these enzymes showed some correlation with induced resistance. Lorito *et al*.,

(1994) reported that chitinase and gluconase have a proven ability to degrade the chitin and glucan matrix integral to the structure of many fungal cell walls. Increased activity of polyphenol oxidase and peroxidase has been reported in plants treated with bioagents (Huang and Backhouse 2005; Raghvendra *et al*., 2007)

Table6.Analysis of variance of peroxidase, polyphenol oxidase and ß 1, 3 glucanase activities in seedlings of Giza 86 grown in soil infested with *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*.

Variable	Source of variation	d.f	M.S	F. value	P> F.
Peroxidase	Replicates	2	0.035	5.143	0.078
	Treatment	2	0.021	2.791	0.174
	Error	4	0.007		
Polyphenol oxidase	Replicates	2	0.00	2.647	0.185
	Treatment	2	0.00	1.612	0.307
	Error	4	0.00		
ß 1,3 glucanase	Replicates	2	0.041	2.919	0.165
	Treatment	2	2.955	210.508	0.00
	Error	4	0.014		

Table 7.Effect of peroxidase, polyphenol oxidase and ß 1,3 glucanase activities in seedlings of Giza 86 grown in soil infested with *Pseudomonas aeruginosa* (B1) and *Stenotrophomonas maltophilia* (B2).

Treatment	Enzyme activity		
	Peroxidase Mg/g fw/min	Polyphenol oxidse mg/g fw/min	ß 1,3 glucanase µg glucose released min-1 mg-1
B1	1.347	0.026	3.344
B2	1.19	0.042	3.026
Control[a]	1.22	0.038	1.488
L.S.D. (P≤ 0.05)	NS	NS	0.27

[a] **soil was free from bacterial strains.**

Each of total sugars, reducing sugars, and free phenols was a significant source of variation in treatments (Table 8). The two strains significantly increased each of total sugars. However, *Pseudomonas* strain was always more effective. *Pseudomonas* strain was only strain which significantly increased free phenols. Non reducing sugars and total phenols were not affected by any strain (Table 9). Phenolic compounds are among the most influential and widely distributed secondary products in the plants. Such compounds govern disease resistance in many crop plants. There are many researches established that higher level of phenolic content was positively proportional to the degree of plant resistant against various fungal diseases (Huang and Backhouse 2005 and Raghvendra *et al*., 2007). Ragab *et al*., 2015 found that adding bioagent to bean plants led to stimulate free phenol production as a self mechanism against pathogens.

Ragab *et al*., (2015) reported that reduced sugar increased in bean plants treated with bioagent due to increase biological activity. The increase in biological activity need reduced sugar to be used in energy production.

Table 8. Analysis of variance of effect of bacteria on plant content of sugars and phenols in cotton seedlings of Giza 86.

Variable	Source of variation	d.f	M.S	F. value	P> F.
Total sugars	Replicates	2	0.059	16.698	0.011
	Treatment	2	0.085	24.030	0.006
	Error	4	0.004		
Reducing sugars	Replicates	2	0.024	8.226	0.038
	Treatment	2	0.051	17.159	0.011
	Error	4	0.003		
Non-reducing sugars	Replicates	2	0.025	9.646	0.029
	Treatment	2	0.005	1.951	0.256
	Error	4	0.003		
Free phenol	Replicates	2	109.156	3.913	0.114
	Treatment	2	169.275	6.068	0.041
	Error	4	27.897		
Total phenol	Replicates	2	15.576	0.331	0.736
	Treatment	2	174.46	3.710	0.123
	Error	4	47.027		

Table 9. Effect of bacteria *Pseudomonas aeruginosa* (B1) and *Stenotrophomonas maltophilia* on plant content of sugars and phenols

Treatment	Biochemical component (mg/g fresh weight)				
	Total sugars	Reducing sugars	Non-reucing sugars	Free phenols	Total phenols
B1	0.587	0.444	0.166	24.380	27.397
B2	0.429	0.305	0.147	14.333	19.433
Control[a]	0.25	0.185	0.087	9.683	12.150
L.S.D. (≤0.05)	0.14	0.12	NS	11.97	NS

[a] **soil was free from bacterial strains.**

CONCLUSION

Each of *Pseudomonas* strain and *Stenotrophomonas* strain was effective as a biocontrol agent against *Fusarium oxysporum* isolates *in vivo*. Both strains reduced the infection of cotton seedlings with damping-off; however, *Pseudomonas* strain was more effective. *Stenotrophomonas* strain increased dry weight of cotton seedlings. *Pseudomonas aeruginosa* strain suppressed growth of *Fusarium oxysporum* isolates *in vitro*, while *Stenotrophomonas maltophilia* failed to inhibit fungal growth. The two bacterial strains significantly increased the activity of ß- 1, 3 glucanase, total and reduced sugar, while *Pseudomonas* strain was the only strain, which significantly increased free phenols. Future research is required to evaluate the efficiency of two bacterial strains in controlling damping-off complex under field condition.

REFERENCE

Abd-El Salam KA, Amal A Asran, Omar MR, Aly AA. Frequency and diversity of *Fusarium* spp. colonizing roots of Egyptian cotton. Arch. Phytopathol. Plant Prot. 2006; 39: 165 - 177.

Aluko R, Ogbadu GH. Analysis of eggplant varieties for enzymes related to their organoleptic properties. Trop. Sci. 1986; 26: 163 - 171.

Aly AA. Incidence of Fusarium wilt of flax as influenced by biotic and abiotic factors. Ph.D. Thesis, South Dokota State Univ., Brookings. 1988; 173 p.

Aly AA, El-Shazly AMM, Youssef RM, Omar MR. Chemical and biological control of charcoal rot of cotton caused by *Macrophomina phaseolina* . J. Agric. Sci. Mansoura Univ., 2001; 26: 7661 - 7674.

Ardakani SS, Heydar A, Khorasani N, Arjmandi R. Development of new bioformulations of *Pseudomonas fluorescens* and evaluation of these products against damping-off of cotton seedlings. J. Plant Pathology. 2010; 92: 83 - 88.

Baker R, Panlitz TC. Theoretical basis for microbial interactions leading to biological control of soil borne plant pathogens in: Hall R (ED). Principals and Practice of managing soil borne plant pathogens. Am . Phythopathol. Soc. St. Paul, Mn. 1996; pp. 50 - 79.

Berg G, Marten P, Ballin G. *Stenotrophomonas maltophilia* in the rhizosphere of oilseed rape-occurrence, characterization and interaction with phytopathologenic fungi. Microbiol. Res. 1996; 151: 19 - 27.

Bharathi R, Vivekananthan R, Harish S. Romanthan and Samiyappan Rhizobacteria-based bio-formulations for the management of fruit rot infection in chilies Crop Protection 2004; 23: 835 -843.

Bloemberg, GV, Lugtenberg BJJ. Molecular basis of plant growth promotion and bio-control by rhizobacteria. Curr. Opin. Plant Biol. 2001; 4: 343 - 350.

Boller T, Metraux JP. Extracellular localization of chitinase in cucumber. Physiol., Mol. PI. Pathol., 1988; 33: 11 - 16.

Chen C, Belanger RR, Benhamou N, Paulitz TC. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizobacteria (PGPR) and *Pythium aphanidermatum*. Physiological and Molecular Plant Pathology 2000; 56: 13 - 23.

Cherrington CA, Elliott LF. Incidence of inhibitory Pseudomonads in the Pacific Northwest. Plant Soil 1987; 101: 159 - 165.

De Weger LA, Van der Bij AJ. Colonization of the rhizosphere of crop plants by plant-beneficial *Peudomonas*. FEMS Microbioligy Ecology 1995; 17: 221 - 228.

Esterbaner H, Schwarzl E, Hayn M. A rapid assay for catechol oxidase and laccase using 2-nitro-5-thio benzoic acid. Anal. Biochem. 1977; 77: 486 - 494.

Fink W, Liefland M, Mendgen K. Chitinases and ß- 1,3-glucanase in the apoplastic compartment of oat leaves (*Avena sativa* L.). PI. Physiol., 1988; 88: 270 - 275.

Flosom D, Friedman BA. *Pseudomonas fluorescens* in relation to certain diseases of potato tubers in Maine. Am. Potato J. 1959; 36: 90 - 97.

He R, Wang G, Liu X. Antagonistic bioactivity of an endophytic bacterium isolated from *Epimedium brevicornu*. African Journal of Biotechnology 2010; 8: 191 - 195.

Heydari A, Naraghi L. Application antagonistic bacteria for the promotion of cotton seedling growth characteristics. International J. Agriculture and Crop Sciences. 2014; 7: 1267 - 1273.

Huang LD, Backhouse D. Induction of defense responses in roots and mesocotyls of sorghum seedlings by inoculation with *Fusarium thapsinum* and *F. proliferatum*. J. Phytopathol. 2005; 153: 522 - 529.

Haas D, Defago G. Regulation of antibiotic production in root-colonizing *Pseudomonas* spp. and relevance for biologyical control of plant disease. Annual Review of Phytopathology. 2005; 41: 117 - 153.

Jakcobi M, Winkelmann G, Kaiser D. Maltophilin: a new antifungal compound produced by *Stenotrophomonas maltophilia* R3089. The Journal of Antibiotics 1996; 49: 1101 - 1104.

Khanuchiya S, Parabia FM, Patel M, Patel V, Patel K, Gami B. Effect of *Pseudomonas fluorescens* , *P. aeruginosa* and *Bacillus subtilis* as biocontrol agent for crop protection Cibtech J. Microbiology 2012; 1 (1): 52 - 59.

Krebs B, Hoding B, Kubart S, Workie MA, Junge H, Schmiedeknecht G, Grosch R, Bochow H, Hevesi M. Use of *Bacillus subtilis* as biocontrol agent. 1. Activities and characterization of *Bacillus subtilis* strains. J. Pl. Dis. Protect 1998; 105: 181 – 197

Kumar RS, Ayyadurai N, Pandiaraja P. Characterization of antifungal metabolite produced by a new strain *Pseudomonas aeruginosa* PUPa3 that exhibits broad-spectrum antifungal activity and biofertilizing traits. Journal of Applied Microbiology 2005; 98:145 - 154.

Lorito M, Harman CK, Di Pietro A, Woo SL, Harman GE. Purification, characterization and synergistic activity of a gulacan 1, 3 - beta glucosidase and an N-acetylglucosaminidase from *Trichoderma harzianum*, Phytopathology 1994; 84: 398 – 405.

Miller GL. Use of dinitrosalicylic acid reagent for the determination of reducing sugar. Anal. Chem. 1959; 31, 426-428.

Misaghi I, Donndelinger C. Endophytic bacteria in symptom-free cotton plants. Phytopathology 1990; 80 (9): 808 - 811.

Ngoma L, Babalola OO, Ahmed F. Ecophysiology of plant growth promoting bacteria. Science Research Essays 2013; 7(47): 4003 - 4013.

Omar MR, Hussein EM, Kasem KHK. Pathogenicity and protein electrophoresis of different cotton *Rhizoctonia solani* isolates. Egypt. J. Phytopathol 2009; 37: 21 – 33.

Qiao H, Huang L, Kang Z. Endophytic bacteria isolated from wheat and their antifungal activities to soil-borne disease pathogens. Journal of Applied Ecology 2006; 17: 690 - 694.

Raaijmakers JM, Vlami M, deSouza JT. Antibiotic production by bacterial biocontrol agents. Antonie van Leeuwenhoek 2002; 81: 537 - 547.

Ragab Mona MM, Abada KA, Maisa L Abd-El-Moneim, Yosra Z Abo-Shosha. Effect of different mixtures of some bioagents and *Rhizobium phaseoli* on bean damping-off under field condition. International Journal of Scientific & Engineering Research 2015; 6: 1099 - 1106.

Raghvendra VB, Lokesh S, Govindappa M, Vasanth KT. Dravya as an organic agent for the management of seed borne fungi of sorghum and its role in the induction of defense enzymes. Pest. Biochem. Physiol 2007; 89: 190 - 197.

Rahman MA, Kadir J, Mahmud TMM, Rahmand RA, Bergum MM. Screening of antagonistic bacteria for biocontrol activities on *Colletotrichum gleosporioides* in Papaya. Asian Journal of Plant Sciences 2007; 6 (1): 12 - 20.

Reddy B, Reddy M, Kumar K. Characterization of antifungal metabolites of *Pseudomonas fluorescens* and their effect on mycelial growth of *Magnaporthe grisea* and *Rhizoctonia solani*. Int. J. Pharm. Technol Res. 2009; 1: 1490 - 1493.

Ryder MH, Yan Z, Terrace TE, Rovira AD, Tang W, Correll RL. Use of strain of *Bacillus* isolated in China to suppress take-all and rhizoctonia root rot, and promote seedling growth of glasshouse grown wheat in Australian soils. Soil Biol Biochem 1999; 31: 19 – 29.

Schulz B, Boyle C. What are endophytes? Microbial Root Endophytes. Springer Berlin Heidelberg, Berlin, Heidelberg 2006; 15: 1 - 13.

Selim, Hend MM, Nafisa M gomaa, Ashraf MM Essa. Comparative *in vitro* study of the antimicrobial activities of some endophytic bacteria. Klagenfrut, Austria Journal 2014; 21(4): 1 - 26.

Shtark O, Shaposhnikov A, Kravchenko L. The production of antifungal metabolites by *Pseudomonas chlororaphis* grown on different nutrient sources. Mikrobiologia 2003; 72: 645 – 650.

Simons, T J, Ross AF. Changes in metabolism associated with enclosed systemic resistance to tobacco. Phytopathology 1971; 61:1261 - 1265.

Thomas W, Dutcher RA. The determination of carbohydrate in plants by picric acid reduction method. The estimation of reducing sugar and sucrose. J. Am. Chem. Soc. 1924; 46: 1662 - 1669.

Van Loon LC. Stress proteins in infected plants. In Plant-Microbe Interactions, Molecular and Genetic Perspectives (Kosuge T, Nester EW. Eds.). Macmillan, New York 1990; 198 - 237.

Van Loon LC. Induced resistance in plants and role of pathogenesis-related proteins. Eur. J. Plant Pathol., 1997; 103: 753 - 765.

Watkins GM. ed. Compendium of Cotton Disease. The Am. Phytopathology. Soc., St. Paul, Minnestola 1981; 87 p.

Whipps J. Microbial interaction and biocontrol in the rhizosphere. J. Exp. Bot. 2001; 52: 487 - 511.

Worthington XX. Enzyme Manual, Worthington Biochemical Corp., Freehold, New Jersey 1972; 41 -45.

استخدام البكتريا الكامنه فى النبات لمقاومة مرض موت بادرات القطن الناجم عن الاصابه بفطر فيوزاريوم اوكسيسبورم

نفيسه جمعة[١] وشيرين السيد النحاس[٢] و ايمان امين محمد عثمان[٢] وماجى السيد محمد حسن[٢]

١ - قسم النبات - كلية العلوم – جامعة الفيوم - مصر

٢ - معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة – مصر.

عند اختبارسلالتين من البكتريا (سودوموناس اوروجينوزا وستينوترفومونس مالتوفيليا) على بادرات القطن صنف جيزه ٨٦ لم تظهر اى من السلالتين اى تاثير معنوى على اطوال البادرات او الوزن الجاف للبادرات. كما كانت السلالتان غير ممرضتان. عزلة السودوموناس كانت فعاله كعامل مقاومه حيويه ضد عزلتى الفيوزاريوم اوكسيسبورم (المسبب لمرض موت البادرات فى القطن) رقم ١ ورقم ٢ بينما كانت عزلة ستينوترفومونس فعالة ضد عزلة الفيوزاريوم رقم ٤. على الرغم من ان عزلتى البكتريا خفضتا الاصابه بعزلة الفيوزاريوم رقم ٣ الا ان عزلة سودوموناس كانت اكثر فاعلية. طول البادرات المصابه بعزلات الفطر كان اكثر انخفاضا فى حالة الاصابة بالعزلتين رقم ١ او رقم ٢ مقارنة بالعزلتين رقم ٣ او رقم ٤. البكتريا ستينوترفومونس ادت الى زيادة الوزن الجاف للبادرات عندما كانت التربه ملوثه باى من عزلتى الفيوزاريوم اوكسيسبورم رقم ٣ او رقم ٤.اختبارات التضاد على الاطباق اظهرت ان بكتريا السودوموناس اوروجينوزا ثبطت نمو عزلات الفيوزاريوم اوكسيسبورم. وهذا التثبيط تراوح مابين ١٩.٣٣ الى ٣١.٠ % على الجانب الاخر فان بكتريا ستينوترفومونس مالتوفيليا لم تظهر اى تثبيط لنمو عزلات الفطر. لم تظهر اى سلاله من سلالتى البكتريا اى تأثير معنوى على نشاط اى من انزيم البيروكسيديز وانزيم البولى فينول اوكسيديز، بينما ادت السلالتين الى الزياده فى نشاط انزيم بيتا ١-٣ جلوكانيز . ادت المعامله بالسلالتين البكتيريتين الى زيادة السكريات الكليه والسكريات المختزله زياده معنويه الا ان سلالة سودوموناس كانت اكثر فاعلية. الزياده المعنوية فى الفينولات الحره حدثت عند المعامله بسلالة السودوموناس فقط.