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Biological Control of Fusarium Dry Rot of Potato Using Some Probiotic Bacteria

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ABSTRACT

In this study, the antagonistic activity of 1,000 bacterial isolates from different areas of Iran and two isolates of *Pseudomonas fluorescens*: CHA0 and UTPf5 were individually assayed against two isolates of *Fusarium solani* and two isolates of *F. oxysporum* that cause potato dry rot in Mashhad, Iran. Using *in vitro* dual culture plate technique, most of the tested isolates significantly reduced mycelia growth of *Fusarium* spp. on PDA after 7 days of incubation at 25ºC, compared to the untreated control. Thirty bacteria isolates with the most inhibition and *P. fluorescens* CHA0 were selected for experiments on potato tubers. All isolates were tested on tuber of potato cultivar Agria under *in vivo* conditions. There was no significant difference between rot diameter by *P. fluorescens* CHA0 and the infected control, but other isolates reduced dry rot development after 21 days of incubation at 25-27ºC, as compared to the untreated control. However, three isolates, namely, VUPf40, VUPf44, and VUPf506 that belong to the *P. fluorescens* species were the most effective. Talc-based formulation of these three effective bacteria and *P. fluorescens* CHA0 were assessed for their *in situ* efficacy against potato dry rot development, caused by *F. solani* FPO67, on treated tubers compared to the control. The formulation of these bacteria significantly decreased dry rot development, but only *P. fluorescens* VUPf506, with 79.8% reduction of fungi development, and 18.57% prevalence, was the most effective in reduction of potato dry rot in natural conditions. Based on these results, *P. fluorescens* VUPf506 can be considered as a promising alternative to chemical fungicides.

Keywords: Antagonistic effects, Dry rot, PGPR, *Pseudomonas.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) and its products are known to be one of the most important source of food for human beings. The world production of potato is 321 million tones, of which 7.5 million tones are produced in Iran (Radmehr, 2010). Fungi and bacteria cause diseases on potato tubers under field and storage condition (Boyd, 1972). Dry rot is one of the most important post-harvest diseases of potato tubers, causing significant economic losses worldwide (Stevenson *et al*., 2001). The disease is caused by various *Fusarium* spp., which include *Fusarium solani*, *F. oxysporum*, *F. Sulphuerum*, *F. sambucinum*, and *F. trichoteciodes*, however, it is majorly caused by *F. solani* and *F. oxysporum* (Eskandari, 2000). The control of dry rot was first achieved by post-harvest application of thiabendazole, a Benzimidazole fungicide (Secor and Gudmestad, 1999). However, indiscriminate use of fungicides may lead to toxic residue, development of fungicide resistance, environmental contaminations, and carcinogenic, teratogenic, and mutagenic

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effects in humans, animals, and plants (Rajavel, 2000). Consequently, many strains of *F. sambucinum* have become resistant to thiabendazole (Desjardins *et al*., 1993; Platt, 1997; Peters *et al*., 2001). No other options for post-harvest diseases management have proven to be sufficiently effective against Fusarium Dry Rot (FDR); this situation prompts the search for new and efficient methods to control fusarium dry rot of potatoes. The use of biocontrol agents in managing post-harvest diseases of fruit and vegetables has been reported to be effective and feasible (Janisiewez, 1988; Wisniewski and Wilson, 1992). So far, biological control of dry and soft rot with different biocontrol agents such as fungi, bacteria, and yeasts have been reported as effective under experimental conditions (Sadfi *et al*., 2002). Among the bio-agents, the Plant Growth Promoting Rhizobacteria (PGPR) *Pseudomonas* spp. and *Bacillus* spp. proved active in suppressing fungal infections (Chen *et al*., 2000). Eighteen bacterial strains that belong to the genera *Pseudomonas*, *Enterobacter* and *Pantoea* consistently suppressed FDR incited by three different strains of *F. sambucinum* in a whole-tuber assay (Schisler and Slininger, 1994). Similarly, in laboratory and commercial storage environments, 18 gram-negative bacteria have been reported to reduce the severity of FDR infection in potato tubers (Schisler and Slininger, 1997; Schisler *et al*., 2000). A storage trial was conducted using 12 different isolates of *P. fluorescens* and *Enterobacter* against FDR (Slininger *et al*., 2003). In one study, a total of 17 PGPR strains, consisting of eight different species (*Bacillus subtilis*, *B. pumilus*, *B. amyloliquefaciens*, *B. atroseptica*, *B. macerans*, *Burkholderia cepacia*, *Pseudomonas putida* and *Flavobacter balastinium*) were tested for antifungal activity *in vitro* and *in vivo* conditions against *F. sambusinum*, *F. oxysporum*, and *F. culmorum*, which are the cause of dry rot disease of potato. Only *B. cepacia* strain OSU-7 had significant effects on controlling potato dry rot by three different fungi species (Recep *et al*. 2009). Field trials conducted in 2005 and 2006 in Canada assessed the efficacy of *P. fluorescens* B21133 and *E. cloaca* B21050, which were applied as a seed treatment in suppressing fusarium dry rot of potato. The results of this study suggest that *P. fluorescens* and *E. cloaca* are capable of controlling potato dry rot (Al-Mughrabi, 2010). Biopesticides have shown increasing promise as tools for safe and sustainable disease management. The identification and registration of effective bio-pesticides is urgently needed to control post-harvest potato diseases and to curb growers' reliance on synthetic chemicals. In the U.S.A., Bio-save 10LP and 11LP (*Pseudomonas syringae*) are registered for control of silver scurf and fusarium dry rot. Al-Mughrabi *et al*. (2013) assessed the efficacy of the biopesticides 10LP and 11LP against silver scurf and dry rot; their findings indicated that the use of *P. syringae* (strains ESC-11 or ESC-10) as a postharvest treatment could contribute to the management of both silver scurf and fusarium dry rot in potato storage. The objective of this study was to evaluate the biocontrol effect of 1,000 bacterial isolates on potato dry rot caused by *F. solani* and *F. oxysporum* under *in vitro*, *in vivo*, and *in situ* conditions.

MATERIALS AND METHODS

Probiotic Bacteria and Pathogenic Fungal Isolates

All bacterial isolates (isolated from different areas of Iran) and two *P. fluorescens* (strain CHA0 and UTPf5) were obtained from the culture collection unit at Vali-e-Asr University of Rafsanjan. Fungal isolates (two isolates of *F. solani* and two isolates of *F. oxysporum*) were obtained from the Plant Protection Research Department, Agricultural and Natural Resources Center of Khorasan-Razavi, Iran. Bacterial isolates were stored at -80°C in 15% glycerol and Luria Broth (LB), and pathogenic fungi

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maintained on sterile sand at 4°C to be used in further studies.

Source of Potato Tubers

The potato cultivar Agria, the most cultivated in Iran, was used in the current study. They were obtained from the Plant Protection Research Department, Agricultural and Natural Resources Center of Khorasan-Razavi, stored in a traditional store and brought to room temperature a couple of hours before use.

Pathogenicity Test

The pathogenicity test was performed for fungal isolates under *in vivo* conditions. Fungi isolates grown on Potato Dextrose Agar (PDA) for 7-10 days, then, one agar disc colonized by fungi was inoculated into wounds (5×5 mm) in potato tubers. The inoculated potato tubers were incubated at 25°C and 50-70% relative humidity for three weeks. After the incubation period, inoculated tubers were evaluated for rot development (Azadvar *et al*., 2007). Each treatment was repeated four times (two tubers×two wounds).

In vitro Assays on Petri Plates

Bacterial isolates were grown on NA at 25°C for 24 hours to obtain fresh culture for petri plate assay. The pathogenic fungi isolates were also cultivated on PDA at 25°C for 7-10 days. Bacterial isolates were cultured in three equidistant sites of a petri dish (containing PDA+King·B), then, a 5-mm disc of the pathogen fungi was simultaneously placed in the center of petri plate. These plates were incubated at 25°C for one week. After 7 days of incubation, antagonistic activities were evaluated by measuring the zones between pathogens and the tested bacteria. Every elementary treatment was repeated four times. The inhibition zones between fungal colonies and bacterial strains were evaluated after 7-10 days. The data was collected as inhibition zones valued as mm in each replication, and was evaluated by one-way Analysis Of Variance (ANOVA) by using SPSS 16.0 for Windows.

In Vivo Activity of Selected Bacteria against Fusarium spp.

Based on the antifungal activities on petri plates, the most effective strains were selected as potential biocontrol agents for the next studies. Cell suspensions of strains were tested for activity against pathogenic fungi on potato tuber under experiment conditions. Bacterial isolates were grown on NA for 24-48 hours at 25°C and suspended in dsH_2O. Concentration of bacterial cell suspension was adjusted to 10^8 CFU mL^{-1}. Pathogenic fungal isolates were grown on PDA and incubated at 25°C for 7 days. The experiments were performed at 25°C and 50-70% humidity. The selected potato tubers of Agria were washed in running water, dipped in sodium hypochlorite (3%) for 10 minutes, rinsed abundantly with sterile distilled water (10 minutes) and air-dried. Cell suspensions of bacteria individually were assayed against the pathogens by co-inoculating (25 µL) of bacteria suspension, and agar disc (5 mm) colonized by fungi into wounds (5×5 mm) in potato tubers. Fungal mycelium disc were used as positive control. Tubers incubation was realized in a growth chamber at 25°C for 21 days at high relative humidity. All treatments consisted of four replicates with two tubers in each replicate, and experiments were repeated two times. After incubation period, tubers were cut longitudinally via sites of inoculation, and after that, parameters of dry rot induced [maximal width (w) and depth (d)] were noted. The pathogen penetration into tubers was calculated following the formula of Lapwood *et al.* (1984) where:

$$\text{Penetration} = [w/2+(d-6)]/2 \quad (1)$$

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All treatments consisted of four replicates of two tubers in each replicate, and experiments were repeated twice. The data were obtained after 21 days and collected as average of penetration in each replication.

Identification of Strains

Biochemical and physiological characterizations of strains Vupf40, Vupf44, and Vupf506 were identified based on Shaad *et al*. (2001). Genomic DNA of strains were extracted and purified according to the method described by Wang *et al*. (2001). 16S rDNA were amplified from genomic DNA with the primers PS16f 5'-TGGCTCAGATTGAACGCTGGCGG-3' and PS16r5'-GATCCAGCCGCAGGTTCCCCT AC-3'. PCR amplification was carried out in 20 µL reaction mixtures containing 4 µL of lysed bacterial suspension, 1×PCR bovine serum albumin, 5% dimethyl sulfoxide, 100 µM each of dATP, dCTP, dGTP and dTTP, 0.40 µM of each primer and 1.4 U of Taq DNA polymerase amplification were performed with a Corpet research g001 cycler. The initial denaturation (2 minutes at 94°C) was followed by 30 PCR cycles (94°C for 30 seconds, 60°C for 30 seconds and 72°C for 60 seconds) and a final extension at 72°C for 10 minutes. The amplified DNA was purified using Polymerase Chain Reaction (PCR) purification kit (promega, Madison, WI, USA), diluted to 200 ng μL^{-1}, and was sequenced at Microsynth Inc. (Balgach, Switzerland).

Preparation of Formulation

Formulation of *Pseudomonas* isolates was prepared on talcum powder carrier. One hundred gram of talcum powder was placed in a metal tray under aseptic conditions, and the pH adjusted to 7 by adding calcium carbonate at the rate of 15 g kg^{-1}. Then, carboxymethyle cellulose was added at the rate of 10 g kg^{-1}, and mixed well. Finally, the mixture was packed into a polythene bag and autoclaved twice for 1 hour at 121°C. A loopful of individual *Pseudomonas* isolates was inoculated into KB broth and incubated on a shake incubator at 150 rpm for 48 hours at room temperature (28±2°C). After 48 hours incubation, the broth containing 9×10^9 CFU mL^{-1} was used for the preparation of Talc-based formulation. 400 mL of bacterial suspension was added to the prepared mixture under sterile condition (Vidhyasekaran and Muthuamilan, 1995); the product was shade-dried to reduce the moisture contest (less than 20%), then, packed in sterile polythene bags and stored at 4°C.

In Situ Activity of Bacterial Formulation *against F. solani* FPO-67

The effect of bacterial formulations on disease incidence and severity of fusarium dry rot was assessed following the manufacturer recommended dose for *P. syringae* ECS10 and *P. syringae* ECS11, under storage condition, using the following treatments: (1) Control; (2) *P. fluorescens* VUPf506 (4.4 g L^{-1}); (3) *P. fluorescens* VUPf44 (4.4 g L^{-1}); (4) *P. fluorescens* VUPf40 (4.4 g L^{-1}); and (5) *P. fluorescens* CHA0 (4.4 g L^{-1}). One month after harvest, potato tubers of Agria were disinfected as mentioned in in vivo test. Then, the disinfected potato tubers were sprayed with the respective treatment solutions. Sterile distilled water was used for the control. Tubers were kept in a sterile place for 12 hours; after that, an agar disc colonized by *F. solani* FPO-67 was inoculated into a slight wound (about 2×2 mm) in potato tubers. Fungi mycelium disc was used in the positive controls. Inoculated tubers were placed in clean plastic crates kept in net bags, and incubated in a traditional storage. After 4 months, disease incidence of fusarium dry rot was assessed as the percentage of infected tubers out of total number of tubers in a replicate; disease severity was assessed as mentioned in *in*

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vivo test. Treatments were replicated four times, each replicate included 3000 g potato. The average of penetration was evaluated by one-way Analysis Of Variance (ANOVA) by using SPSS 16.0 for Windows.

Statistical Analysis

An Analysis Of Variance (ANOVA) and Duncan's multiple range test (at P= 0.05) were performed to analyze statistical differences and to discriminate among means.

RESULTS

Pathogenicity Test

In the pathogenicity test, all fungal isolates were able to cause rot with 7.5-20.5 mm penetration into potato tubers after three weeks.

In Vitro Assays

In *in vitro* assay results, 1,000 bacterial isolates had inhibitory effects on the development of at least one fungal isolate; among them 29 isolates that reduced the mycelium growth of four fungal isolates were selected. Although *P. fluorescence* CHA0 had no effect on the reduction of mycelium growth, it was chosen because of its antimicrobial metabolites. The influence of each bacterial isolate was different not only on two fungal species, but also on four fungal isolates. Five of thirty isolates (506, 347, 646 and 351, 194) with inhibition zone values of 12, 12, 14, and 14.25 mm were the stronger antagonists against *F. solani* FPO-67, *F. solani* FPO-19, and *F. oxysporum* FPO-35 and *F. oxysporum* FPO-39, respectively (Table1). The remaining isolates did not show significant inhibitory effect against pathogenic fungi. For this reason, they were not tested for *in vivo* assays.

In Vivo Assays

Table 2 shows the mean pathogen penetration in tubers of cv. Agria, individually inoculated by two isolates of *F. solani* and *F. oxysporum*, noted after 21 days of incubation at 25-27^{o}C, depending on different treatments. Almost all bacterial isolates caused a significant reduction in dry rot development when compared to the control. Bacterial antagonistic effect on different fungal isolates varied. For example, in the case of *F. solani* FPO-19, *P. fluorescens* VUPf506 inhibited fungi penetration completely, but for *F. solani* FPO-67, thorough inhibition was reached by *P. fluorescens* VUPf44. *P. fluorescens* VUPf506 had also significant effect on controlling potato dry rot caused by *F. solani* FPO-67 compared to *P. fluorescens* VUPf44. Strains *P. fluorescens* VUPf365 and *P. fluorescens* VUPf506 provided 100 and 97.49% inhibition of dry rot caused by *F. oxysporum* FPO-35 and *F. oxysporum* FPO-39, respectively. *F. fluorescens* CHA0 did not inhibit dry rot when compared to the control (Table 2).

Identification of Strains

The result of biochemical and physiological testing showed that strains Vupf40, Vupf44 and Vupf506 were related to *Pseudomonas fluorescens*. Primers, PS16f and PS16r amplified a complete DNA fragment of 1.49 kb 16s rDNA when the total genomic DNA of strains was used as template in PCR. The 16s rRNA gene sequence of these strains (Vupf40, Vupf44 and Vupf506) exhibited, respectively, 99.3, 99.1 and 99.6% similarity to that of *P. fluorescens.*

In Situ Assays

All treatments caused a significant reduction in dry rot when compared to the control (Figure 1). *P. fluorescens* VUPf506

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Table 1. *In vitro* efficacy of bacterial isolates against *F. solani* (FPO-67 and FPO-19), and *F. oxysporum* (FPO-35 and FPO-39).[a]

Treatment	Inhibition (mm)			
	F. solani		*F. oxysporum*	
	FPO-19	FPO-67	FPO-35	FPO-39
VUPf506	9.5cd	12a	12.25a-c	12.75ab
VUPf355	11ab	11.25ab	10.5cd	9.25de
VUPf347	12a	11.25ab	11.75b-d	10.25cd
VUPf194	10b-d	9.75bc	10.25d	14.25a
VUPf40	7ef	9.5bc	11b-d	10.25cd
VUPf365	6f-h	9.5bc	12.75ab	11.75bc
VUPf646	10.5a-c	9c	14a	10cd
VUPf351	8.5de	9c	14a	7.25fg
VUPf692	5.75f-i	6.75d	8.25e	7.25fg
VUPf689	5.5f-j	6.5d	7.25ef	7.25fg
VUPf786	4i-k	6.25de	5g-i	3.75i-k
VUPf350	7.25ef	6.25de	8.25e	9.25de
VUPf353	6.5fg	5.75d-f	3.5ij	7.5ef
VUPf621	4.75g-k	5.25d-g	5g-i	5.25g-j
VUPf394	5g-k	5.25d-g	5g-i	12.75ab
VUPf674	7ef	5d-h	7.25ef	5.25g-j
VUPf399	8.5de	5d-h	6.75e-g	5.75f-i
VUPf368	5.75f-i	4.75d-h	5g-i	6fgh
VUPF785	5g-k	4.25e-i	5g-i	2.75k
VUPf50	5.75f-i	4.25e-i	5g-i	4.75h-k
VUPf44	6f-h	4f-i	4.25h-j	4.75h-k
VUPf359	5g-k	4f-i	5g-i	6.5f-h
VUPf354	5g-k	4f-i	5.25g-i	3.5jk
VUPf709	7ef	3.75f-i	4.25h-j	4.5h-k
VUPf693	8.5de	3.75f-i	3.25ij	4.5h-k
VUPf401	3.75jk	3.75f-i	6fgh	4.75h-k
VUPf734	4i-k	3.5g-i	4.75g-i	5.5f-j
VUPf402	4i-k	3.5g-i	6.25f-h	5.5f-j
VUPf718	4.25h-k	3hi	5g-i	3.5jk
BPP1	3.5k	2.25i	2.75j	2.75k
CHA0	0l	0j	0k	0l
CONTROL	0l	0j	0k	0l

[a] Values (means of four replicates) followed by the same letter within each column are not significantly different from each other according to Duncan's multiple range test (P= 0.05).

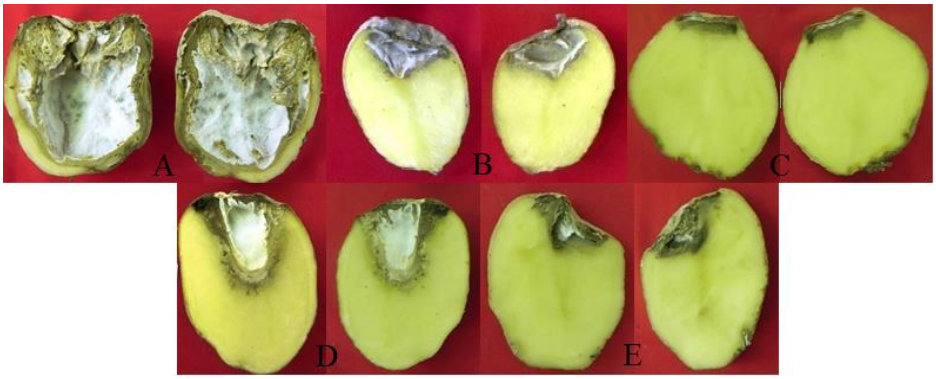

Figure 1. Effect of bacterial formulation on development of dry rot in storage condition. (A) Control, (B) *P. fluorescens* VUPf44, (C) *P. fluorescens* VUPf506, (D) *P. fluorescens* VUPf40, and *P. fluorescens* CHA0.

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Table 2. *In vivo* efficacy of bacterial isolates against *F. solani* (FPO-67 and FPO-19), and *F. oxysporum* (FPO-35 and FPO-39).[a]

Treatment	Penetration(mm)			
	F. solani		*F. oxysporum*	
	FPO-19	FPO-67	FPO-35	FPO-39
Negative control	0a	0a	0a	0a
VUPf44	0.62a-c	0a	0.8a	0.8ab
VUPf40	0.94a-e	0.19a	4.09b-h	1.06a-d
VUPf506	0a	0.25a	1.37ab	0.31a
VUPf354	0.5ab	0.56a	2.08a-c	3.6d-i
VUPf394	1.75a-h	1.37ab	2.25a-d	0.9a-c
VUPf353	0.5ab	1.68a-c	2.7a-f	2.9b-f
VUPf674	2.2a-i	2.3a-d	2.56a-f	3.12b-h
VUPf347	3c-i	3.8a-e	5.4g-i	3.7d-i
VUPf194	3.12d-j	4.06a-f	2.6a-f	4.9f-i
VUPf351	0.3ab	4.06a-f	2.06a-c	1.4a-e
VUPf355	0.62a-c	4.6a-g	2.8a-g	3.5c-h
VUPf368	0.75a-d	4.9a-g	6.6hi	4.4f-i
VUPf734	1.3a-f	5.8b-h	0.75a	3.56c-h
VUPf50	0.8a-e	6.2b-h	4.9c-i	4.4f-i
VUPf689	4.3ij	6.2b-h	7hi	6.25ij
VUPf621	2a-i	6.5b-h	5.7g-i	5.7g-j
VUPf402	2a-i	6.8c-h	1.87ab	1.56a-e
VUPf646	0.62a-c	7.2d-h	5.06d-i	1.3a-d
BPP1	4h-j	7.25d-h	6.9hi	3.4b-h
VUPf350	0.75a-d	7.56d-i	4.9c-i	2.4a-f
VUPf401	3.75g-j	7.8e-i	5.2e-i	3.7d-i
VUPf709	0.25ab	7.8e-i	4.8c-i	2.7a-f
VUPf399	4.9j	8.12e-i	4.06b-h	4.06e-i
VUPf365	1.37a-g	8.4e-i	0a	3b-g
VUPf359	3.12d-j	8.4e-i	6.4hi	4.8f-i
VUPf693	1.6a-h	8.4e-i	7.12i	5.06f-i
VUPf786	3.2e-j	8.9ei	5d-i	5fg-i
VUPf785	1.2a-e	9e-i	2.5a-e	7.8j
VUPf692	2.3a-i	9.3e-i	0.62a	3.06b-h
CHA0	2.6b-j	10g-i	5.75g-i	5.75h-j
VUPf718	3.6f-j	11hi	6.12hi	3.3b-h
Positive control	9.8k	12.75i	15.62j	12.37k

[a] Values (means of four replicates) followed by the same letter within each column are not significantly different from each other according to Duncan's multiple range test (P= 0.05).

inhibited the development of *F. solani* FPO-67 by 80%, but *P. fluorescens* VUPf40 did not significantly inhibit dry rot development when compared to other treatments (Figure 2).

Figure 3 shows the percentage of infected tubers in storage condition. *P. fluorescens* VUPf506 and *F. fluorescens* CHA0 had a significant effect on dry rot when compared to the control, but *fluorescens* VUPf44 and *fluorescens* VUPf40 did not reduce disease incidence compared to the control. *P. fluorescens* VUPf506 had a significant effect on severity and disease incidence at this concentration (Figures 2 and 3).

DISCUSSION

Biological control using postharvest biocontrol products has a great potential because postharvest environmental

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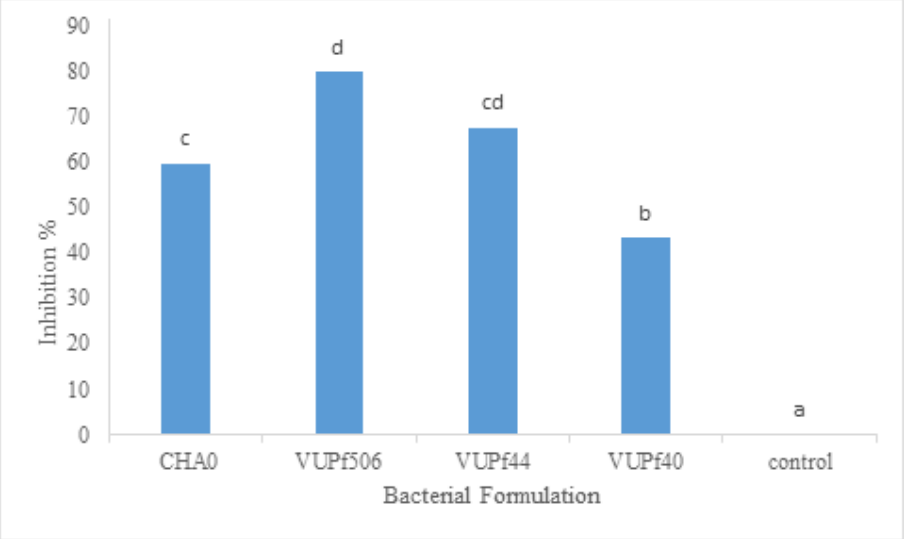

Figure 2. Effect of 4.4 g L^{-1} bacterial formulation on the development of dry rot caused by *F. solani* FPO-67 in storage condition. Inhibitions with the same letter are not significantly different according to Duncan's multiple range test (P= 0.05).

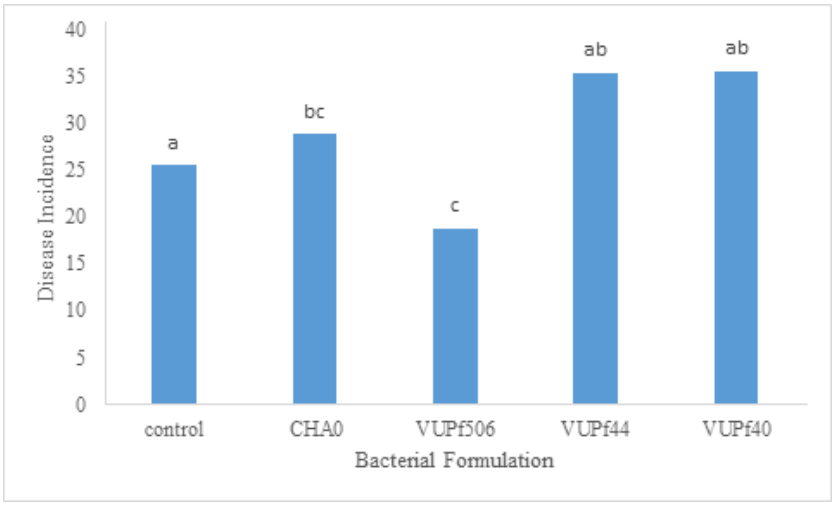

Figure 3. Effect of bacterial formulations on the potato dry rot incidence. Disease incidences with the same letter are not significantly different according to Duncan's multiple range test (P= 0.05).

parameters, namely, temperature and humidity, can be rigidly controlled to suit the needs of the biocontrol agents. The use of biocontrol agents in managing postharvest diseases of fruits and vegetables has been reported to be effective and feasible (Janisiewez, 1988; Wisniewski and Wilson, 1992). The present study was conducted to evaluate the efficacy of bacterial antagonists, applied in *in vitro*, *in vivo*, and *in situ* trials, against fusarium dry rot of potato tubers. Most bacterial isolates effectively suppressed the *in vitro* growth of *F. solani* FPO-19, *F. solani* FPO-67, *F. oxysporum* FPO-35, and *F. oxysporum* FPO-39, the casual agents of potato dry rot in storage. It is also to note that all *in vivo* biocontrol assays were conducted on entire tubers and the results reflected interactions between tuber defense, antagonists, and

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pathogens. Among 30 tested isolates, *P. fluorescens* (VUPf506, VUPf40, and VUPf44) had a significant effect on controlling all four fungi isolates under *in vivo* condition. There was no correlation between *in vivo* and *in vitro* results in *P. fluorescens* CHA0, as *P. fluorescens* CHA0 reduced the penetration of four fungal isolates under *in vivo* condition. There are many studies in literature indicating that strains of species *Pseudomonas* sp., *Pantoea* sp, *Enterobacter* sp, *Bacillus* sp, and *Trichoderma* sp, may be used as potential biocontrol agents against potato dry rot (Schisler, 1994; Schisler *et al*. 1997; Sadfi *et al*. 2001, 2002; Van den Boogert and Luttikholt, 2004). The effectiveness of biological agents against fusarium dry rot pathogens has been previously documented. The treatment of potato tubers with *Pseudomonas fluorescens* S22: T: 04 prior to storage reduced the severity of fusarium dry rot, caused by *Gibberella pulicaris*, by 19% when compared to the control and the Thiabendazole treatments (Schisler *et al*. 2000). In a storage trial conducted using 12 different isolates of *P. fluorescens* and Enterobacter, nine bacterial isolates were able to reduce the severity of fusarium dry rot more than Thiabendazole treatments. Among the different tested isolates, *P. fluorescens* bv. V P22: Y: 05 gave the best control (Slininger *et al*., 2003). The use of *P. fluorescens* and *Enterobacter cloacae* applied to potato tubers entering storage has been shown to reduce severity of fusarium dry rot significantly compared to the control treatments (AL-Mughrabi, 2010). In this study, we observed that the biocontrol agents can show different activities against different species and different isolates. The different efficacy of the biocontrol agents could be due to the influence of several factors. These factors include the efficiency of the type or strain of biocontrol agent, the type or aggressiveness of pathogens, the susceptibility of the host to the pathogen, and the environment (Frances *et al*., 2006). In our storage studies, the disease control achieved with *P. fluorescens* was noticeable. Our results indicated that the inhibition of dry rot varied between 43.24 and 79.8%. This level of inhibition is paramount when compared to the results achieved previously in controlling fusarium dry rot of potatoes using *P. fluorescens* and Enterobacter by Schisler *et al*. (2000), who reported only a 19% reduction of Fusarium dry rot. In another study, a significant reduction in dry rot between 35 and 95% was observed when using *P. syringae* ESC10 and *P. syringae* ESC11 individually or in combination with thiabendazole. However, when *P. syringae* ESC10 and *P. syringae* ESC11 were used individually, the inhibition was between 37 and 88% (Al-Mughrabi *et al*., 2013). *P. fluorescens* CHA0 had a better effect on *F. solani* FPO-67 when compared to *in vivo* condition, because sometimes the substances in the formulation can have a positive effect on bacterial efficacy. In conclusion, our results show that *P. fluorescens* VUPf506 have great potential to be used as biocontrol agents to manage Fusarium species causing dry rot on potato as well as many other fungi and bacterial pathogens (Kotan *et al*., 1999, Kotan *et al*., 2009, Al-Mughrabi, 2010, AL-Mughrabi *et al*., 2013).

Storage trials conducted in a traditional storage showed the effectiveness of *P. fluorescens* against *F. solani* in reducing potato dry rot disease incident and severity. The in vitro, in vivo, and in situ data are supporting that antagonism is the main mechanism of *P. fluorescens* VUPf506 for biological control of potato dry rot, but the antimicrobial compounds have not been isolated and identified yet. Thus, further study is necessary for determining the mechanisms of antagonistic action of *P. fluorescens* VUPf506.

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کنترل بیولوژیک پوسیدگی خشک فوزاریومی سیب‌زمینی با استفاده از باکتری‌های پروبیوتیک

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چکیده

در این تحقیق، فعالیت آنتاگونیستی ۱۰۰۰ جدایه باکتریایی (جدا شده از مناطق مختلف ایران) و دو جدایه‌ی *Pseudomonas fluorescens CHA0* و UTPf5 به طور جداگانه روی دو جدایه قارچ *Fusarium solani* و *F. oxysporum*، که عامل پوسیدگی خشک سیب‌زمینی در منطقه‌ی مشهد می‌باشند، مورد بررسی قرار گرفت. در آزمون هاله بازدارنده، اکثر جدایه‌ها به طور قابل توجهی باعث کاهش رشد میسلیومی جدایه‌های فوزاریوم روی محیط PDA بعد از ۷ روز نگهداری در دمای ۲۵Ċ، در مقایسه با شاهد شدند.۳۰ جدایه باکتریایی با بیشترین بازدارندگی به همراه باکتری *P. fluorescens* CHA0 برای آزمایش روی غده سیب‌زمینی انتخاب شدند. تمام باکتری‌ها روی

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غده‌های سیب‌زمینی رقم آگریا در شرایط آزمایشگاه بررسی شدند. اختلاف قابل توجهی بین قطر پوسیدگی در نمونه‌های تیمار شده با*P. fluorescens* CHA0 و کنترل منفی وجود نداشت، اما سایر جدایه‌ها باعث کاهش گسترش پوسیدگی خشک بعد از ۲۱ روز نگهداری در دمای ۲۷-۲۵ Ċ ، در مقایسه با شاهد شدند. اگرچه ۳ جدایه‌ی VUPf40، VUPf44 و VUPf506 که متعلق به گونه‌ی*P. fluorescens* می‌باشند، بیشترین تاثیر را داشتند. فرمولاسیون ساخته شده‌ی پودر تالک این سه باکتری موثر و همچنین*P. fluorescens* CHA0 روی گسترش پوسیدگی خشک سیب‌زمینی با عامل *F. solani* FPO67 در شرایط انبار فنی ارزیابی شد. فرمولاسیون همه‌ی باکتری‌های موثر به طور قابل توجهی گسترش پوسیدگی خشک را محدود کردند، ولی فقط *P.* VUPf506 *fluorescens*، با ۷۹/۸٪ کاهش گسترش قارچ و۱۸/۵۷٪ بازدارندگی، موثرترین جدایه در کاهش پوسیدگی خشک سیب‌زمینی در شرایط طبیعی بود. بر اساس نتایج، *P. fluorescens* VUPf506 می‌تواند بعنوان یک جایگزین امید بخش برای محصولات شیمیایی در نظر گرفته شود.

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