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SUPPRESSION OF BACTERIAL WILT DISEASE OF POTATO, USING BIOCONTROL AGENTS AND COMPOST

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ABSTRACT: ***Ralstonia solanacearum (Yabucchi) Race 3 (biovar. II) is the causal organism of potato bacterial wilt disease (brown rot) in Egypt. The obtained isolate was pathogenic to both tomato and potato plants. Bacillus subtilis , Pseudomonas fluorescens , Streptomyces griseus and Trichoderma harzianum were in association with symptomless potato plant roots and tubers, grown in naturally heavily infested fields. Under laboratory conditions ; inhibition zones were recorded between the biocontrol agents and Ralstonia solanacearum. Under greenhouse conditions and artificial soil infestation, the application of compost and /or the biocontrol agents significantly reduced incidence potato wilt disease and rotten tubers. These treatments improved significantly potato vegetative growth, increased the average number of tubers / plants, fresh and dry weight of tubers plant and total carbohydrate .***

Key Words: ***Ralstonia solanacearum , Potato bacterial wilt (brown – rot), biological control, Bacillus subtilis, Pseudomonas fluorescens, Streptomyces griseus , Trichoderma harzinaum and Soil amendment.***

INTRODUCTION

Potato brown rot disease caused by *Ralstonia solanacearum* (Yabucchi) is one of the limiting factors affecting production and exportation of this crop, (Abd El-Ghaffar *et al*., 1995 and Gabr and Saleh 1997). Race 3 (biovar . II) which called " Potato race " is the only strain of *R.solanaceaum* recorded in Egypt (Schaad, 1988). Serious outbreak of the disease have been reported in Europe (Grousset *et al*. 1998).

Maggie Assan *et al*. (2003) and Balabel *et al*. (2005) indicated that the bacterial isolates obtained from Spunta and Nicola tuber cultivars were pathogenic to tomato seedlings. Farag *et al*,. (2004) and Gerges *et al*. (2006) reported that the most pathogenic isolates of *R. solanacearum* were recovered from potato tubers, while those of weeds, soil, water and potato stem were moderately pathogenic. Mahmoud and Gomah (2007) demonstrated that potato cultivars, currently cultivated in Egypt, i.e., Desiree, Spunta, Valor, the cv. Nicola and Dimant are susceptible to the disease. the

cv. Nicola. Is was reported as less susceptible, to brown –rot disease , under the Egyptian conditions.

Biological control of plant diseases is considered a successful and environmental acceptable method. *Bacillus subtilis* has been showed to be effective biocontrol agent to *R. solanacearum* and other pathogens, as reported by Phae *et al*., (1992), Silveria *et al*., (1995), Hassanein (1997) and Abd El-Sayed *et al*., (2003). The bioeffect of *Pseudomonas fluorescens* against potato bacterial wilt was recoded by Abd El-Ghaffar *et al*., (1995), Hassanein (1997) and Abd El-Sayed *et al*., (2003). The bioeffect of *Pseudomonas fluorescens* against potato bacterial wilt pathogen was recoded by Abd El-Ghaffar *et al*., (1995), Ciampi *et al*., (1997)., Tolba (1998) , Abd El-Sayed *et al*., (2003) , Rodrigo –Costa *et al*., (2006) and Virginia and Stockwell (2007) .

Certion strains of *Streptomyces* spp were found to be effective antagonistis to many soil borne pathogens by (Kun *et al*., 2002, Andrew 2004, Javad *et al*., 2006 and Minguo-Wan *et al*., 2008. *Trichoderma harzianum* is an important antagonist to the fugal and bacterial pathogens as reported by Khalifa 1997, Amer and El-Shennawy 2007, Khalifa 2008 and Monchan 2008).

Such biocontrol agents are soil inhabitants but need organic matter to increase their populations in the soil (Ammar, 2003) . Therefore, application of compost to the soil decreases various diseases beside it's fertilization role. This was indicated by Widmer and Graham (1998), Dissanayake and Hoy (1999), Matthew *et al*., (2001), Abbasi *et al*., (2002) , Diab *et al*., (2003) Zhou and Everts (2004) and Ochiai and Powelson (2007).

The aim of this work was to evaluate the role of some biocontrol agents alone or in combination with compost for controlling bacterial wilt disease of potato, under artificial soil infestation in greenhouse.

MATERIALS AND METHODS

This work was conducted in greenhouses at Faculty of Agriculture, Minufiya University and Sakha Agricultural Research Station , Kafr El-Sheikh, in 2006 and 2007 growing seasons.

I-Isolation of the *R.solanacearum*:

Diseased samples of potato tubers (*Solanum tubersum* L.)which showed typical symptoms of brown –rot were collected from Gharbiya, Minufiya , Beheira, Dakhliya and Kafr El-Sheikh governorates to isolate the causal organism. Isolation was carried out from cvs. Nicola and Spunta where tubers were surface sterilized by 75% ethanol for min. and rinsed twice in sterilized distilled water. The tubers were individually cut across and a loopful of bacterial oozes exuding from vascular rings was suspended in 5 ml. sterilized distilled water. A loopful of the resulted suspension was streaked on plates of glycerol agar medium (GAM) contained 0.5 , 2.3 and 5%

tetrozolium triphenyl chloride (TTC)and incubated at 30◦ C for 48-72h. Single distinct colonies were selected and stored separately on slants of NGA or into 5 ml. sterile water at room temperature. Routine checks were made at 15 and 90 days intervals in for all bacterial slants and water suspensions , respectively, by streaking them on TTC medium to determine the stability of virulent types. Eight isolates from each cultivar were identified and tested for their pathogenicity. Avirulent isolates of *R. solanacearum* were distigushed by growing virulent strains on GAM and streaking a loopful of old bacterial growth on TTC for 48-72 h at 30◦ C where individual small red butyrous colonies typical of the avirulent variants were observed .

II-Isolation of the biocontrol agents microorganisms :

Healthy roots and tubers were collected from potato plants , grown in naturally infested fields, at the abovementioned five governorates. The samples were individually cut into small pieces and one gram of each sample was added to 99 ml sterilized distilled water in sterilized bottles (600 ml. vol.). The bottles were shaked for 2 hrs on electric shaker and isolation of rhizoplane microogranisms was carried out according to the methods of Abd El-Moity (1976). One ml of each suspension was added to 99 ml of sterile water, and repeated to obtain different dilutions(10^{-2} – 10^{-6}). Peptone dextrose agar medium plus rose Bengal and streptomycin was used to isolate fungi at the dilution of 1 : 10.000; glycerol agar medium for *Bacillus* spp. (the same dilution), King's Bagar medium (KBA) for *Pseudomonas* spp. (1 : 100.000 dilution) and Jensen's agar medium for actinomycetes (*Streptomyces* spp.) at the dilution of 1 : 1000.1000. Dilute plate method was used for purification and obtaining separate colonies of the isolated microorganisms. Different isolates were selected, especially those belong to *Bacillus* spp., *P. fluorescens*, *Streptomyces* spp. and *Trichoderma* spp.

III-Identification of bacterial and fungal isolates:

The isolated bacteria were identified according to their cultural, morphological and physiological characteristics (Bergey's Manual of Determinative Bacteriology, Holt *et al*., 1994). Actinomycets were identified according to Waksman and Henrici (1943) whereas *Trichoderma* spp., isolates were identified according to Rifai (1969).

IV-Pathogenicity tests :

Pathogenicity tests of the causal organism were carried out , both for the phathogenic isolates and biocontrol agent ones, to make sure that the later are not pathogenic. Clay pots (30 cm in diameter) were sterilized by 5% formalin solution for 5 minutes and left for a week in open air to get rid of formalin. Clay loam soil was autoclaved for 3hr at three consecutive days. Sterilized soil was separately infested with each isolate at the rate of 2.5% of

soil weight (8kg/pot). Inocula were prepared by growing each bacterial isolates , individually for 48 h on NB medium while actinomycete and fungal isolates were grown for 7 days , respectively on Jensen and PDA media. Healthy , equal size and surface sterilized potato tubers (Spunta and Nicola cvs.) were planted (one tuber / pot) and three pots were used as replicates for each treatment. Three untreated pots were used as control for each tested cultivar. One month later , the plants were uprooted and examined for disease incidence. In the meantime, tomato seedlings (*Lycopersicon esculentum* Mill., Super –Marmoud cv.) were planted in sterilized potted soil. Six weeks later , the plants were inoculated by stem puncture technique using suspension of 48 h old bacterial growth (10^6 CFU/ml). Three weeks later ; tomato plants were examined for bacterial with incidence.

V-Biological control studies:

A-Laboratory tests :

Isolates of *Bacillus*, *Pseudomonas*, sp., *Streptomyces* sp, and *Trichoderma* sp., were tested for their antagonistic potentials against the virulent isolates of *Ralstonia solanacearum*. The control agent isolates were separately grown on, glycerol agar for *Bacillus* spp., King's B for *Pseudomonas* spp., Jensen's agar for Streptomyces spp. And Peptone dextrose agar for *Trichoderma* spp. The virulent isolates of *R.solanacearum* were grown on Nutrient glucose broth (NG) medium for 48h . Ten ml of *R. solanacearum* cell suspension were added to 90 ml of NGA sterilized medium, mixed thoroughly and poured into sterilized Petri dishes (10 ml/dish). After solidification, a loopful of each antagonist or a filter paper disk (5mm in diameter) saturated with the biocontrol agent filtrate was placed in the center of earth Petri-dish. Plates with 10 ml of *R.solanacearum* were grow only as control. Five plates of each treatment were used as replicates and all of them were incubated at 30◦C. After 3 days, the plates were examined and the resulted inhibition zones were estimated.

B-Greenhouse experiment:

The biocontrol agents isolates, alone or in combination with compost, were tested for their efficiency in controlling the bacterial wilt disease of potato, under greenhouse conditions, using the technique of Michel and Mew (1998). Sterilized pots and soil, as mentioned in pathogencity test experiment, were infested with *R.solanacearum* and / or any of the tested biocontrol agents at the rate of 200 ml of 10^8-10^{-9} CFU/ml. All tested microorganisms were grown on the suitable media for the required time, as mentioned before to obtain inocula. Compost was added and incorporated at the rate of 15 gm /pot. Three pots were used for each particular treatment and three untreated ones were used as control. Spunta and Nicola potato

cultivars were sown (one tuber / pot) after surface sterilization and sprouting stimulation. Fertilization with NPK was added once as the recommended doses.

Percentage of wilted leaves, were recorded after 70, 85 and 100 days after sowing, according to the formula;

$$\text{Wilt severity : WS} = \frac{\text{No. of wilted leaves / plant}}{\text{Total No. of leaves /plant}} \times 100$$

Meanwhile , average shoot length and number of branches fresh and dry weight of shoot / plant, were recorded after 100 days from sowing. At harvest, average number of tubers / plant, percentage of brown rot disease/plant tuber and total carbohydrates % were recorded . Average fresh and dry weight of a tuber / plant and tubers yield / plant.

All The obtained data were statistically analyzed according to Sndecor and Cochran (1967) as a complete block design experiment.

Carbohydrate content was determined according to the method described by Dubois *et al*., (1956).

RESULTS AND DISCUSSION

Isolates of *R.solanacearum*:

Twenty isolates of *R.solanacearum* were isolated from disease potato tubers (cvs. Nicola and Spunta), collected from the five governorates, i.e. Gharbiya, Minufiya, Dakahliya, Beheira and Kafr El-Sheikh in Egypt.

The cultural and morphological characteristics of the obtained isolates were compared to those described by Bergey's Manual of Determinative Bacteriology, (Holt *et al*., 1994 ; Abd El-Ghaffar *et al*., 1995; and Gabr and Saleh , 1997). All the obtained isolates belong to race 3 (biovar , II, is the only strain which was recoded in Egypt, (Schaad, 1988).

I-The biocontrol agents microorganisms:

Fourty isolates of two bacterial genera, an actinomycete and a fungus were isolated from the rhizosphere of healthy potato cvs. Spunta and Nicola grown in naturally infested fields. Ten isolate were identified as *Bacillus subtilis* and other ten were identified as *Pseudomonas fluorescens* according to Bergey's Manual of Determinative Bacteriology, (Holt *et al*., 1994). However, ten isolates were identified as *Streptomyces griseus* according to Waksman and Henrici (1943). The fungal isolates (10) were identified as *Trichoderma harzianum* (Rifai , 1969).

These results indicate the presence of the biocontrol agent (s) in potato rhizosphere may reduce infection with *R.solanacearum* invasion, because these isolates were observed from healthy roots and tubers.

II-Pathogenicity tests:

Eight isolates of *R.solanacearum* were tested for their pathogeicity to potato (c.v. Spunta and Nicola) and tomato seedlings cv.Super Marmoua c.v. According to leaf wilt symptoms, isolates 1 , 2, 4,5,7 and 8 considered as highly pathogenic while isolates 3 and 6 were moderately pathogenic. Results illustrated in Figures (1-3) clear the aggressiveness of isolate No (1) on tomato seedlings (Figure 1) and potato plants (Figures 2 and 3). Bacterial wilt symptoms could be easily noticed on the plants grown in the pots infested with *R.solanacearum* in comparison with healthy ones grown in sterilized soil. The obtained results were in agreement with those recorded by Assan *et al*., (2003), Farag *et al*., (2004), Balabel (2005), Gerges *et al*., (2006) and Mahmoud and Gomah,(2007). who reported that the most pathogenic isolates of *R.solanacearum* recovered from potato tubers and they were pathogenic to both tomato and potato plant. On the other hand , all tested biocontrol agents were not pathogenic to potato plants.

III-Biological control studies:

A-Assay of antagonism, in vitro:

Result presented in Table (1) showed that all tested biocontrol agents were effective in inhibiting the growth of *R.solanacearum* , grown on different media. *Bacillus subtilis* isolates were the most effective bioagent; where the inhibition was 7.3 mm (Gharbiya isolate 2) and 7.0-7.1 mm(Kafr El-Shekh isolates 7 and 8) respectively. The antagonistic effect of *B.subtilis* against *R.solanacearum* was also reported by Phae *et al*., (1992), Silveria *et al*., (1995), Hassanein (1997) and Abd El-Sayed (2003). *Pseudomonas fluorescens* also showed antagonistic effect to *R.solanacearum*, where it resulted inhibition zones ranged from 5.2 to 6.8 mm, respectively with Dakahliya isolate 3 and Kafr El-Sheikh isolate 4. This antagonistic relationship was also recorded by Abd El-Ghaffar *et al*., (1995); Ciampi *et al*., (1997), Tolba (1998) , Abd El-Sayed *et al*., (2003), Rodrigo –Costa *et al*., (2006) and Virginia and Stockwell (2007).

***Streptomyces griseus* showed less efficiency in antagonistic relationship with *R.solanacearum*, where the most observed inhibition zone was 5.6 mm(Dakahliya isolate 7). The antagonistic role of *Streptomyces* spp. against soil borne pathogens was also reported by Kun *et al*., (2002), Andrew (2004), Javad *et al*., (2006) and Minguo *et al*., (2008). *Trichoderma harzianum* (isolate 1), obtained from Gharbiya governorate, was the most effective antagonist to *R.solanacearum* (7.4 mm inhibition zone). Other *T.harzianum* isolates showed satisfactory effects. Such results were recorded also by Khalifa (1997), Amer and El-Shennawy (2007), Khalifa (2008) and Monchan (2008).**

Generally ; the antagonistic interrelationship may take the form of exploitation, antibiosis and competition , (Ammar , 2003).

Figure (1): Effect of *Ralstonia solanacearum* on Tomato plants (pathogenicity test).

Figure (2) : Effect of *Ralstonia solanacearum* on Potato (Spunta cultivar).

Figure (3): Effect of *Ralstonia solanacearum* on Potato (Nicola cultivar).

Table (1): Antagonisitic reaction between biocontrol agents and *R.solanacearum.*

Biocontrol agent	Isolate Code No.	Source	Inhibition zone in mm
Bacillus subtilis	6	Gharbiya	5.6
B. subtilis	2	Gharbiya	7.3
B. subtilis	1	Minufiya	6.2
B. subtilis	5	Minufiya	6.1
B. subtilis	4	Beheira	5.4
B. subtilis	3	Beheira	5.6
B. subtilis	2	Dakahlia	4.0
B. subtilis	1	Dakahlia	6.8
B. subtilis	7	Kafr El-Sheikh	7.0
B. subtilis	8	Kafr El-Sheikh	7.1
Pseudomonas fluorescnes	6	Gharbiya	6.5
P.fluorescnes	7	Gharbiya	6.3
P.fluorescnes	1	Minufiya	5.8
P.fluorescnes	8	Minufiya	5.6
P.fluorescnes	1	Beheira	5.8
P.fluorescnes	5	Beheira	6.0
P.fluorescnes	2	Dakahlia	6.1
P.fluorescnes	3	Dakahlia	5.2
P.fluorescnes	6	Kafr El-Sheikh	6.3
P.fluorescnes	4	Kafr El-Sheikh	6.8
Streptomyces grisues	3	Gharbiya	5.0
St. griseus	2	Gharbiya	4.8
St. griseus	5	Minufiya	4.6
St. griseus	8	Minufiya	5.2
St. griseus	4	Beheira	5.3
St. griseus	6	Beheira	5.0
St. griseus	7	Dakahlia	5.6
St. griseus	3	Dakahlia	5.3
St. griseus	8	Kafr El-Sheikh	4.0
St. griseus	2	Kafr El-Sheikh	4.6
Trichoderma harzianum	1	Gharbiya	7.4
T.harzianum	7	Gharbiya	7.0
T.harzianum	3	Minufiya	6.6
T.harzianum	5	Munufiya	7.1
T.harzianum	8	Beheira	7.0
T.harzianum	6	Beheira	6.5
T.harzianum	7	Dakahlia	6.0
T.harzianum	4	Dakahlia	6.1
T.harzianum	2	Kafr El-Sheikh	5.4
T.harzianum	4	Kafr El-Sheikh	5.8

B-Greenhouse Experiments:

1-Effect of biocontrol agents and compost on bacterial wilt disease incidence:

Results in Table (2) indicate that Spunta cv. was more susceptible to the bacterial wilt disease than cv. Nicola. Data also show that application of either tested biocontrol agent and / or compost significantly reduced wilt disease incidence. After 100 days of tuber planting, the percentage of wilted leaves / plant decreased from 100% non – treated control to 5.2 , 5.7 , 5.2 , 0 and 0 , respectively when *B.subtilis, S.griseus, P.fluorescens, T.harzianum* and compost were individually applied to soil infested with the pathogen *R.solanacearum* and cultivated with Spunta cultivar. These were 3.9 , 4.1 , 3.9, 0 and 0 for Nicola cultivar , in the same respect with biocontrol agents.

On the other hand, couple applications of the tested biocontrol agents showed less efficiency in disease reduction. This could be due to the competition interrelationships between the biocontrol agents. However , application of compost and *T.harzianum* , individually or on combination, to the infested soil weith *R.solanacearum* led to complete disease reduction. Compost can be considered as a good substratum which enhance growth of *T.harzianum* increasing its population in the soil and consequently affect the pathogen (Ammar , 2003).

The results obtained support many other investigations about biological control of *R.solanacearum* and other pathogens. Of those Phae *et al*., (1992), silveria *et al*., (1995), Hassanein (1997) and Abd El-Sayed (2003), who explained the role of *B.subtilis* in disease reduction. The role of P.fluorescens in reducing potato bacterial wilt was reported by Abd El-Ghaffar *et al*., (1995), ciampi *et al*., (1997), Tolba (1998), Abd El-Sayed *et al*., (2003) , Rodrigo –Costa *et al*., (2006) and Virginia and Stockwell (2007). *Streptomyces* spp, are so good antagonists to many soil born pathogens as reported by Kun *et al*., (2002), Andrew (2004), Javad *et al*., (2006) and Minguo-Wan *et al*., (2008) *Trichoderma harzianum* was recorded to be an important biocontrol agent to certain fungal and bacterial diseases by Khalifa (1997), Amer and El Shennawy (2007), Khalifa (2008) and Monchan (2008).

2-Effect of biocontrol agents and compost on certain growth characters of potato :

Data in Table (3) show clearly that both shoot length and number of branches potato plants were significantly increased compared to those of control (blank). Treatment with compost and *T.harzianum* produced the highest values of both Spunta and Nicola cultivars. However, individual application of compost or any biocontrol agents to the soil infested with *R.solancearum* gave higher values of shoot length and number of branches compared with plants grown in soil infested with *R.solancearum*. However , applications of compost and / or biocontrol agents showed less efficiency than single applications.

Table (2): Percentage of wilted leaves of potato plants (Spunta and Nicola cultivars) as affected by different biocontrol agents and compost treatments

Treatment	Percentage of wilted leaves					
	Days after planting					
	70		85		100 days	
	Spunta	Nicola	Spunta	Nicola	Spunta	Nicola
Control (blank)	0.000f**	0.000 h	0.000 h	0.000e	0.000i	0.000i
*R.solancearum**	82.650a	76.930a	95.750a	93.20a	100.00a	100.000a
B.subtilis	0.000 f	0.000 h	0.000 h	0.000e	0.000i	0.000i
S.griseus	0.000 f	0.000 h	0.000 h	0.000e	0.000i	0.000i
P.fuoresence	0.000 f	0.000 h	0.000 h	0.000e	0.000i	0.000i
T.harizanum	0.000 f	0.000 h	0.000 h	0.000e	0.000i	0.000i
Compost	0.000 f	0.000 h	0.000 h	0.000e	0.000i	0.000i
R.solancearum+B.subtilis	2.417e	1.837 f	4.360f	2.903d	5.293h	3.957h
R.solancearum+B.grisues	3.353c	2.210 e	5.390a	3.863d	5.787g	4.147h
R.solancearum+P.fluoresence	2.987 d	1.127 g	4.820g	2.930d	5.290h	3.923h
R.solancearum+T.harizanum	0.000 f	0.000 h	0.000h	0.000e	0.000i	0.000i
R.solancearum.+comp.	0.000 f	0.000 h	0.000h	0.000e	0.000i	0.000i
R.solancearum+S.grisues+P.fluoresence	4.160 b	3.250 c	6.333c	5.300b	10.557d	8.623b
R.solancearum+S.grisues+B.subtilis	4.200 b	3.010 c	7.130b	5.183b	11.277c	8.557bcd
R.solancearum+S.grisues+T.harzianum	3.967 b	2.953 c	6.210c	4.427c	10.207de	8.287bcd
R.solancearum+S.grisues+compost	3.307 c	3.193 c	6.100c	4.343c	10.67e	7.943de
R.solancearum+P.fluoresence+B.subtilis	4.223 b	3.803 b	7.333b	5.437b	11.830b	8.537be
R.solancearum+P.fluoresence+T.harzianum	3.923 b	2.973 c	6.130c	5.437b	10.237de	8.130cde
R.solancearum+P.fluoresence+compost	3.933	2.943	5.940c	4.283c	9.237f	7.780e
R.solancearum+B.subtilis+T.harzianum	3.937 b	2.950 c	6.010c	4.500c	10.013e	8.233bcd
R.solancearum+B.subtilis+compost	3.520 c	2.610d	5.853d	4.220c	9.247f	6.777f
R.solancearum+T.harzianum+compost	3.400 c	0.000 h	5.603d	4.077c	8.850f	5.200 g

*** :*R.solanacearum*: means artificial soil infestation with *R.solanacearum* (diseased control)**

****: There are significant differences between means carrying different litters and vice versa.**

Table (3): Effect of compost application and soil infestation with biocontrol agents and *Ralstonia Solancearum* on shoot length (cm) and number of branches / potato plants (cvs. Spunta and Nicola), under greenhouse conditions

Treatment	Spunta		Nicola	
	Shoot length (cm)	No. of branches	Shoot length (cm)	No. of branches
Control (blank)	47.323**	7.0 ab	30.273 e	8.00abc
*R.solancearum**	30.727 n	3.333 i	21.713 l	3.667 l
B.subtilis	49.030c	7.33 a	32.323c	8.333 ab
S.griseus	48.00 cd	7.0 ab	31.180 de	6.667bcd
P.fluoresence	48.200 cd	7.0 ab	32.013 cd	6.667bcd
T.harizanum	50.717 b	7.33 a	34.083 b	8.333ab
Compost	54.173 a	7.667a	36.217a	8.667a
R.solancearum+B.subtilis	40.770 g	5.667 a	26.863g	6.667efg
R.solancearum+B.grisues	40.603 g	5.667 cde	25.557hi	6.333fgh
R.solancearum+P.fluoresence	43.197 f	5.333 def	26.487gh	6.667efg
R.solancearum+T.harizanum	43.187 f	6.00 cd	26.913g	7.000def
R.solancearum.+comp.	46.620 e	6.00 cd	28.833f	7.333cde
R.solancearum+S.grisues+P.fluoresence	35.853 kl	6.333 bc	23.247jk	4.667k
R.solancearum+S.grisues+B.subtilis	34.900lm	4.00 hi	23.620jk	5.000jk
R.solancearum+S.grisues+T.harzianum	36.490 jk	4.333 gh	24.500ij	5.333ijk
R.solancearum+S.grisues+compost	38.313h	4.667 fgh	24.153jk	5.667hj
R.solancearum+P.fluoresence+B.subtilis	33.957m	5.00 efg	23.107k	4.667k
R.solancearum+P.fluoresence+T.harzianum	37.110 ij	4.333 gh	23.250jk	4.667k
R.solancearum+P.fluoresence+compost	36.753 jk	4.667 fgh	24.083 jk	5.333ijk
R.solancearum+B.subtilis+T.harzianum	37.177hij	4.667 fgh	23.653 jk	5.667hij
R.solancearum+B.subtilis+compost	38.137 hi	5.00 efg	24.060 jk	5.667hij
R.solancearum+T.harzianum+compost	37.517hij	5.333 def	24.103jk	6.000ghi

*** :*R.solanacearum*: means artificial soil infestation with *R.solanacearum* (diseased control)**

****: There are significant differences between means carrying different litters and vice versa.**

Results presented in Table (4) showed clearly that fresh and dry weight of shoot / potato plant were significantly increased than control when any of the biocontrol agents and /or compost were applied, either individually or in combinations. The best values, however, were obtained when compost and /or *T.harzianum* were applied individually, both to the sterilized soil or that infested with *R.solancearum*.

The obtained results demonstrated that the vegetative growth of Spunta and Nicola potato cultivars were improved significantly by compost application and / or the biocontrol agents soil infestation, However , this could be attributed to the fertilization role of compost and antagonistic effects of these biocontrol agents.

3-Effects on yield components :

Data shown in Tables (5 and 6) indicate that all investigated yield components of potato tubers were significantly higher than the diseased control ones , in response to compost and / or any tested biocontrol agent applications. The average number of tubers /plant , average weight of a tuber /plant , average fresh and dry weight of the tubers/plant and total carbohydrates were all significantly increased. The obtained results indicate that compost and *T.harzianum* individually or in combination with *R.solanacearum* resulted higher number of tubers/plant than other treatments . Meantime, the percentage of diseased tubers with brown rot was reduced by application of any tested treatment to the soil, previsouly infested with *R.solanacearum*. The best results were observed when either compost or *T.harzianum* was applied. .

The results indicated that compost and / or the biocontrol agents improved both quantity and quality of potato yield and reduced brown rot disease incidence. This could be due to the fertilizer role of compost, in side, as reported by Widmer and Graham (1998), Dissanayake and Hoy (1999), Matthew *et al*., (2001), Abbasi *et al*., (2001), Ammar (2003), Diab *et al*., (2003) Zhou and Everts (2004) and Ochiai and Powelson (2007).

On the other hand , the antagonisitic role of the tested biocontrol agents was also reported by Kun *et al*., (2002), Abd El-Sayed (2003), Andrew (2004), Gray (2006) , Javad *et al*., (2006), Rodrigo –Costa (2006), Amer and El-Shennawy(2007), Virginia and Stackwell (2007), Khalifa (2008) and Minguo *et al*., (2008).

Table (4): Effect of compost application and soil infestation with biocontrol agents and *Ralstonia Salancearum* on fresh and dry weight of shoot/potato plant of (cvs. Spunta and Nicola), under green house conditions

Treatment	Spunta		Nicola	
	Fresh weight (g)	Dry Weight(g)	Fresh Weight(g)	Dry Weight(g)
Control (blank)	64.620e**	13.307c	62.237c	12.460b
*R.solancearum**	39.887o	7.740 k	38.197m	7.010k
B.subtilis	65.633c	14.493b	62.137c	11.993b
S.griseus	63.803 d	13.680c	62.483c	11.177c
P.fuoresence	65.500 c	14.357b	64.410b	12.047b
T.harizanum	69.130 b	14.613b	64.603b	12.310b
Compost	77.233 a	16.377a	67.937a	15.500 a
R.solancearum+B.subtilis	55.170 g	11.183e	54.900f	9.397 e
R.solancearum+B.grisues	54.237 g	10.220f	51.500g	9.010ef
R.solancearum+P.fluoresence	55.113 g	10.357f	55.500f	10.123d
R.solancearum+T.harizanum	59.842 f	12.557f	58.617e	10.440o
R.solancearum.+comp.	62.490d	13.59c	60.127d	10.420d
R.solancearum+S.grisues+P.fluoresence	42.813 n	8.850hi	43.713k	8.130ghi
R.solancearum+S.grisues+B.subtilis	40.660o	8.913hi	41.140l	8.153ghi
R.solancearum+S.grisues+T.harzianum	43.237n	8.813hi	44.280k	8.747f
R.solancearum+S.grisues+compost	46.997l	10.237f	45.963j	9.000hij
R.solancearum+P.fluoresence+B.subtilis	42.350n	8.517ij	43.820k	7.373ij
R.solancearum+P.fluoresence+T.harzianum	44.873m	8.760ij	44.673k	8.000 hij
R.solancearum+P.fluoresence+compost	48.030k	9.377gh	47.957j	8.803f
R.solancearum+B.subtilis+T.harzianum	44.560j	8.210jk	48.48j	7.530jk
R.solancearum+B.subtilis+compost	52.820h	10.087f	48.990j	8.663 fg
R.solancearum+T.harzianum+compost	51.067 i	9.870fg	50.220h	8.500fgh

*** :*R.solanacearum*: means artificial soil infestation with *R.solanacearum* (diseased control)**

****: There are significant differences between means carrying different litters and vice versa.**

Table 5

Table 6

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تثبيط الذبول البكتيرى فى البطاطس
باستخدام الكائنات الدقيقة المضادة والكومبوست

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الملخص العربى

أجريت تجارب هذه الدراسة بكلية الزراعة ، جامعة المنوفية ومحطة البحوث الزراعية بسخا، كفر الشيخ ،خلال عامى ٢٠٠٦ ، ٢٠٠٧ فى العروة الشتوية . تم إجراء عزل الميكروب المسبب لمرض العفن البنى فى البطاطس وكذلك الكائنات الحية الدقيقة الآخرى من عينات مأخوذة من محافظات الغربية ، المنوفية ، الدقهلية ، البحيرة ، كفر الشيخ ، من نباتات البطاطس السليمة والمصابة (صنفى سبونتا ونيكولا).

أثبتت الدراسة أن ميكروب ***Ralstonia salanacearum*** – السلالة ٣ هو المسبب لمرض الذبول البكتيرى (العفن البنى) للبطاطس فى مصر ، كما أثبتت أن العزلات المتحصل عليها كانت ذات قدرة إمراضية متباينة لنباتات البطاطس والطماطم وكان صنف البطاطس سبونتا أكثر قابلية للإصابة بالمرض من الصنف نيكولا ، كما أثبت العزل مصاحبة ميكروبات ***Bcaillus subtilis , Pseudomonas fluorescens , Streptomyces griseus and Trichoderma harziamum)*** لجذور ودرنات نباتات البطاطس السليمة المأخوذة من حقول ملوثة بشدة بالميكروب الممرض. وتحت ظروف المعمل ، أثبتت هذه العزلات مقدرتها التضادية لميكروب ***Ralstonia solanacearum*** . وتحت ظروف الصوبة والعدوى الصناعية وقد وجد أن إضافة الكومبوست أو أى من كائنات التضاد الحيوى السابقة تؤدى إلى نقص معنوى فى نسبة حدوث مرض الذبول البكتيرى لنباتات البطاطس وكذلك العفن البنى فى الدرنات. كما أدت المعاملات إلى زيادة معنوية

فى ارتفاع النباتات ، الوزن الغض والجاف للمجموع الخضرى وكذلك عدد الأفرع المتكونة لكل نبات. وسجلت أيضاً زيادة معنوية فى متوسط عدد الدرنات التى ينتجها النبات ، الوزن الغض والجاف للدرنة الواحدة وكذلك محتواها من الكربوهيدرات الكلية.

ومن ثم ، فإن هذه الدراسة تؤكد أهمية دراسة تطبيق العزلات لمكافحة المرض تحت ظروف الحقل.

Table (5) : Effect of compost application and soil infestation with biocontrol agents on tuber number , percentage of diseased tubers / plant and total charohydrates of tuber % of Spunta and Nicola cvs. Grown in soil infested with *R.Solancearum* under greenhouse conditions

Treatments	Spunta			Nicola		
	No. of tuber/ plant	%Diseased tubers /plant	%total charbohydrates	No. of tuber/plant	%Diseased tubers /plant	%total charbohydrates
Control (blank)	4.0 bc	0.00 f	81.50 bcd	7.66 cf	0.00 b	80.87 bed
*R.solancearum**	1.3 i	100.00 a	77.80 l	5.0 j	100.0 a	76.30 j
B.subtilis	4.3 abc	0.0 f	81.66 bcd	8.0 cde	0.0 b	81.49 b
S.griseus	4.3 abc	0.0 f	81.147 cde	8.0 cde	00. b	81.47 b
P.fluoresence	4.6 ab	0.0 f	81.653 bcd	8.33 bcd	00. b	82.64 a
T.harizanum	5.0 b	0.0 f	82.13 b	8.667 bc	00. b	82.78 a
Compost	5.3 a	0.0 f	8397 a	10.33 a	00. b	83.63 a
R.solancearum+B.subtilis	3.6 c	6.66 ef	81.107 cde	7.66 c	8.33 b	80.53 a
R.solancearum+B.grisues	3.3 def	6.16 def	80.63 d	7.33 dg	8.927 b	80.53 cd
R.solancearum+P.fluoresence	3.6 c	16.66 def	80.88 def	8.0 cde	3.700 b	80.77 bcd
R.solancearum+T.harizanum	3.6 c	0.0 f	81.513 bcd	8.66 bc	0.0 b	80.40 bc
R.solancearum.+comp.	4.00 bc	0.0 f	82.65 b	9.33 ab	0.0 b	80.450 bc
R.solancearum+S.grisues+P.fluoresence	1.66 hi	33.33 bcd	79.17 h	7.0 ef	23.8 b	81.127 bc
R.solancearum+S.grisues+B.subtilis	1.66 hi	33.33 bcd	79.15 h	6.66 fgh	19.83 b	78.50 hi
R.solancearum+S.grisues+T.harzianum	2.66 fgh	27.27 bc	79.81 fgh	7.0 eh	18.64 b	79.15 fi
R.solancearum+S.grisues+compost	3.0 efg	22.226 f	80.23 eh	7.33 d	18.45 b	79.54 be
R.solancearum+P.fluoresence+B.subtilis	2.0 ghi	38.88 bcd	79.19 h	5.33 id	17.77 b	78.19 i
R.solancearum+P.fluoresence+T.harzianum	3.0 efg	44.44 b	80.16 de	6.0 hid	22.217 b	78.56 hi
R.solancearum+P.fluoresence+compost	3.0 efg	36.11 bcd	80.29 de	6.33 ghi	21.42 b	79.23 fi
R.solancearum+B.subtilis+T.harzianum	3.33 def	41.66 b	79.59 gh	6.33 ghi	15.87 b	78.81 ghi
R.solancearum+B.subtilis+compost	3.33 def	19.44 cd	80.13 de	6.66fgh	15.07 b	80.153 cd
R.solancearum+T.harzianum+compost	3.33 def	27.77 bc	80.24 de	7.0 ef	14.28 b	80.417 be

*** :*R.solanacearum*: means artificial soil infestation with R.solanacearum (diseased control)**

****: There are significant differences between means carrying different litters and vice versa.**

Table (6): Effect of compost application and soil infestation with biocontrol agents on fresh and dry of average and yield / plant of Spunta and Nicola , grown in soil infested with *Ralstonia Solancearum* under green house conditions

Treatments	Spunta				Nicola			
	Av.tuber weight/plant(g)		Yield/plant		Av.tuber weight/plant(g)		Yield/plant	
	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Drgy
Control (blank)	64.9 ab	10.3 b	213.0 cde	34.5def	34.4 abc	5.1 bc	42.1cd	42.1 cd
*R.solancearum**	32.0 j	5.0 h	410.0 g	6.7 k	21.4 f	3.4 l	17.2g	17.2 g
B.subtilis	61.2 bc	10.5 b	253.7 bc	39.7 bcd	32.0 bcd	5.0 cde	47.1 c	37.5 b
S.griseus	65.5 cd	9.4 c	213.7 cde	35.3 cf	33.8 abc	4.9 cf	40.1 d	38.0 d
P.fuoresence	59.8 bc	9.0 c	220.9 cde	33.1 def	33.7 abc	5.1 bc	53.5 b	38.4 d
T.harizanum	64.6 a	9.6 c	277.8 b	44.1 b	33.6 abc	5.7 ab	55.9 b	53.5 b
Compost	67.6 a	11.3 a	338.3 a	250.5 a	38.9 a	6.2 a	66.9 a	66.9 a
R.solancearum+B.subtilis	56.8 a	8.0 d	226.888 bcd	30.5 g	31.7 cd	4.7 cg	34.5 d	40.1 d
R.solancearum+B.grisues	53.8	8.1 d	196.9 def	31.9 ef	31.9 bcd	4.4 dh	37.8 d	37.8 b
R.solancearum+P.fluoresence	53.4 de	8.0 d	208.1 cde	36.0 cde	32.9 bc	5.0 bcd	37.8 d	37.8 d
R.solancearum+T.harizanum	58.5 cd	8.2 d	235.4 bcd	36.0 cde	33.7 abc	5.1 ab	38.0 d	47.1 c
R.solancearum.+comp.	64.3 ab	9.1 c	259.222 bc	40.9 bc	37.4 ab	6.1 ab	38.7 d	55.9 b
R.solancearum+S.grisues+P.fluoresence	47.2 fg	6.9 f	78.6 ig	50.9 a	26.0 ef	4.2 fgh	29.2 f	29.0 f
R.solancearum+S.grisues+B.subtilis	44.4 gh	6.4 g	74.8 ig	18.4 id	26.7 de	3.8 hi	26.2 f	26.9 f
R.solancearum+S.grisues+T.harzianum	50.7 ef	4.2 ef	135.3 gh	16.8 g	30.5 cde	4.1 gh	31.1 ef	31.1 f
R.solancearum+S.grisues+compost	47.2 gh	4.7 de	141.8 gh	20.2 hid	31.2 cde	4.3 dh	30.5 ef	36.1 de
R.solancearum+P.fluoresence+B.subtilis	4.5 hi	6.4 ge	81.3 ig	24.8 gh	29.5 cde	3.9 hi	28.1 f	28.1 f
R.solancearum+P.fluoresence+T.harzianum	38.2 i	6.3 g	102.2 hi	14.5 ga	29.3 cde	4.3 eh	30.4 ef	30.4 ef
R.solancearum+P.fluoresence+compost	50.2 ef	7.2 ef	148.777 fgh	18.3 id	31.0 cde	4.0 hi	30.7 ef	30.7 ef
R.solancearum+B.subtilis+T.harzianum	40.6 hi	6.6 fg	134.8 gh	24.8 gh	30.9 cde	4.2 gh	29.4 f	29.4 f
R.solancearum+B.subtilis+compost	50.7 ef	7.9 d	168.8 efg	22.8hi	31.4 cde	4.1 gh	31.4 ef	31.4 ef
R.solancearum+T.harzianum+compost	51.2 ef	7.2 ef	170.4 efg	31.7 of	31.1 cde	4.3 eh	36.1 de	30.5 ef

:R.solanacearum: means artificial soil infestation with R.solanacearum (diseased control)

****: There are significant differences between means carrying different litters and vice versa.**