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BIOLOGICAL CONTROL OF POTATO BACTERIAL WILT DISEASE UNDER EGYPTIAN CONDITIONS

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ABSTRACT

The biological control of potato bacterial wilt disease is caused by *Ralstonia solanacearum* (Smith) Yabunchi *et al.* race 3, biovar II has been investigated under Egyptian conditions. The present work was planned to control the disease biologically using three isolates of *Pseudomonas fluorescens* (pf), three isolates of *Streptomyces* spp. and one isolate of *Bacillus subtilis* (Bs). All bioagents tested inhibited growth of *R. solanacearum, in vitro*. Isolates of *P. fluorescens*, *Streptomyces* spp. and *B. subtilis* were the most inhibitors on King's B (KB), starch nitrate agar (SNA) and nutrient agar (NA) media, respectively. Bioagents populations increased in the rhizosphere potato plants with increasing the period after planting. Meanwhile, populations of *P. fluorescens* and *B. subtilis* were higher than *Streptomyces* spp. population at the end of 80 days from planting. Application of bioagents of a soil drench decreased the population of *R. solanacearum* in potato plants rhizosphere. However, severity of potato bacterial wilt disease was reduced with application of bioagents as tuber treatment or soil drench treatment, as compared with the control. Application of bioagents as soil drench treatment was better than tuber treatment. *Pseudomonas fluorescens* (Pf5) and *Streptomyces griseoviridis* (Sg) isolates were the most effective in the reduction of the disease severity and in increasing disease biocontrol. However, increasing a soil drench for three applications significantly reduced disease severity and thus increased the disease biocontrol comparable to only one application under the same specified Egyptian conditions.

Key words: Potato; Bacterial wilt; Biological control; *Ralstonia solancearum*; Fluorescent Pseudomonads; *Streptomyces* spp.; *Bacillus subtilis*

INTRODUCTION

Potato bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabunch *et al.* is a major soil borne disease in the tropics and subtropics (**Hayward, 1991**). The disease is considered as one of the limiting factors to potato production in Egypt and exportation of potatoes (**Abd El-Ghafar *et al* 1995 and Gabr and**

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Saleh, 1997). It is difficult to control is disease because its pathogen can exist in the soil for a long period **(Hsu, 1977)**.

Biological control strategies may provide alternatives for management of bacterial wilt or they may be integrated with other practices for its practical field management. Several organisms including avirulent strain of *R. solanacearum* **(Lee *et al* 1986, Hsu *et al* 1992, Arwiyanto *et al* 1994, Bora *et al* 2000 and Toyota and Kimura, 2000)**, fluorescent pseudomonads **(Kempe & Sequeira, 1983; Aspira & Cruz, 1986; Hsu *et al* 1992; Shekhawat *et al* 1992; Abd Alla *et al* 1999 and Anith *et al* 2000)**, *Bacillus* spp. **(Aspira & Cruz, 1986; Shekhawat *et al* 1992; Bora *et al* 2000 and Abd Alla *et al* 1999)** and actinomycetes **(Gao *et al* 11983; Shekhawat *et al* 1992; Moura & Romeiro, 2000 and Bora *et al* 2000)** have been tried with variable success for biocontrol of bacterial wilt.

The present work aimed to study the ability of some bioagents to inhibit the growth of *R. solanacearum in vitro* and to investigate their potentiality in suppression of potato bacterial wilt under artificial inoculation conditions.

MATERIAL AND METHODS

Preparation of inoculum and soil infestation

Virulent isolate of *R. solanacearum* previously isolated and identified from infected potato plants **(Abd El-Ghafar *et al* 1995)** was used through this study. The bacterium was grown on sucrose peptone agar (SPA) medium at 28°C for 48h. Bacterial growth was suspended in sterile saline solution (0.85 % NaCl) and optical density adjusted at A600 nm =0.3 to 10^7 colony forming units (CFU)/ml according to **Michel and Mew (1998)**. Soil infestation was carried out by adding 200 ml of the previous bacterial suspension per pot (30 cm^2 diameter) containing sterilized sandy – clay soil (1:1 v:v) .The bacterial inoculum was mixed with the soil thoroughly. These pots were irrigated at intervals.

Seed tubers and sowing

Potato tubers (daimant, CV.) were obtained from International Potato Center (IPC), Kafr El-Zayat, Gharbia governorate. Tubers previously stored at 4°C, were placed in moistened trays at room temperature in the dark for 7 days to stimulate germination. These tubers were planted in pots containing soil infestation by the pathogen. One tuber was sown in each pot. Ten pots were used as replicates for each treatment.

Source of Bioagents and inocula preparation

Three isolates of *P. fluorescens* (Pf3, Pf4 and Pf5) and one isolate of *Bacillus subtilis* (Bs3) were previously isolated and evaluated as biocontrol agents against phytopathogenic bacteria **(Abd El-Ghafar & Abd El-Sayed, 1997; Abd El-Ghafar, 2000 and Abd El-Ghafar & Mosa, 2001)**. Four isolates of actinomycetes namely: *Streptomyces fumigatiscleroticus* (Sf1), *S. violaceusniger* (Sv1), *S. rochei* (Sr1) and *S.griseoviridis* (Sg1) were kindly obtained from Microbial Taxonomy and Physiology Laboratory, Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt. *Pseudomonas fluorescens* and *B. subtilis* isolates were

grown on Tryptic soy agar (TSA) medium for 48 h. at 28°C. Meantime, actinomycetes isolates were grown on starch nitrates agar (SNA) medium for seven days at 28°C. All bioagents were suspended in sterile distilled water (SDW) and centrifuged at 3000 g for 30 min. The precipitant was resuspended in SDW to reach the concentration of 10^8 CFU/ml as determined from a standard curve based on absorbance at 620nm in case of soil drench treatment. Meanwhile, in case of tuber treatment, bioagents suspensions were resuspended in equal volume of 1 % methylcellulose supplemented with 0.1 M $MgSO_4$ to reach the same pervious concentration.

Assay of antagonism

All previously mentioned bioagents were tested *in vitro* for their ability to inhibit the growth of *R. solanacearum* on Nutrient agar (NA), King's B (KB) and starch nitrate agar (SNA) media. Isolates of *Pseudomonas fluorescens* and *Bacillus subtilis* (24 h. –old growth) and actinomycetes isolates (7 -day old growth) were streaked at the center of plates containing the previous media and then incubation at 28°C. After 48 h., the pathogen (24 –h. old growth) was streaked vertically on the bioagent and incubated at 28°C. inhibition length was measured after 5 days from the second inoculation.

Population of bioagents and *R. solanacearum* in potato rhizosphere soil

For each treatment, ten grams of soil were taken at 0, 20, 40, 60, and 80 days after planting. Each sample was placed into flask (250 ml) containing 90 ml of SDW and shaken for 30 min. using horizontal shaker. Serial dilutions were prepared and one ml of 10^5 dilution was placed on NA, KB, SNA and tetra zolium agar (TZA) media to detected *Bacillus subtilis*, *P. fluorescens*, *Streptomyces* spp. and *R. solanacearum*, respectively. Colonies of bioagents and *R. solanacearum* were estimated after 3 days of incubation at 28°C and proportioned to one gram of soil. Five plates were used as replicates for each treatment.

Disease assessment

Disease severity was assessed after 90 days from planting. Severity of potato bacterial wilt was calculated as percentage of sprouts showing wilt symptoms per treatment and as disease index (%) using the following scale: 0= no symptoms 1=1- 10% of the foliage wilted, 2=11-30% of the foliage wilted, 3=31-60% of the foliage wilted, 4=more than 60 % but less than 100 % of the foliage wilted and 5= all leaves wilted. Disease index (DI) was calculated by the following formula:

$$DI = \frac{\Sigma R.T}{5XN} \; X\,100$$

Where T = Number of plants with disease severity scale; R (R= 1,2,3,4, and 5);

N = Total number of plants inoculated.

Percentage of disease control (PDC) was calculated as follows:

$$PDC = \frac{(DI_{ck} - DI_{tr})}{DI_{ck}} \; X\,100$$

Where, DI_{ck} = Disease index in check
DI_{tr} = disease index in treatment.

Data were statistically analyzed using the (F) test and the value of LSD ($P < 0.05$) was calculated.

RESULTS

Effect of different bioagents on growth of *R. solanacearum in vitro*

Data in Fig. (1) show that all bioagents isolates tested were effective in inhibiting the growth of *R. solanacearum* on both nutrient agar (NA), King's B (KB) and starch nitrate agar (SNA) media, compared with the control. *Streptomyces* spp. isolates were the most effective in inhibiting the growth of *R. solanacearum* on SNA medium, where inhibition length between ranged 8-9.9 mm corresponding to 3-3.9 and 1.8-2.2 mm, in case of NA and KB media respectively. Meanwhile, *P. fluorescens* isolates were the most effective on KB medium, where inhibition length ranged between corresponding 9.6-9.9 mm were least effective, where inhibition length ran ged to 3.8-3.9 and 1.2-1.3 mm on NA and SNA media respectively. On the other hand, *B. subtilis* isolate was the most effective on NA medium, where inhibition length was 9.1 mm corresponding to and on KB and SNA media 4.4 and 1.2 mm, respectively.

Population of different bioagents and *R. solancearum* in potato rhizosphere soil

Population of different bioagents increased in the rhizosphere potato with increasing the period after planting. Meantime, population of *P. fluorescens* and *B. subtilis* were higher than *Streptomyces* spp. population at the end of incubation period. But in the first phase of inoculation, no significant differences among different bioagents were detected. After 80 days, there were significant differences among *Streptomyces* spp and *P. fluorescens* or *B. subtilis* (Fig. 2).

Application of different bioagents as soil drench for potato plants led to a decrease in the population of *R. solanacearum* in potato rhizosphere soil, compared with the control. Population of *R solanacearum* was less effective in potato rhizosphere soil with increasing the period after plating. No significant differences among all bioagents isolates were observed on population of *R. solanacearum* (Fig. 2).

Influence of bioagents on disease severity of artificially inoculated plants

(A) Method of treatment

Results in Table (1) show that application of different bioagents as tuber and soil drench treatments reduced severity of potato bacterial wilt disease and to increase disease control, compared with the control. However, soil drench treatment was better than tuber treatment, where percentage of disease control ranged between 20.6-39.2 % and 10.3-23.5%. respectively. In case of soil drench treatment, *P. fluorescens* (pf3, pf4 and pf5) and *S. griseoviridis* (Sg1) the most effective reduction in the disease was recorded, where percentage of disease control was 32.4- 35.6 and 39.2 % respectively. Meantime, isolates of *S. violaceasniger* (Sv1), *B. subtilis* (Bs2) and *S. fumigatiscleroticus* (Sf1) were less

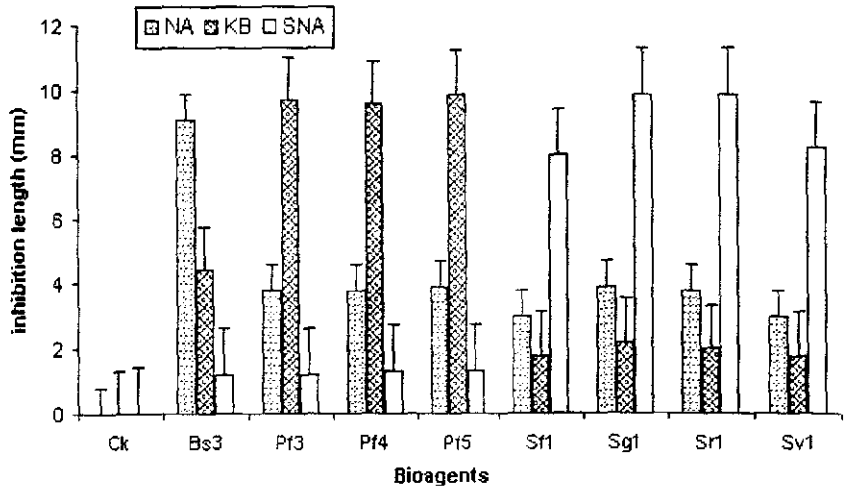

Fig. 1. Inhibitory effects of different bioagents on growth of *Ralstonia solanacearum* on nutrient Agar (NA), King,s B (KB) and Starch nitrate agart (SNA) media, where. Check (Ck), *Bacillus subtilis* (Bs3), *Pseudomonas fluorescen* (Pf 3, 4, 5), *Streptomyces fumigatiscleroticus* (Sf1), *S. griseovirids* (S.g1), *S. rochei* (Sr1) and *S. violaceusniger* (Sv1). Bars indicate SE.

Table 1. Efficiency of tubers and soil drench treatments by different bioagents on severity of potato bacterial wilt disease, after 90 days from planting under artificial inoculation conditions

Bioagent	Tuber treatment			Soil drench		
	A	B	C	A	B	C
Check	99.7	89.6	0.0	99.7	89.6	0.0
B. subtilis (Bs3)	90.8	80.0	10.7	75.4	70.3	21.5
P. fluorescens (pf3)	80.2	70.7	21.1	67.3	60.5	32.5
P. fluorescens (pf4)	78.5	70.0	21.9	67.5	60.6	32.4
P. fluorescens (pf5)	75.7	68.5	23.5	64.3	57.7	35.6
P.fumigatiscleroticus(Sf1)	90.0	79.7	11.0	80.2	70.2	21.7
S. griseoviridis (Sg1)	76.8	69.2	22.8	63.8	54.4	39.2
S. rochei (Sr1)	83.4	75.8	15.4	72.6	66.2	26.1
S.violaceusniger (Sv1)	91.7	80.4	10.3	82.6	71.1	20.6
LSD at 5%	6.4	4.9		6.5	4.5	

A= Percentage of wilted sprouts.
B= Disease index (%) according to disease ratting on the scale from 0 to 5.
C= Percentage of disease control.

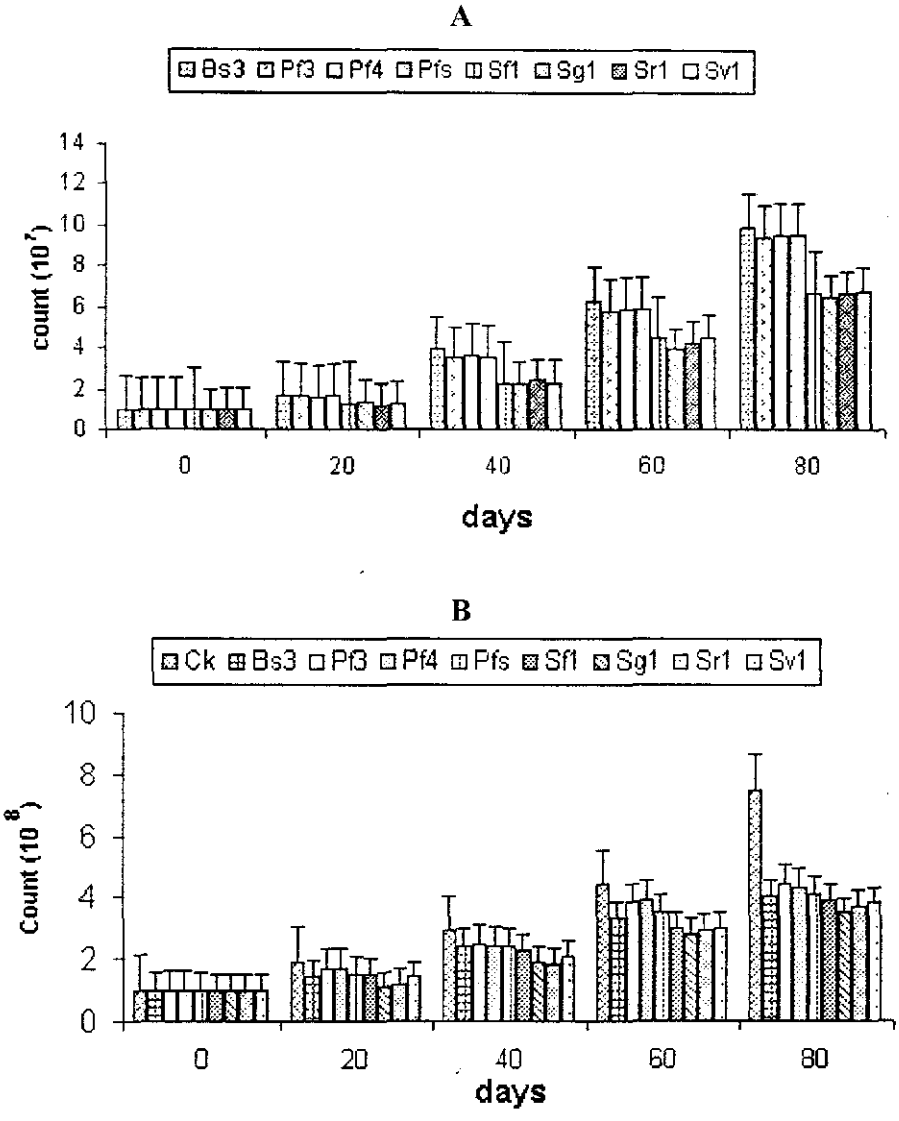

Fig. 2. Populations of different bioagents (A) and *Ralstonia sol nacearum* (B) in potato rhizospheric soil at different periods,using the bioagents as soil drench,under artificial inoculation conditions,where *Pseudomonas fluorescens* (Pf 3,4,5);*Bacillus subtilis*(Bs3); *Streptomyces fumigatiscleroticus* (Sf1) *S. griseovirids (Sg1),S.rochei (Sr1)and S.violaceusniger(Sv*1).Bars indicate SE.

effective, where percentage of disease control was 20.6, 21.5 and 21.7 %, respectively. Similar results were obtained from tubers treatment.

(B) Number of applications as soil drenches treatment

In previous experiment, results showed that *S. griseoviridis* (Sg1) and *P. fluorescens* (pf5) isolates were the most potent in reducing the disease severity, when applied as soil drench treatment. In this experiment, the same isolates were applied one or two or three times as soil drench treatment, at intervals every 20 days from planting. Data in Table (2) indicated that application of *S. griseoviridis* (Sg1) and *P. fluorescens* (pf5) isolates were the most effective in the reduction of the severity of potato bacterial wilt disease, when these isolates were applied for three times, where percentages of disease control ware 66.9 and 71.8 %, respectively. However, these isolates were less effective, when applied for only one time, where the percentages of disease control were 39.2 and 35.6% respectively.

DISCUSSION

All bioagents tested inhibited growth of *R. solanacearum* compared with the control. Meanwhile, fluorescent pseudomonads, actinomycetes and *B. subtilis* isolates were most effective on KB, SNA and NA media, respectively. These results are in agreement with those reported by **Abo El-Dahab & El-Goorani, (1969)**. **Shekhawat *et al* (1992), Abd Alla *et al* (1999) and Moura & Romeiro, (2000)**. **Vidaver (1983) and Fravel (1988)** who mentioned that growth inhibition zones on agar media may be due to chemical factors, to antibiotic substances or to more specific bacteriocin and the antibiotic like substances. However, the ability of antagonistic isolates to inhibit pathogen growth and to produce certain secondary metabolites has been claimed to be important for biological control **(Weller, 1988)**. While, fluorescent pseudomonads strains on KB and PDA media produced a diverse array of inhibitory compounds (siderophores), which inhibited growth of phytopathogens **(Kloepper *et al* 1980).**

Application of different bioagents as tubers and soil drench treatments led to reduce severity of potato bacterial wilt disease and increased disease control. However, soil drench treatments were the most effective, compared with the control. Population of *R. solanacearum* was decreased in potato rhizosphere soil, when different bioagents isolates were applied as soil drench treatment. Fluorescent pseudomonads and *S. griseoviridis* were more effective than *S. violaceasniger*, *S. fumigatiscleroticus* and *B. subtilis* isolates in reduction disease severity and increasing disease control. Application of *S.griseoviridis* (Sg1) and *P. fluorescens* (Pf5) isolates were the most effective in increasing disease control, when these isolates were applied for three time compared with only one time. **Abd Alla *et al* (1999)** found a significant reduction in severity of tomato bacterial wilt disease that was achieved in greenhouse tests, when the antagonistic bacteria were applied to tomato bacteria, they were promising candidates for the biological control of *R. solanacearum*. Actinomycetes with a positive growth promotion effect were effective for biological

Table 2. Influence of number of soil drench treatments by different bioagents on severity of potato bacterial wilt discase, after 90 days from planting, under artificial inoculation conditions

Bioagent	No. of soil drench Treatment	Wilted sprouts (%)	Disease index (%)	Disease control (%)
Check	0	99.7	89.6	0.0
P. fluorescens (pf5)	1	64.3	57.7	35.6
	2	48.0	40.2	55.1
	3	33.7	25.6	71.8
S.griseoviridis (sg1)	1	63.8	54.4	39.2
	2	50.7	43.6	51.3
	3	36.5	29.7	66.9
LSD at 5%		10.2	11.4	

control of bacterial wilt of tomato (**Moura and Romeiro, 2000**). Plant growth promoting rhizobacteria (PGPR) stimulated plant growth and increased yield indirectly by their aggressive colonization of root system. Production of both antibiotics and siderophores has been cited as a factor relation to the ability of PGPR to displace or exclude soil borne pathogens and deleterious rhizosphere microorganisms (**Suslow and Schroth, 1982**). Colonization of the rhizosphere with fluorescent pseudomonads has been successfully employed to reduce the amount of pathogen inoculum reaching the roots and they promote plant growth (**Fravel & Engelkes, 1994 and Toyota & Kimura, 2000**). There are three main approaches to achieve control of a wide spectrum of pathogens by application of antagonists largely for biological control (1) modify the genetics of the biocontrol agent to add mechanisms of disease suppression that are operable against more than the pathogen, (2) alter the environment microflora and (3) develop strain mixtures with superior biocontrol activity (**Janisiewicz, 1988**).**Palleroni** (1984) observed that fluorescent pseudomonads usually coexist with other microorganisms in adverse environments including soil, water and biomaterials.

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مجلة حوليات العلوم الزراعية ، كلية الزراعة ، جامعة عين شمس ، القاهرة ، م٤٨ ، ع(١) ، ٣٥٣-٣٦٤ ، ٢٠٠٣

المكافحة الحيوية لمرض الذبول البكتيرى فى البطاطس تحت الظروف المصرية

[٢٦]

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يعتبر مرض الذبول البكتيرى فى البطاطس والمتسبب عن بكتريا *رالستونيا سولاناسيرم* من الأمراض المحددة لتصدير البطاطس المصرية الى دول العالم المختلفة وتتواجد هذه البكتريا فى التربه بصفه أساسيه. أجريت هذه الدراسه بغرض إمكانية الإستفادة من بعض عزلات الكائنات الدقيقة المضادة والمتواجدة فى التربة بصفة أساسيه فى مكافحة هذا المرض حيويا تحت ظروف العدوى الصناعية. ومن اهم النتائج التى تم الحصول عليها إن جميع العزلات البكتيرية المضادة والمختبرة كانت ذات قدرة عاليه على تثبيط نمو بكتريا *رالستونيا سولاناسيرم* معمليا بالمقارنة مع التجربة المقارنة. فى حين ان عزلات كل من *السيدوموناس فلورسنس* والأكتينو ميسيتات *والباسيلس ستلس* أظهرت كفاءة عالية للتثبيط على بيئات كينج ب وأجار نترات النشادر والأجار المغذى على الترتيب وكانت عزلات *السيدوموناس فلورسينس* اقل تثبيطا على بيئات آجار النترات والنشا والأجار المغذى وعزلات الأكتينوميسيتات أقل تثبيطا على بيئات كينج ب والأجار المغذى وعزلة *الباسيلس ستلس* أقل تثبيطا على بيئات كينج ب وأجار نترات النشا وأدى استخدام العزلات المضادة كمعاملة للتربة الى خفض معنوى فى تعداد بكتريا *رالستونيا سولاناسيرم* فى حين ان تعداد العزلات المضادة زادت مع زيادة الفترة بعد الزراعة وقد وجد ان تعداد كلا من عزلات بكتريا *سيدوموناس فلورسينس* وعزلات بكتريا *الباسيلس ستلس* زادت بدرجة اكبر من عزلات الأكتينوميسيتات بعد ٨٠ يوم من الزراعة وا نخفضت شدة المرض وزادت المقاومة بدرجة معنوية عند استخدام العزلات المضادة كمعاملة للتربة أو الدرنات فى حين أن معاملة التربة كانت أكثر فاعلية من معاملة الدرنات ولقد وجد ان العزلة رقم ٥ من *السيدوموناس فلورسنس* وعزلة *الإستربتوميسس جريسيو فيريدس* (س ح

١) كانتا أكثر فاعية من العزلات الأخـــرى المختبرة وزادت مقاومة المرض وخفضـــت شدة الاصابه بدرجـــة كبـيرة جـدا عنـد استخدامهما كمعاملة للتربة على ثلاث مرات خلال فترة نمو المحصول على فترات زمنية ثابتة (كل ٢٠ يوم مـــن بدايـــة الزراعـــة) بالمقارنة مع استخدامهـــــا مـــرة واحـــدة فقط .

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