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SELECTION OF BIOCONTROL AGENTS FOR CONTROL OF ONION WHITE ROT DISEASE

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ABSTRACT

Isolation of bioagents was carried out from rhizoplane of healthy onion plants, 70 and 120 days after transplanting from different governorates, i.e. El-Behera; El-Sharkia; Beni-Swif and El-Menia. Only 21 isolates from the obtained 331 isolates have prevented sclerotial formation of *Sclerotium cepivorum* on NYDA medium. The inhibition zones of mycelial growth of *S. cepivorum* after 14 days were 13, 9, 7, 4 and 2 ml for isolates *Pseudomonas flourescens; Bacillus licheniformis* 10D14; *Bacillus licheniformis* 6D11; 7D12 and *Chaetomium globosum* respectively. Most of treatments of potting medium, with bacterial isolates soon after onion seeds sowing, increased the percentage of onion seed germination. The highest increase 19.5 % was achieved by isolates *B. licheniformis* 6D11 and *B. amyloliquefaciens* 1D9. The promising biocontrol agents that had promotion toward onion seed germination and plant growth were tested in greenhouse for their potential control of onion white rot. At 50 or 100 sclerotia/ Kg potting medium, the best reduction in dead plants was achieved by treatment with *P. flourescens, B. licheniformis* 6D11, *T. harzianum, B. amyloliquefaciens* 1D9, *C. globosum* or *Paenibacillus polymyxa* 9D14. Clear significant increase in plant height was occurred by treatment with *P. flourescens* followed by *B. licheniformis* 6D11, *C. globosum, B. amyloliquefaciens* 1D9, *Trichoderma harzianum* and *Paenibacillus polymyxa* 9D14. However, in field experiment all applications were highly effective in reducing the percentage of dead onion plants due to white rot infection till the end of growing season (4.5 months from transplanting). The isolates, *P. flourescens, B. licheniformis* 6D11, *B. amyloliquefaciens* 1D9 or *T. harzianum* isolates were the most effective on disease control in the field. Significant increase in bulb yield was recorded with all tested isolates especially those of bacteria.

Keywords: Biological control, Onion, White rot, *Sclerotium cepivorum, Pseudomonas flourescens, Bacillus licheniformis, B. amyloliquefaciens, Trichoderma harzianum, Chaetomium globosum*

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important crops grown in Egypt, not only for local consumption, but also for exportation. However, onion in most areas of the world is affected by white rot disease caused by the soil-borne fungus *Sclerotium cepivorum* Berk (Entwistle,1990). In Egypt, onion white rot is considered a serious disease and some areas have been forced out of production because of this pathogen. Lack of resistant onion cultivars (Van der Meer *et al.*, 1983; Brix & Zinkernagel, 1992 and Hunger *et al.*, 2002), in addition to problems of fungicidal treatments namely fungicide residue (Norman,1988), microbial degradation of dicarboximide fungicides

and developing resistance against dicarboximides (Littley and Rahe, 1984; Walker *et al.*, 1986; Slade *et al.*, 1992) have generated an urgent need for alternative control measures for onion white rot. Biological control could be incorporated into an integrated disease-management programme with regards to reduce chemical applications. The use of biological agents for onion white rot control was first investigated by Ghaffar (1969). Many antagonistic micro-organisms against *S. cepivorum* were studied, namely *Trichoderma harzianum*; *T. virens; T. pseudokoningii, T. atroviride and T. koningii* (Abd-El-Moity *et al.* 1982; De-Oliveira *et al.*, 1984; Abd-Elrazik *et al.*, 1986; Harrison & Stewart 1988; Mohamed & Fahmy, 1990; Kay & Stewart 1994; McLean & Stewart, 2000; Melero-Vara *et al.*, 2000; Clarkson *et al.*, 2002; Clarkson *et al.*, 2004; McLean *et al.*, 2004 and Metcalf *et al.*, 2004); *Chaetomium globosum*, (Harrison & Stewart, 1988; Stewart & Harrison, 1989; Kay & Stewart 1994 and McLean & Stewart, 2000); *Gliocladium roseum* and *G. virens* (Harrison and Stewart, 1988; Stewart and Harrison, 1989 and Jackson *et al.*, 1991) and *Bacillus* spp (Utkhede and Rahe 1980,1983; Abd-Elrazik *et al.*, 1986; Mousa *et al.*, 1987; Ismail *et al.*, 1990 and Melero-Vara *et al.*, 2000).

Targets in the disease cycle for such microbial treatments include eradication of sclerotia in the soil before planting, suppression of formation or degradation of sclerotia on infected plants, and protection of the growing root systems. Although there have been some promising results with biocontrol agents that address these stages in the pathogen life cycle, results are generally variable (Kay & Stewart, 1994). This is perhaps due to inappropriate screening systems, as suggested by Jackson *et al.*, 1991, but biological control of *S. cepivorum* will also vary with environmental conditions, and the origin of both biological control agent and *S. cepivorum* isolate (Coley-Smith, 1987). This work describes a screening programme (in vitro and greenhouse) designed to select Egyptian biocontrol agents that have the ability to suppress sclerotial formation of *S. cepivorum* and their ability to control white rot disease in the field.

MATERIAL AND METHODS

Plant material

Onion (*Allium cepa* L.) seeds cv. Giza 20 and its transplants (45 days old, uniform in shape, size and free from wounds or infection) kindly obtained from Department of Onion, Field Crops Research Institute, Agricultural Research Center, and used in the following experiments.

Pathogen and mass production of sclerotia

A highly virulent isolate of *Sclerotium cepivorum* among many isolates isolated and tested for their pathogenicity was used in this study. The culture was maintained on malt extract agar (MEA) medium (per litre: malt extract 20 gm, peptone 5 gm and agar 15 gm) and covered by phosphate buffer (pH 6.5) at 4±1°C. Sclerotia of *S. cepivorum* were produced on whole barley grains using the technique described by Van der Meer *et al.*, 1983. After 6 weeks incubation at 20±1°C, the sclerotia were harvested using progressive wet sieving through 500 and 212 mesh sieves. Thereafter, the

collected sclerotia were air dried at room temperature on sterile Whatman No. 1 filter paper for 24 h before they were "conditioned" to overcome dormancy. Sclerotia were contained in nylon bags and buried in plastic box (60 x 40 x 30 cm) filled with silt loam soil. The box was incubated between 16 and 23°C, the moisture content of the soil maintained between 40 and 50% for two months, by which time dormancy was overcome (Coley- Smith 1960). Such nylon bags with sclerotia were obtained again and stored in open vessels at 4 ± 1° C till needed.

Isolation of biocontrol agents from rhizoplane of onion plants

Healthy onion plants cvs Giza 6 and Giza 20 grown after corn or cotton crops at different governorates, i.e. El-Behera; El-Sharkia; Beni-Swif and El-Menia were collected at two ages (70 and 120 days after transplanting) for isolation of antagonists. Roots with adherent fine soil were set into conical flasks each contained 100 ml sterile phosphate buffer (pH 6.5) and 0.05 % tween 20. Flasks were shaked on a rotary shaker at 120 rpm for 15 min. Serial dilution technique was applied with subsequent plating on nutrient yeast dextrose agar (NYDA) medium (per litre: nutrient broth 8 g, yeast extract 5 g, dextrose 10 g and 15 g of agar) for isolation of bacteria. Plates were incubated at 23 ±1° C for 2-4 days. After colonies appeared, isolation was made at random based on the visual characteristics of colonies. Purification of isolated bacteria were made by triple restreaking and transferred on NYDA medium slants. All isolated microorganisms were stored under a phosphate buffer at 4±1° C for future use (Knudsen *et al.*, 1997).

Testing antagonistic effect *in vitro*

The collected 331 bacterial isolates that isolated from rhizoplane of onion plants as well as one isolates of *Pseudomonas flourescens*, previously isolated by the authors (Mosa *et al.*, 1997) were tested for their antagonistic effect against *S. cepivorum*. In addition, an isolate of *Trichoderma harzianum* was kindly supplied by Abd-Elmoity, T. H.; and an isolate of each of *Chaetomium globosum*, *Gliocladium roseum* were kindly obtained from Type Culture Collection of Egyptian Fungi, Mycology Research Section, Plant Pathology Research Institute, Agricultural Research Center, Giza, also were tested for their antagonistic effect against *S. cepivorum*. Dual culture technique (Whipps, 1987) was followed. Equal discs 5 mm of 7 days old of *S. cepivorum* grown on MEA media were set in Petri dishes containing NYDA medium for testing antagonistic bacteria and MEA medium for testing antagonistic fungi. The antagonistic bacteria and fungi were inoculated on such media simultaneously with *S. cepivorum* inoculation. Bacteria were streaked 4 cm apart from *S. cepivorum* disc while fungi were inoculated as culture disc at the same distance. Three dishes were used as replicates for each isolate, and then incubated at 20±1° C. Sclerotial formation and the inhibition zones were recorded after 7 and 14 days from inocubation.

Preparation of bioagents inocula

Bacterial isolates were cultured in 250 ml conical flasks each containing 50 ml of NYDB medium for bacterial growth in 1st experiment. In the 2nd and 3rd experiments, the bacterial isolates in addition to the three fungal isolates mentioned before were cultured in 500 ml conical flasks each containing 120 ml of NYDB medium for bacterial growth or 120 ml malt

extract broth medium (MEB) for fungal growth. Flasks were incubated on a rotary shaker (120 rpm) at 23 ± 2° C for two days for bacterial isolates and twelve days for fungal isolates. However, In the 2nd and 3rd experiments, 100 ml of sterilized distilled water were added to each flask of fungal isolates, the contents was blended in a blender twice for 30s at 500 rpm to produce spores / mycelium homogenate. While, the bacterial isolates were used without adding water or blending. Both of fungal and bacterial isolates were mixed with 20 gm of autoclaved ground wheat bran (particle diameter about 500 µm) and 1 gm carboxy methylcellulose for 100 ml of mixture and mixed using a magnetic stirrer. In the 3rd experiment (field experiment) the previous biocontrol isolates formulations were incubated for 48 hr at 23±1°C before application (Ahmed and Tripe, 1977).

Greenhouse experiments

Preparation of potting medium

Peat-moss and vermiculite mixture (1:1,v:v), plus basic fertilizers, containing the following: 250 g ammonium nitrate, 150 g potassium sulfate, 24 g magnesium sulfate, 400 g super phosphate, 4 kg calcium carbonate and 75 cc foliar fertilizer containing micronutrients per 50 kg peat moss was used as potting medium. The infested and non-infested potting media were potted in pots (15 cm) at the rate of 330 g in each pot. Pots were irrigated when needed.

Effect of biocontrol agents on onion seed germination and plant growth parameters in greenhouse.

The most effective bacterial isolates that exhibited wide inhibition zone or prevented sclerotial formation of *S. cepivorum* were tested for their effect on onion seed germination and plant growth under greenhouse conditions.

New foam punnets (seedling trays) each consisted of eighty four inverted pyramid shape cells 4 x 4 cm at the top, 6 cm deep, and 77 cc capacity were filled with potting medium. Three onion seeds were sown in each cell. Three ml of bacterial suspension containing $1x10^9$ cfu/ml for each isolate individually was inoculated onto the surface of the potting medium in each cell of punnets. Nine cells were used for each isolate as replicates. Nine cells treated with three ml of sterilized NYDB were used as control (check). Punnets were irrigated as needed .The percentage of seeds germination, plant height, number of leaves and fresh weight per plant were recorded after 45 days from sowing.

Effect of biocontrol agents on onion white rot incidence in greenhouse.

Healthy onion transplants were treated with suspension of different bioagents by immersing them in the suspension containing $0.2\text{-}1x10^8$ cfu / ml for 5 min. Treated transplants were transplanted in infested potted media with *S. cepivorum* 50 or 100 sclerotia / kg potting medium. The control treatments included two sets of pots, the first was by cultivated transplants treated with sterile NYDB or MEB in infested potted medium with *S. cepivorum* (control) and the second set was planted onion transplants without treatment in non-infested potted medium (Healthy). Five pots (replications), each with three onion transplants were used for each treatment. Percentage

of dead plants after 90 days and plant length after 45 days from transplanting were recorded in all treatments.

Identification procedure

The most effective bacterial isolates were identified with GP Biolog microplates of the Biolog system (Biolog Inc., Hayward, CA) according to the recommended procedure. The plates were inoculated with the bacterial suspensions made from cultures grown in standard Biolog Universal Growth Medium (BUGM) for 36h, which were washed twice in sterile distilled water before application to the plates. The data from the GP plates were analyzed with the MLCLUST program (Biolog Inc.).

Field experiment

The experiment was carried out in natural infested soil at El-Der province, Kalubia governorate, to investigate the effect of three fungal isolates, i.e. *C. globosum, G. roseum* and *T. harzianum* and three bacterial isolates, i.e. *P. flouresence, Bacillus licheniformis* 6D11 *and B. amyloliquefaciens* 1D9 on percentage of white rot disease of onion and bulbs yield at harvest. Onion transplants were immersed for 5 min before transplanting in suspension ($0.2\text{-}1x10^8$ cfu / ml) of each isolate. Treatments were distributed in complete randomized block's design. Three plots were used as replicates for each treatment in addition to the control (check). The area of each plot was 16 m^2 contained 400 transplants. All treatments received the same normal agricultural practices till harvest at the end of growing season. Data of white rot disease incidence were recorded throughout the season till harvest. Percentage of dead plants was calculated as number of dead plants/total number of plants x100. The yield bulbs were weighed and average weight of bulbs was calculated.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA), and mean values were compared using least significant difference (LSD) at 5% (SAS Institute 1989, SAS/STAT User's Guide, Version 6, Cary, North Carolina).

RESULTS

Isolation of biocontrol agents from rhizoplane of healthy onion plants and antagonistic effect *in vitro*

Isolation from healthy onion plants grown after corn or cotton crops at different governorates, i.e. El-Behera, El-Sharkia, Beni-Swif and El-Menia resulted 331 bacterial isolates Table (1). The obtained isolates were tested by dual culture technique, results indicated that no obvious relationship between the previous crop of onion and the efficiency of bacterial isolates, meanwhile isolates achieved from middle Egypt (6D11, 7D14 and 10D14) were more effective and isolates obtained after 120 days from transplants were more effective than those of 70 days.

Table 1: Sources and number of bacterial isolates that isolated, 70 and 120 days after transplanting, from rhizoplane of healthy onion plants grown after corn or cotton crops at different locations.

Governorate	Location	Previous crop	Total number of bacterial isolates	
			After 70 days	After 120 days
El-Behera	Kom-Hamada	corn	10	8
		cotton	12	10
	Etay-Elbaroud	corn	9	11
		cotton	7	15
El-Sharkia	Dyarb -Negm	corn	15	7
		cotton	11	9
	Mashtool	corn	8	11
		cotton	7	6
Beni-Swif	Beba	corn	9	14
		cotton	13	9
	Somsta	corn	7	14
		cotton	15	15
El-Menia	Maghagha	corn	8	7
		cotton	12	16
	Beni-Mazar	corn	10	6
		cotton	13	7
Total	-	-	166	165

Only 21 isolates prevented sclerotial formation up to 14 days by dual culture technique. The results of these isolates as well as other three isolates that had obvious inhibition zone effect present in Table (2).

Table 2: Width of inhibition zone and formation of sclerotia on NYDA medium for bacterial isolates(1) or MEA for fungal isolates after incubation at 20±1° C for 14 days.

Tested isolate	Inhibition zone (mm)		Sclerotial formation after 14 days	Tested isolate	Inhibition zone (mm)		Sclerotial formation after 14 days
	7 days	14 days			7 days	14 days	
3A(2)1	0.0	0.0	--(3)	3D11	0.0	0.0	--
1A4(4)	1	0.0	--	6D11	10	7	+
1A5	1	1	--	8D11	0.0	0.0	--
4A5	0.0	0.0	--	3A12	0.0	0.0	--
1A6	0.0	0.0	--	1D12	0.0	0.0	--
1A7	1	0.0	--	7D12	4	4	+
1A8	1	0.0	--	3D14	0.0	0.0	--
2A8	0.0	0.0	--	4D14	0.0	0.0	--
7A8	0.0	0.0	--	5D14	0.0	0.0	--
4D8	0.0	0.0	--	9D14	0.0	0.0	--
1D9	2	0.0	--	10D14	11	9	+
3D9	0.0	0.0	--	*P. flourescens*	15	13	--
3A10	0.0	0.0	--	*C. globosum*	2	2	--
6A10	0.0	0.0	--	*G. roseum*	1	0.0	--
1D11	0.0	0.0	--	*T. harzianum*	0.0	0.0	--

(1) Isolated from rhizoplane of healthy onion plants except *P. flourescens.*
(2) The isolate number contains (A) letter was isolated after 70 days from transplanting and (D) letter was isolated after 120 days.
(3) The negative mark (--) means that sclerotia were absent and the positive mark (+) means that sclerotia were present.
(4) The right number indicates the location of isolates 1-4 El-Behera; 5-8 El-Sharkia; 9-12 Beni-Swif and 13-14 El-Menia.

The distances of inhibition zones reached 11, 10 and 4 mm for isolates 10D14, 6D11 and 7D12 respectively. However, *Pseudomonas flourescens*, *Chaetomium globosum* and *Gliocladium roseum* caused inhibition zones amounting to 15, 2, and 1 mm on NYDA or MEA media respectively, while *Trichoderma harzianum* did not exhibit effect after seven days. On the other hand, with extending the incubation period the inhibition zones were slightly reduced to 13, 9, 7, 4, 2 and zero for isolates *P. flourescens;* 10D14; 6D11; 7D12; *C. globosum* and *G. roseum* respectively.

Effect of biocontrol agents on onion seed germination and plant growth parameters in un-infested potting medium.

The effect of treating potting medium with different bacterial isolates on seed germination, plant height, number of leaves/plant and fresh weight/plant was studied. Results (Table 3) indicated that most bacterial isolates increased the percentage of onion seed germination over the untreated control. Such increase in onion seed germination ranged from 2.8% with isolates 1A4 and 6A10 up to 19.5 % with isolates 6D11 and 1D9.

Table 3: Effect of treating potting medium[1] with different bacterial isolates[2] on onion seed germination and growth parameters of seedlings after 45 days from seed sowing[3] in seedling trays under greenhouse conditions.

Isolates	Seed germination (%)	plant height (cm)	Number of leaves/plant	Fresh weight (gm/plant)
2A[4]1[5]	58.3	29.18	2.38	0.38
1A4	55.5	25.93	2.59	0.43
1A5	66.6	29.92	2.37	0.50
4A5	66.6	28.01	2.43	0.46
1A6	33.3	29.00	2.78	0.48
1A7	52.7	23.50	2.29	0.27
1A8	61.1	27.93	2.50	0.35
2A8	44.4	25.68	2.11	0.35
1D9	72.2	29.27	2.37	0.64
3D9	66.6	27.92	2.75	0.38
6A10	55.5	28.30	2.48	0.43
6D11	72.2	26.50	2.20	0.44
8D11	44.4	26.10	2.33	0.28
1D12	58.3	27.77	2.33	0.53
7D12	61.1	28.88	2.06	0.46
4D14	59.3	25.25	2.51	0.40
9D14	70.3	29.1	2.46	0.52
10D14	69.4	30.47	2.33	0.55
Control	52.7	27.54	2.28	0.41
LSD at 5%	8.7	Ns	Ns	0.2

(1) Peat-moss vermiculite mixture inoculated with 3 ml of bacterial suspension ($1x10^8$ cfu/ml) for each isolate onto the surface of each tray-cell.
(2) Isolated from rhizoplane of healthy onion plants.
(3) Three onion seeds (Giza 20) were sown in each cell and Nine cells were used for each isolate.
(4) The isolate number contains (A) letter was isolated after 70 days from transplanting and (D) letter was isolated after 120 days.
(5) The right number indicates the location of isolates 1-4 El-Behera; 5-8 El-Sharkia; 9-12 Beni-Swif and 13-14 El-Menia.

On the other hand, isolates 1A6, 2A8 and 8D11 showed an inhibitory effect in onion seed germination reached, 19.4 % for 1A6 and 8.3 % for the later two isolates respectively. However, the parameters of seedling, i.e. plant height, number of leaves/plant and fresh weight of onion plants did not show clear variances between different bacterial isolates and the control when determined after 45 days from seed sowing.

Effect of biocontrol agents on onion white rot incidence in greenhouse.

In this experiment the most effective bacterial isolates, that had not inhibitory effect on onion growth, in addition to three isolates of fungi were tested by treating transplants. Treated transplants were sowed in infested potting media containing 50 or 100 sclerotia/kg. Data presented in Table 4 showed that clear reduction in percentage of dead onion plants, compared with the control after 90 days from transplanting, except *Bacillus* sp. 4A5 that was equal with untreated control.

Table 4: Effect of application[1] of onion transplants[2] by different antagonistic bacterial and fungal isolates before transplanting in infested potting medium[3] with *S. cepivorum*[4], on percentage of dead plants and plant height under greenhouse conditions.

Tested Isolate[5]	Percentage of dead plants, 90 days from transplanting		Plant height (cm), 45 days from transplanting	
	50 S/KG	100 S/KG	50 S/KG	100 S/KG
Bacillus fastidiosus 1A5	60.00	93.33	28.3	10.6
Bacillus sp. 4A5	80.00	100.00	21.0	10.0
Bacillus amyloliquefaciens 1D9	40.00	46.67	32.9	29.1
Bacillus licheniformis 6D11	20.00	33.33	36.8	33.9
Bacillus licheniformis 1D12	60.00	93.33	22.1	10.3
Micrococcus luteus 4D14	60.00	73.33	24.3	22.0
Paenibacillus polymyxa 9D14	46.67	53.33	30.8	27.5
Bacillus licheniformis 10D14	53.3	86.70	24.0	13.9
Pseudomonas flourescens	13.33	26.67	39.6	37.0
Chaetomium globosum	40.00	53.33	33.2	29.7
Gliocladium roseum	66.67	73.33	25.5	20.0
Trichoderma harzianum	33.33	46.67	32.9	24.5
Control	80.00	100.00	20.7	10.0
LSD. (5%)	16.2	17.7	5.3	5.1
Healthy	0.00		45.4	

(1) Ttransplants were immersed 5 min in suspension containing 0.2-1x10^8 cfu / ml for fungi or bacteria, 20 gm autoclaved ground wheat bran and 1gm carboxy methylcellulose/ 100 ml were added.
(2) Onion transplants (cv. Giza 20) 45 days old were used.
(3) Peat-moss vermiculite mixture, (1:1,v:v) plus basic fertilizers was used.
(4) potting medium contained 50 or 100 sclerotia of *S. cepivorum* / kg.
(5) All bacterial isolates except *Pseudomonas flourescens* isolated from rhizoplane of healthy onion plants.

The highest reduction in dead plants was obtained with treatments by *P. flourescens*, *B. licheniformis* 6D11, *T. harzianum*, *B. amyloliquefaciens* 1D9, *C. globosum* and *Paenibacillus polymyxa* 9D14 either potting media contained 50 or 100 sclerotia / kg. As for plant length, clear significant increase in plant height was occurred with treatment by *P. flourescens* followed by *B. licheniformis* 6D11, *C. globosum*, *B. amyloliquefaciens* 1D9, *T. harzianum* and *Paenibacillus polymyxa* 9D14 and sowed in potting media containing 50 or 100 sclerotia/ kg. However, there was not significant variance in plant height between treatment with the isolates *Bacillus* sp. 4A5, *B. licheniformis* 1D12, *B. licheniformis* 10D14, *Micrococcus luteus* 4D14 and *Gliocladium roseum* compared with the control in both tested pathogen inocula.

Field experiment

The experiment was carried out in natural infested soil at El-Der province, Kalubia governorate, to investigate the effect of three bacterial isolates and three fungal isolates on percentage of white rot disease of onion and bulbs yield at harvest. Results presented in Table (5) indicated that all applications highly effective in reducing the percentage of dead onion plants due to white rot infection till the end of growth season (4.5 months after transplanting). The three fungal isolates tested were less effective than bacterial isolates. *P. flourescens*, *B. licheniformis* **6D11** isolates were the most effective on disease control at the field. Significant increase in bulb yield was recorded with all the tested isolates especially those of bacteria.

Table 5: Effect of application(1) of onion transplants(2) by bacterial and fungal isolates before transplanting in in natural infested field(3) on percentage of dead plants and fresh weight of yield at El-Der, Kalubia governorate.

Tested Isolate	Percentage of dead plants	Fresh weight (gm) of one bulb	Fresh weight (kg) of yield/m^2	Yield increase (%)
Bacillus amyloliquefaciens 1D9	6.9	88.5	2.05	84.7
Bacillus licheniformis 6D11	5.4	92.3	2.18	96.4
Pseudomonas flourescens	4.5	91.4	2.18	96.4
Chaetomium globosum	12.5	86.3	1.88	69.4
Gliocladium roseum	8.5	80.7	1.84	65.8
Trichoderma harzianum	9.8	83.5	1.88	69.4
Control	38.7	73.0	1.11	
LSD . (5%)	2.0	9.5	0.7	

(1) Ttransplants were immersed 5 min in suspension containing 0.2-1x10^9 cfu / ml for fungi or bacteria, 20 gm autoclaved ground wheat bran and 1 gm carboxy methylcellulose/ 100 ml were added. Mixtures were incubated for 48 hr at 23 ± 1° C before used.

(2) Onion transplants (cv. Giza 20) 45 days old were used.

(3) The estimated density of mature black sclerotia in field soil was 192 sclerotia/kg soil using wet sieving method (Crowe *et al.*, 1980).

DISCUSSION

The present work proposed that the appropriate site to search for a biocontrol strain is at the site of infection by the pathogen. Thus, we isolated a collection of rhizoplane bacteria, which associated healthy onion plants in different geographical locations in Egypt. We preferred the isolation from rhizoplane to isolate micro-organisms tolerant to the toxic effect of *Allium* and any exudates from onion roots. In this respect, The toxic effect of *Allium* and its derived sulfide compounds for microbial agents in humans has been recognized for a long time (Cavallito and Bailey 1944). However there is still a lack of data on the toxic effects of these substances on plant microbial agents. Noteworthy results have been achieved on *Pseudomonas solannacearum* (Yu, 1999) in associated crops. Indeed, growing tomatoes with Chinese chives has been observed to reduce the number of potato plants that wither due to infection. *Allium sativum* is also effective against several species of *Pseudomonas* and *Xanthomonas*.

A low percentage (6.35%) of the isolated bacteria which were tested as potential antagonists against *S. cepivorum* prevented sclerotial formation and 1.2% of isolated bacteria showed inhibitory zone of growth against *S. cepivorum* up to 14 days under dual culture technique. This inhibition is mostly due to presence of fungitoxic or antibiotic substances produced by tested promising isolates which could interfere with the growth, enzymatic activity or cell membrane of the pathogen causing leakage of sugars and electrolytes. This phenomenon can be supported by Becker *et al.*, 1985 who stated that some of the bacterial isolates produced potent antifungal compounds with a wide spectrum of activity.

Eighteen bacterial isolates, isolated from onion rhizoplane, were selected for studying their effect on growth of onion plants. Sex isolates of them significantly increased at least one aspect of growth parameters. Previous researches suggest that mechanisms by which microorganisms promote plant growth are not fully understood, but are thought to include: (i) the ability to produce or change the concentration of the plant hormones indol eacetic acid (IAA) (Mordukhova *et al.*, 1991); gibberellic acid (Mahmoud *et al.*, 1984); cytokinins (Tien *et al.*, 1979) and ethylene (Arshad & Frankenberger, 1991 and Glick *et al.*, 1995); (ii) a symbiotic N2 fixation (Boddey & Dobereiner, 1995 and Kennedy *et al.*, 1997); (iii) antagonism against phytopathogenic microorganisms: by production of siderophores (Scher & Baker, 1982); b-1,3-glucanase (Fridlender *et al.*, 1993); chitinases (Renwick *et al.*, 1991); antibiotics (Shanahan *et al.*, 1992); and cyanide (Flaishman *et al.*, 1996); and (iv) solubilization of mineral phosphates and other nutrients (Sperber 1958a, 1958b; De Freitas *et al.*, 1997). Many of the studies with PGPR show plant growth promotion, but only under gnotobiotic conditions (Shenbagarathai, 1993 and Glick *et al.*, 1995) or in potting media (Polonenko *et al.*, 1987).

During our reaserch, the best reduction in dead plants was achieved by treatments with *Pseudomonas flourescens*, *Bacillus licheniformis* 6D11, *Trichoderma harzianum*, *B. amyloliquefaciens* 1D9, and *Chaetomium globosum* either in greenhouse or in the field. Successes of using isolates of *Trichoderma* to control white rot have been reported in the literature. (De

Oliveria *et al.*, 1984 and Abd-El-Moity & Shatla 1981). *Chaetomium globosum* (A53) was able to colonise and degrade *S. cepivorum* sclerotia in vitro and capable of reducing the incidence of onion white rot (Kay and Stewart 1994). Mechanisms of biocontrol of fungal isolates are sclerotial degradation by mycoparasitism, which has been observed for *S. cepivorum* by *Trichoderma* spp. (Abd-El-Moity & Shatla, 1981), and *Chaetomium globosum* (Kay & Stewart,1994), competition for space and nutrients (Harrison & Stewart,1988 and Metcalf & Wilson, 2001) and antibiosis (Abd-El-Moity & Shatla, 1981 and Harrison & Stewart, 1988). Pseudomonads has been shown to be involved in the suppression of seedling diseases (Weller 1988 and Keel *et al.*, 1990). Pseudomonads may affect plant diseases through different mechanisms: a) competition in the rhizosphere for nutrients and preferred colonization sites (Foster, 1986 and Paulitz,1990); b) the production of iron-chelating siderophores (Baker *et al.*, 1986 and Becker & Cook 1988); and c) the production of antibiotic metabolites, such as phenazine carboxylic acid (Thomashow & Weller 1988), pyoluteorin (Plt) (Natsch *et al.*, 1998), pyrrolnitrin (Homma *et al.*, 1989), 2,4-diacetylphloroglucinol (PHl) (Keel *et al.*, 1990,1996), oomycin A (Howie & Suslow 1991), and cyanide (Voisard *et al.*, 1989). On the other hand, *Bacillus* spp. affect plant diseases through different mechanisms, competition for nutrients (Mari *et al.*, 1996 and Yu and Sinclair 1996) rather than to production of antibiotics (Yu & Sinclair 1996 and Yoshida *et al.*, 2001). Members of the genus *Bacillus* produce a variety of antibacterial and antifungal peptide antibiotics (Katz & Demain, 1977; Zuber *et al.*, 1993). Strains of *B. licheniformis* are capable of producing licheniformin (Callow & Hart, 1946), bacitracin (Johnson *et al.*, 1945) and proticin (Woolford, 1972 and Katz & Demain, 1977). Strains of *B. amyloliquefaciens* produce iturin A and its isomers (Yu *et al.*, 2002).

Further studies are needed to incorporate our promising tested isolates into an integrated disease-management programme of onion white rot.

REFERENCES

Abd-El-Moity, T. H. and M. N. Shatla (1981). Biological control of white rot disease of onion (*Sclerotium cepivorum*) by *Trichoderma harzianum*. Phytopathologische Zeitshrift 100: 29–35.

Abd-El-Moity, T. H.; G. C. Papavizas and M. N. Shatla (1982). Induction of new isolates of *Trichoderma harzianum* tolerant to fungicides and their experimental use for control of white rot of onion. Phytopathology 72: 396-400.

Abd-Elrazik, A. A.; A. M. El-Shabrawy; M. A. Sellam, and M. H. A. El-Rehim (1986). Effectiveness of certain fungi and bacteria associated with sclerotia of *Sclerotium cepivorum* in Upper Egypt soil on controlling white rot of onion. Egypt. J. of Phytopathol. 7: 107-114.

Ahmed, A. H. M. and H. T. Tribe. (1977). Biological control of white rot of onion (*Sclerotiium cepivorum*) by *Coniothyrium minitans*. Plant Pathology 26: 75-78.

Arshad, M. and W. T. Frankenberger, Jr. (1991). Microbial production of plant hormones. Plant Soil 133:1–8.

Baker, R.; Y. Elad and B. Sneh. (1986). Physical, biological and host factors in iron competition in soils, pp. 77-85. In: T. R. Swinburne (ed.), Iron, Siderophores, and Plant Disease. Plenum Press, New York.

Becker, J. O. and R. J. Cook (1988). Role of siderophores in suppression of *Pythium* species and production of increased growth response of wheat by fluorescent pseudomonads. Phytopathology 78:778-782.

Becker, J. O. ; R. W. Hedges; L. V. Royen; E. Messens; M. F. Outryve; B. Lambert; F. Leyns; P. Tenning; V. Gossele; M. Zabeau and F. Gossele (1985). The production of antifungal compounds by phytosphere bacteria. Mededelingen van de Faculteit van Landbouwwetenschappen Univ. Ghent 50:(3b)1135-1139.

Boddey, R. M. and J. Dobereiner (1995). Nitrogen fixation associated with grasses and cereals: Recent progress and perspectives for the future. Fert. Res. 42:241–250.

Brix, H. D. and V. Zinkernagel (1992). Screening for resistance of *Allium* species to *Sclerotium cepivorum* with special reference to non-stimulatory resistance. Plant Pathology 41:308–16.

Callow, R. K. and P. D. Hart (1946). Antibiotic material from *Bacillus licheniformis* (Weigmann, emend. Gibson) active against species of mycobacteria. Nature 157:334.

Cavallito, C.J. and J. H. Bailey (1944). Allicin, the antibacterial principale of *Allium sativum* L. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. 66: 1950-1952.

Clarkson, J. P.; T. Payne; A. Mead and J. M. Whipps (2002). Selection of fungal biological control agents of *Sclerotium cepivorum* for control of white rot by sclerotial degradation in a UK soil. Plant Pathology 51: 735–745.

Clarkson, J. P.; A. Mead; T. Payne and J. M. Whipps (2004). Effect of environmental factors and *Sclerotium cepivorum* isolate on sclerotial degradation and biological control of white rot by *Trichoderma.* Plant Pathology 53: 353–362.

Coley-Smith, J. R. (1960). Studies of the biology of *Sclerotium cepivorum* Berk. IV Germination of sclerotia. Ann. Appl. Biol. 48: 8-18.

Coley-Smith, J. R. (1987). Alternative methods of controlling white rot disease of *Allium.* pp.161–77. In: Chet, I. ed. Innovative Approaches to Plant Disease Control. John Wiley, New York, USA.

Crowe, F. J.; D. H. Hall; A. S. Greathead and K. G. Baghott (1980). Inoculum density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. Phytopathology 70:64-69.

De Freitas, J. R.; M. R. Banerjee and J. J. Germida (1997). Phosphate solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biol. Fertil. Soils 24:358–364.

De-Oliveira, V. L.; M. M. De-Belle and A. C. Borges (1984). Control of white rot of garlic by antagonistic fungi under controlled environmental conditions. Can. J. Microbiol. 30: 884-889.

Entwistle, A. R. (1990). Root diseases. In: Rabinowitch, H.D., J. L. Brewster, eds. Onions and Allied Crops, Vol. II. pp. 105–154, CRC Press, Boca Raton, FL. USA.

Flaishman, M. A.; Z. Eyal; A. Zilberstein; C. Voisard and D. Hass (1996). Suppression of *Septoria tritici* blotch and leaf rust of wheat by recombinant cyanide-producing strains of *Pseudomonas putida*. Mol. Plant-Microbe Interact. 9:642–645.

Foster, R. C. (1986). The ultrastructure of the rhizoplane and rhizosphere. Annu. Rev. Phytopathol. 24:211-234.

Fridlender, M.; J. Inbar, and I. Chet (1993). Biological control of soil-borne plant pathogens by a b-1,3-glucanase–producing *Pseudomonas cepacia*. Soil Biol. Biochem. 25:1211–1221.

Ghaffar, A. (1969). Biological control of white rot of onion I. Interactions of soil microorganisms with *Sclerotium cepivorum* Berk. Mycopathologia et Mycologia Applicata 38: 101-111.

Glick, B. R.; D. M. Karaturovic and P. C. Newell (1995). A novel procedure for rapid isolation of plant growth promoting pseudomonads. Can. J. Microbiol. 41:533–536.

Harrison, Y. A. and A. Stewart (1988). Selection of fungal antagonists for biological control of onion white rot in New Zealand. New-Zealand Journal of Experimental Agriculture **16**: 249–56.

Homma, Y.; Z. Sato; F. Hirayama; K. Konno; H. Shirahama and T. Suzui (1989). Production of antibiotics by *Pseudomonas cepacia* as an agent for biological control of soilborne plant pathogens. Soil Biol. Biochem. 21:723-728.

Howie, W. J. and T. V. Suslow (1991). Role of antibiotic biosynthesis in the inhibition of *Pythium ultimum* in the cotton spermosphere and rhizosphere by *Pseudomonas fluorescens*. Mol. Plant Microbe Interact. 4: 393-399.

Hunger, S. A.; K. L. Mclean; C. C. Eady and A. Stewart (2002). Seedling infection assay for resistance to *Sclerotium cepivorum* in *Allium* species. New Zealand Plant Protection 55:193-196.

Ismail, I. M. K.; A. M. Salama; M. A. Ali and S. A. Ouf (1990). Effect of *Aspergillas terreus* and *Bacillus pumilus* culture filtrates on *Sclerotium cepivorum in vitro*. Egyptian Journal of Microbiology 25: 335-348.

Jackson, A. M.; J. M. Whipps and J. M. Lynch (1991). *In vitro* screening for the identification of potential biocontrol agents of *Allium* white rot. Mycological Research 95: 430-434.

Johnson, B.A.; H. Anker and F.L. Meleney (1945). Bacitracin: A new antibiotic produced by a member of the *B. subtilis* group. Science 102:376-377.

Katz, E. and A. L. Demain (1977). The peptide antibiotics of Bacillus: chemistry, biogenesis, and possible functions. Bacteriol. Rev. **41**:449–474.

Kay, S. J. and A. Stewart (1994). Evaluation of fungal antagonists for control of onion white rot in soil box trials. Plant Pathology 43: 371-377.

Keel, C.; P. Wirthner; T. Oberhansli; C. Voisard; U. Burger; D. Haas and G. Defago (1990). Pseudomonads as antagonists of plant pathogens in the rhizosphere: role of the antibiotic 2,4-diacetylphloroglucinol in the suppression of black rot of tobacco. Symbiosis 9:327-341.

Keel, C.; D. M. Weller; A. Natsch; G. Defago; R. J. Cook and L. S. Thomashow (1996). Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. Appl. Environ. Microbiol. 62: 552-563.

Kennedy, I. R.; L. L. Pereg-Gerk; C. Wood; R. Deaker; K. Gilchrist, and S. Katupitiya (1997). Biological nitrogen fixation in non-leguminous field crops: Facilitating the evolution of an effective association between *Azospirillum* and wheat. Plant Soil 194:65–79.

Knudsen, I. M. B.; J. Hockenhull; D. Funck Jensen; B. Gerhardson; M. Hökeberg; R. Thavonen; E. Teperi; L. Sundheim and B. Henriksen (1997). Selection of biological control agents for controlling soil and seed-borne diseases in the field. Eur. J. Plant Pathol. 103:775-784.

Littley, E. R. and J. E. Rahe (1984). Specific tolerance of *Sclerotium cepivorum* to dicarboximide fungicides. Plant Dis. 68: 371-374.

Mahmoud, S. A. Z.; E. M. Ramadan; F. M. Thabet and T. Khater (1984). Production of plant growth promoting substances by rhizosphere microorganisms. Zbl. Mikrobiol. 139:227–232.

Mari, M.; M. Guizzardi and G. C. Pratella (1996). Biological control of gray mold in pears by antagonistic bacteria. Biol. Control 7:30-37.

McLean, K. L. and A. Stewart (2000). Application strategies for control of onion white rot by fungal antagonists. New Zealand Journal of Crop and Horticultural Science 28:115–22.

McLean, K. L.; S. L. Dodd; B. E. Sleight; R. A. Hill and A. Stewart (2004). Comparison of the behaviour of a transformed hygromycin resistant strain of *Trichoderma atroviride* (M1057-hygr) with the wild-type strain (M1057). New Zealand Plant Protection 57:72-76.

Melero-Vara, J. M.; A.M. Prados-Ligero; M. J. Basallote-Ureba (2000). Comparison of physical, chemical and biological methods of controlling garlic white rot. European J. of Pl. Pathol. 106: 581–588.

Metcalf, D. A. and C. R. Wilson (2001). The process of antagonism of *Sclerotium cepivorum* in white rot affected onion roots by *Trichoderma koningii*. Plant Pathology **50**, 249–57.

Metcalf, D. A.; J. J. C. Dennis and C. R. Wilson (2004). Effect of inoculum density of *Sclerotium cepivorum* on the ability of *Trichoderma koningii* to suppress white rot of onion. Plant Dis. 88:287-291.

Mohamed, M .S. and F. G. Fahmy (1990). Improvement of the biologcal control of *Trichoderma harzianum* Rifai to onion white rot. Egypt J. Phytopathol. 20: 117-125.

Mordukhova, E. A.; N. P. Skvortsova; V. V. Kochetkov; A. N. Dubei-kovskii and A. M. Boronin (1991). Synthesis of the phytohormone indole-3-acetic acid by rhizosphere bacteria of the genus *Pseudomonas*. Mikrobiologiya 60:494–500.

Mosa, A. A.; S. T. Shehata and S. M. Abd-Allah (1997). Biocontrol of cucumber damping off by fluorescent Pseudomonads. Egypt. J. Appl. Sci. 12:268-286.

Mousa, O. M.; F. A. Khalil and M. M. Ragab (1987). Variability of *Sclerotium cepivorum* Berk., microflora of unviable sclerotia and their antagonistic effect to this pathogen. Egyptian Journal of phytopathology 19: 1-14.

Natsch, A.; C. Keel; N. Hebecker; E. Laasik and G. Defago (1998) Impact of *Pseudomonas fluorescens* strain CHAO and a derivative with improved biocontrol activity on the culturable resident bacterial community on cucumber roots. FEMS Microbiology Ecology 27: 365-380

Norman, C. (1988). EPA sets new policy on pesticides risks. Science 242:366-367.

Paulitz, T. C. (1990). Biochemical and ecological aspects of competition in biological control, pp. 713-724. In: R. R. Baker and P. E. Dunn (ed.), New Directions in Biological Control. Alan R. Liss, Inc., New York.

Polonenko, D. R.; F. M. Scher; J. W. Kloepper; C. A. Singleton; M. Laliberte and I. Zaleska. (1987). Effects of root colonizing bacteria on nodulation of soybeans roots by *Bradyrhizobium japonicum*. Can. J. Microbiol. 33:498–503.

Renwick, A.; R. Campbell and S. Coe (1991). Assessment of *in vivo* screening systems for potential biocontrol agents of *Gaeumannomyces graminis*. Plant Pathology 40:524–532.

Scher, F. M. and R. Baker. (1982). Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium wilt pathogens*. Phytopathology 72:1567–1573.

Shanahan, P.; D.J. O'Sullivan; P. Simpson; J.D. Glennon and F. O'Gara. (1992). Isolation of 2,4-Diacetylphloroglucinol from a fluorescent pseudomonad and investigation of physiological parame-ters influencing its production. Appl. Environ. Microbiol. 58: 353–358.

Shenbagarathai, R. (1993). Isolation and characterization of mutants of *Rhizobium* SBS-R100 symbiotic with *Sesbania procumbens*. Soil Biol. Biochem. 25:1339–1342.

Slade, E. A.; R. A. Fullerton; A. Stewart and H. Young (1992). Degradation of the dicarboximide fungicides iprodione, vinclozolin and procymidone in Paturmahoe clay loam soil, New Zealand. *Pesticide Science* 35: 95–100.

Sperber, J. I. (1958a). The incidence of apatite solubilizing organisms in the rhizosphere and soil. Aust. J. Agric. Res. 9:778–781.

Sperber, J. I. (1958b). Solution of apatite by soil microorganisms producing organic acids. Aust. J. Agric. Res. 9:782–787.

Stewart, A. and Y. A. Harrison (1989). Mycoparasitism of sclerotia of *Sclerotium cepivorum*. Australasian plant pathology 18: 10-14.

Thomashow, L. S., and D. M. Weller. (1988). Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J. Bacteriol. 170: 3499-3508.

Tien, T. M.; M. H. Gaskins and D. H. Hubbell (1979). Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.). Appl. Environ. Microbiol. 37:1016–1024.

Utkhede, R. S. and J. E. Rahe (1980). Biological control of onion white rot. Soil Biology and Biochemistry 12: 101-104.

Utkhede, R. S. and J. E. Rahe (1983). Interaction of antagonist and pathogen in biological control of onion white rot. Phytopathology 73: 890-893.

Van der Meer, Q. P.; J. L. Van Bennekom and A. C. Van der Giessen (1983). Screening for resistance to white rot caused by *Sclerotium cepivorum* Berk. in onions (*Allium cepa* L.)and leek (*Allium porrum* L.) Euphytica 32: 697-701.

Voisard, C.; C. Keel; D. Haas and G. Defago. (1989). Cyanide production by *Pseudomonas fluorescens* helps suppress black rot of tobacco under gnotobiotic conditions. EMBO J. 8:351-358.

Walker, A.; P. A. Brown and A. R. Entwistle (1986). Enhanced degradation of iprodione and vinclozolin in soil. Pesticide Science 1: 183–193.

Weller, D. M. (1988). Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annu. Rev. Phytopathol. 26:379-407.

Whipps, J. M. (1987). Effect of media on growth and interaction between a range of soilborne glasshouse pathogens and antagonistic fungi. New Phytologist 107: 127-142.

Woolford, M. K. (1972). The semi large-scale production, extraction, purification, and properties of an antibiotic produced by *Bacillus licheniformis* strain 2725. J. Appl. Bacteriol. 35:227-231.

Yoshida, S.; S. Hiradate; T. Tsukamoto; K. Hatakeda and A. Shirata (2001). Antimicrobial activity of culture filtrate of *Bacillus amyloliquefaciens* RC-2 isolated from mulberry leaves. Phytopathology 91:181-187.

Yu, G. Y., and J. B. Sinclair (1996). Evaluation of *Bacillus amyloliquefaciens* B94 for control of *Rhizoctonia* seedling disease on soybeans. (Abstr.) Phytopathology 86(suppl.):S54.

Yu, G. Y.; J. B. Sinclair; G. L. Hartman and B. L. Bertagnolli (2002). Production of iturin A by *Bacillus amyloliquefaciens* suppressing *Rhizoctoria solani* . Soil Biol. Biochem. 34:955–963.

Yu J. Q. (1999). Allelopathic suppression of *Pseudomonas solanacearum* infection of tomato (*Lycopersicon esculentum*) in tomato-chinese chive (*Allium tuberosum*) intercropping system. Journal of Chemical Ecology, 25: 2409-2417.

Zuber, P.; M. M. Nakano and M.A. Marahiel (1993). Peptide antibiotics of Bacilli and other Gram-positive Bacteria. Pp. 897-916, In: *Bacillus subtilis* and other Gram-positive Bacteria: Physiology, Biochemistry and Molecular Biology. A. L. Sonenshein, R. Losick, and J. A. Hoch (eds.) American Society for Microbiology, Washington D.C.

انتقاء عوامل المكافحة الحيوية لمكافحة مرض العفن الأبيض في البصل

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تم إختيار ٢١ عزلة منعت تكون الأجسام الحجرية للفطر *Sclerotium cepivorum* من بين ٣٣١ عزله بكتيرية عزلت من على سطح جذور نباتات بصل سليمة بعد ٧٠ و١٢٠ يوم من الشتل جمعت من اربع محافظات هى البحيرة ،الشرقية، بنى سويف، المنيا. كانت هالة التثبيط للنمو الميسليومي للفطر *S. cepivorum* ١٣، ٩، ٧، ٤ و ٢ مليمتر لكل من العزلات *Pseudomonas flourescens* ، 10D14 *Bacillus licheniformis* ،6D11 *Bacillus licheniformis* ،7D12 و *Chaetomium globosum* علي الترتيب حتي ١٤ يوم من التحضين. أدت معاملة سطح بيئة الزراعة بممثل العزلات منفردة بعد زراعة البذور مباشرة إلى زيادة النسبة المئوية لإنبات بذور البصل وذلك فى معظم العزلات البكتيرية. وهذه الزيادة بلغت ١٩,٥ % بالنسبة للعزلتين *B. licheniformis* 6D11 و *B. amyloliquefaciens* 1D9 . تم اختبار عوامل المكافحة الحيوية المبشرة والمحفزة لإنبات بذور البصل ونموه في الصوبة لدراسة قدرتها علي مكافحة العفن الأبيض في البصل فى بيئات الزراعة المحتوية على ٥٠ أو ١٠٠ جسم حجري/ كجم من بيئة الزراعة. أحدثت المعاملة بكل من *P. flourescens*، *T. harzianum* ، *B. licheniformis* 6D11، *B. amyloliquefaciens* 1D9 ، *C. globosum* أو *Paenibacillus polymyxa* 9D14 افضل اختزال للنباتات الميتة مقارنة بالغير معامل. كما كانت هناك زيادة معنوية في طول النباتات من خلال المعاملة بكل من *P. flourescens*، *B. licheniformis* 6D11، C. *globosum* ، *B. amyloliquefaciens* 1D9 ، *T. harzianum* و *Paenibacillus polymyxa* 9D14 فى بيئات الزراعة المحتوية على ٥٠ أو ١٠٠ جسم حجري/ كجم .

و بالنسبة للدراسة الحقلية كانت جميع المعاملات ذات كفاءة عالية في خفض النسبة المئوية لنباتات الميتة بسبب الإصابة بالعفن الأبيض وذلك حتي نهاية موسم النمو (أربعة أشهر ونصف من الشتل) و كانت العزلات *P. flourescens* ، *Bacillus licheniformis* 10D14 ، *B. amyloliquefaciens* 1D9 و *T. harzianum* أكثر العزلات كفاءة في مكافحة المرض في الحقل، كما أدت المعاملات إلى زيادة عالية في محصول الأبصال مع جميع العزلات المختبرة وبخاصة البكتيرية منها.