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Biological control of *Pectobacterium carotovorum* subsp. *carotovorum*, the causal agent of bacterial soft rot in vegetables, in vitro and in vivo tests

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Abstract

Background: Several chemical bactericides were applied for controlling soft rot bacteria, *Pectobacterium carotovorum* subsp. *carotovorum*, which causes the destructive soft rot disease to many economically important vegetables, but because of their toxic hazards on human and environment became limit. The biocontrol was applied to control many plant pathogens. Therefore, this work is aimed to study the antagonistic activity of bacterial agents, i.e. *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus megaterium* and *Pseudomonas fluorescens*, and fugal agents, i.e. *Trichoderma harzianum*, *Trichoderma viride* and *Trichoderma virens*, to control bacterial soft rot disease under in vitro and in vivo tests.

Results: The tested treatments could protect the potato tubers against the development of soft rot. *T. viride* and *T. virens* were highly effective in reducing soft rot symptoms on inoculated potato tuber slices, when applied at the same time or 2 h before pathogen inoculation, while *B. megaterium* and *T. harzianum* were highly effective when applied at the same time or 2 h after pathogen inoculation. In whole potato tubers technique, *B.pumilus* highly protected the stored potato tuber under artificially infection conditions, than *P. fluorescens*, *T. harzianum*, *B. subtilis*, *T. viride*, *T. virens* and *B. megaterium*, respectively.

Conclusion: Application of fungal agents or specify the bacterial species can play an important role in controlling bacterial soft rot disease in vegetables and increase the stored periods of potato tubers under storage conditions without any toxic effects.

Keywords: Biological control, *Pectobacterium carotovorum*, Soft rot disease, Vegetables

Background

Pectobacterium carotovorum subsp. *carotovorum* (Syn. *Erwinia carotovora* subsp. *carotovora*) causes the destructive soft rot disease to many economically important vegetables such as carrot, cabbage, cucumber, eggplant, garlic, onion, pepper, potato, radish, sweet potato, squash and tomato (Opara and Asuquo 2016), where the disease can be detected in the field, transmit, storage and market. The soft rot bacteria can successfully penetrate the plant, through the wounds or natural openings, causing economic damage to fleshy vegetables by producing many cell wall-degrading enzymes (Péromblon 2002; Bhat et al. 2010). Application of chemical bactericides for controlling soft rot bacteria is not favored because of their non-persistence, side toxic effects, high cost as well as development of resistance in bacterial populations (Jones et al. 1996; Vanneste 2000).

Therefore, biological control may be one of the good crop protection methods for controlling bacterial soft

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rot disease by application of *Trichoderma* spp., *Bacillus* spp. or *Pseudomonas* spp. which widely applied as biological agents against many soil-borne pathogens (Wulff et al. 2003; Alabouvette et al. 2006). Applications of *B. cereus*, *B. subtilis*, *B. megaterium* or *B. pumilus* showed good activity against *P. carotovorum* subsp. *carotovorum* in vitro tests using the disk plate method (Issazadeh et al. 2012). *Pseudomonas* spp. also controlled the bacterial soft rot disease in Valerian rhizome as well as significantly increased the fresh and dry weights of root (Ghods-Alavi et al. 2012). When neem cake applied in soil with *P. putida* as seeds treatment, it could reduce *E. carotovora* infection as well as significantly increased the carrot yield under field conditions (Sowmya et al. 2012). *Pseudomonas fluorescens* and *B. subtilis* showed the highest inhibitory effects against *E. carotovora* subsp. *carotovora* in vitro and in the pot experiment (Algeblawi and Adam 2013). *Bacillus* spp. and *B. pumilus* controlled *P. carotovorum* subsp. *carotovorum* in chili in vivo, where all treatments showed a higher reduction in disease severity than the controls (Silva et al. 2014). *Trichoderma asperellum* also could reduce the pathogenic effect of *E. carotovora* on the young seedlings of okra (Idowu et al. 2016).

Therefore, this work aimed to test seven biocontrol agents, *i.e.* four bacterial agents, namely *B. subtilis*, *B. pumilus*, *B. megaterium* and *P. fluorescens*, and three fungal agents, namely *T. harzianum*, *T.viride* and *T. virens*, to control *P. carotovorum* subsp. *carotovorum* in vitro and in vivo tests.

Methods

Soft rot pathogen

Fifteen bacterial soft rot strains isolated from some vegetables showing naturally typical bacterial soft rot symptoms were collected from some marketing and storage locations in Egypt (Table 1). All bacterial strains were identified as *P.carotovorum* subsp. *carotovorum* according to pathological, cultural, morphological and biochemical characters using standard bacteriological methods in pervious study by Mikhail et al. (2019).

Preparation of soft rot bacteria inoculum

The bacterial inoculums of soft rot bacterial strains were prepared by growing of each bacterial strain on nutrient glucose (2%) agar medium in slant tubes. All inoculated slants were incubated at 28 °C ± 2 for 48 h. The bacterial suspension for each strain was done by scraping the bacterial growth in 5 ml of sterile 0.2 M phosphate buffer (pH 7.2). The bacterial inoculums were adjusted to a standard inoculums density [ca. 10^{7-9} colony forming unit (CFU)/ml] by measuring the turbidity using a Prim light spectrophotometer at 610 nm and then kept at cool conditions until used within 12 h (Moh et al. 2012).

Table 1 Soft rot bacteria strains isolated from some vegetables

Strains code	Vegetables	Soften plant parts
Pcc_1	potato (*Solanum tuberosum* L.)	Tubers
Pcc_2		
Pcc_3		
Pcc_4		
Pcc_5	Sweet potato (*Ipomoea batatas* L.)	Roots
Pcc_6	Cucumber (*Cucumis sativus* L.)	Fruits
Pcc_7		
Pcc_8		
Pcc_9	Carrot (*Daucus carotovora* L.)	Roots
Pcc_{10}		
Pcc_{11}		
Pcc_{12}	Eggplant (*Solanum melongena* L.)	Fruits
Pcc_{13}		
Pcc_{14}	Chili (*Capsicum frutescens* L.)	Fruits
Pcc_{15}		

Biocontrol agents

Four bacterial agents namely *B. subtilis*, *B. pumilus*, *B. megaterium* and *P. fluorescens*, and three fungal agents namely *T. harzianum*, *T. viride* and *T. virens*, were tested in this work. All biocontrol agents were obtained from Plant Pathology Department, National Research Centre.

Preparation of the biocontrol agents

Preparation of cultural filtrates of each bacterial biocontrol agent was carried out using sterilized nutrient glucose (2%) broth medium (3 g beef extract, 5 g peptone, 20 g glucose in one liter distilled water, pH 7.2) in 250 ml flasks. Each flask was separately inoculated with 1 ml of 48-h-old-culture of each bacterial agent. Three flasks were used as replicates for each bacterial antagonist. The inoculated flasks were incubated at 28 ± 2 °C for 48 h under static conditions. Each bacterial culture was centrifugated at 2038 × g for 15 min; then, the supernatant was filtered through filter paper (Whatman No.1) and finally sterilized by filtration through sterile 0.45 µm membrane filter (cellulose nitrate, Whatman). The bacterial filtrates were then kept at − 20 °C until used (Abd-El-Khair and Haggag 2007).

Preparation of cultural filtrates of each *Trichoderma* spp. was made using sterilized potato glucose 2% broth medium (200 ml potato extract and 20 g glucose in one liter distilled water) in 250 ml flasks. Each flask was separately inoculated with 1 cm-diameter disc of one-week-old culture of each *Trichoderma* spp. The inoculated

flasks were incubated at 28 ± 2 °C for one week under static conditions. Then, the *Trichoderma* spp. mycelial mats were separated by filtration with filter paper (Whatman No.1) and finally the fungal cultural filtrates were sterilized by filtration through sterile 0.45 µm membrane filter. The fungal cultural filtrate of each *Trichoderma* spp. was separately kept at − 20 °C until used (Abd-El-Khair and Haggag 2007).

Screening for antagonistic activity in vitro *tests*

The inhibitory activity of cultural filtrates of biocontrol agents against all *P.carotovorum* subsp. *carotovorum* strains were tested by using filter paper disc plate method (Thornberry 1950). All tests were laid in complete randomized design with four replicates. Twenty milliliters of nutrient glucose (2%) agar medium were poured in each sterile Petri dish (9 cm-diameter) and allowed to solidify. Each Petri dish was separately inoculated with 0.1 ml of each soft rot bacterial strain suspension (10^{7-9} CFU/ml) onto the surface of the plate in the center by pipette. The bacterial inoculums were spread over the surface of the plate using a sterile L-shaped spatula and let for 5 min. The filter paper discs (5 mm-diameter) were immersed individually for 1 min in each cultural filtrate of biocontrol agent which prepared before. For control, the filter paper discs were soaked in sterilized distilled water. Four filter paper discs were used as replicates for each treatment as well as the controls. The inoculated Petri dishes were incubated at 28 ± 2 °C for 48 h. The antibacterial activity was recorded by measuring the diameter of the zones of inhibition around the filter paper disc (Mills et al. 2006; Rashid et al. 2013).

Screening for antagonistic activity in vivo *tests*

a. Potato slices method

The inhibitory activity of cultural filtrates of applied biocontrol agents, against high pathogenic *P.carotovorum* subsp. *carotovorum* strains, *i.e.* Pcc_3, Pcc_4 and Pcc_5, were tested by using potato tuber slices method. Healthy potato tubers cv. Diamond was surface-sterilized by sodium hypochlorite solution at concentration of 5% for 3 min. The potato tubers were washed in serial of sterile distilled water and left for drying. The potato tubers were cut into slices (2 cm-thick) by sterile knife under sterile conditions. One potato slice was put in each Petri dish containing sterilized filter paper and impregnated with 3 ml of sterile distilled water. Then, each potato slice was separately inoculated with 0.1 ml of each soft rot bacteria suspension onto the center. Each cultural filtrate of biocontrol agent was applied at the same time of pathogen inoculation and 2 h before or after pathogen inoculation. Potato slices were separately treated with pathogenic bacteria suspension and distilled water for controls. Three potato slices were used as replicates for each treatment. Inoculated potato slices were incubated at 30 °C ± 2 for 72 h. The soft rot symptoms were evaluated according to the scale described by Bartz (1999), as the following:— no rotting; + restricted rot < 1 cm; + + small active rot 1–2 cm and + + + highly active rot > 2 cm.

b. Potato tubers method

The inhibitory activity of cultural filtrates of applied biocontrol agents against the highest pathogenic *P. carotovorum* subsp. *carotovorum* (Pcc_3 strain) were tested by using potato tubers method. Healthy potato tubers cv. Diamond were surface sterilized by sodium hypochlorite solution as mentioned before. Then, each potato tuber was wounded in three places using sterile knife (cross hale). Firstly, potato tubers were separately treated with each cultural filtrate by spraying with atomizer and then allowed 2 h for drying. Then, the treated tubers were inoculated with bacterial pathogen inoculums by spraying with atomizer and then air-dried. For controls, potato tubers were either treated with bacterial pathogen inoculums or distilled water. Ten inoculated potato tubers were weighed and stored in plastic net at room temperature. Three batches were employed as replicates for each treatment as well as the controls. Data of soft rot incidence were weekly recorded for 3 months. Weight of soft rot infected tubers were recorded and expressed in percentage using the following modified formula described by Abd-El-Khair and Haggag (2007).

$$\text{Soft rot incidence \%} = \frac{\text{Initial weight of tubers} - \text{Weight of healthy tubers}}{\text{Initial weight of tubers}} \times 100$$

Statistical analysis

Data were subjected to analysis of variance using Computer Statistical Package (CO-STATE) version 3.03, Barkley Co., USA, and means were compared using the Least Significant Difference (LSD) test at $P = 0.05$ (Snedecor and Cochran 1980). The significance of the treatment effects, concentration, exposure time and their interactions were also analyzed.

Results

In vitro *tests*

The cultural filtrates of each *B. subtilis*, *B. pumilus*, *B. megaterium* and *P. fluorescens* could inhibit the bacterial growth of all tested *P. carotovorum* subsp. *carotovorum* strains, except Pcc_6 & Pcc_8 strains and Pcc_9 & Pcc_{10} strains, which isolated from cucumber fruits and carrot roots, respectively (Table 2). The cultural filtrates of

Table 2 Inhibitory activity of the crude cultural filtrates of bacterial agents against the bacterial growth of *Pectobacterium carotovorum* subsp. *carotovorum* strains in vitro tests

P. c. subsp. *carotovorum*	Inhibition zone (cm) of bacterial growth				
	Control	*Bacillus subtilis*	*Bacillus pumilus*	*Bacillus megaterium*	*Psudomonas fluorescens*
Potato strains					
Pcc_1	0.00	0.90	0.95	0.73	0.70
Pcc_2	0.00	0.91	0.96	0.00	0.00
Pcc_3	0.00	0.95	0.73	0.86	0.85
Pcc_4	0.00	0.88	0.71	0.70	0.81
Sweet potato strain					
Pcc_5	0.00	0.68	0.70	0.70	0.70
Cucumber strains					
Pcc_6	0.00	0.00	0.00	0.00	0.00
Pcc_7	0.00	0.70	0.70	0.65	0.68
Pcc_8	0.00	0.00	0.00	0.00	0.00
Carrot strains					
Pcc_9	0.00	0.00	0.00	0.00	0.00
Pcc_{10}	0.00	0.00	0.00	0.00	0.00
Pcc_{11}	0.00	0.81	0.68	1.00	0.70
Eggplants strains					
Pcc_{12}	0.00	0.66	0.73	0.71	0.00
Pcc_{13}	0.00	0.00	0.70	0.00	0.00
Chili fruits strains					
Pcc_{14}	0.00	0.78	0.85	0.85	0.78
Pcc_{15}	0.00	0.70	0.71	0.66	0.71
Total mean		0.53	0.56	0.46	0.40

L.S.D. 0.05 Strains (I) = 0.02 Bioagent (B) = 0.02I x B = 0.11

above bacterial biocontrol agents inhibited the bacterial growth of *P. carotovorum* subsp. *carotovorum* in the ranges of 0.66–0.95 cm, 0.68–0.96 cm, 0.65 to1.00 cm and 0.68–0.85 cm, respectively (Table 2). Results revealed that strain Pcc_3 showed the highest inhibition by the cultural filtrates of *B. subtilis*, followed by Pcc_2, Pcc_1, Pcc_4, Pcc_{11}, Pcc_{14}, Pcc_7, Pcc_{15}, Pcc_5 and Pcc_{12}, respectively. Strain Pcc_3 also was highly affected the cultural filtrates of *P. fluorescens*, followed by Pcc_4, Pcc_{14}, Pcc_{15}, Pcc_1, Pcc_5, Pcc_{11} and Pcc_7, respectively. Results showed that strain Pcc_2 was highly inhibited by the cultural filtrates of *B. pumilus*, followed by Pcc_1, Pcc_{14}, Pcc_3, Pcc_{12}, Pcc_4, Pcc_{15}, Pcc_5, Pcc_7, Pcc_{13} and Pcc_{11}, respectively, while strain Pcc_{11} was highly inhibited by the cultural filtrates of *B. megaterium*, followed by Pcc_3, Pcc_{14} Pcc_1, Pcc_{12}, Pcc_4, Pcc_5, Pcc_{15} and Pcc_7, respectively (Table 2).

Results revealed that the cultural filtrates of fungal agents, *i.e., T. harzianum, T. viride* and *T. virens*, could inhibit the bacterial growth of all tested *P. carotovorum* subsp. *carotovorum* strains, except Pcc_6 and Pcc_{10} isolated from cucumber and carrot rots, receptively. The above *Trichoderma* spp. inhibited the bacterial growth of soft rot bacteria strains in the ranges of 0.66–1.08 cm, 0.70–0.93 and 0.70–1.1.10 cm, respectively (Table 3). *T. harzianum* was the most effective against strain Pcc_8, followed by Pcc_2, Pcc_{11}, Pcc_1, Pcc_4, Pcc_5, Pcc_7, Pcc_3, Pcc_{13}, Pcc_{14} and Pcc_{15}, respectively. *T. viride* was the most effective against strain Pcc_{15}, followed by Pcc_{11}, Pcc_{14}, Pcc_8, Pcc_7, Pcc_3, Pcc_5, Pcc_4 and Pcc_{12}, respectively. Results showed that *T. virens* was the most effective against strain Pcc_8, followed by Pcc_1, Pcc_{11}, Pcc_2, Pcc_4, Pcc_{14}, Pcc_{15}, Pcc_5, Pcc_3, Pcc_7 and Pcc_9, respectively (Table 3).

In vivo *tests*

a. Potato tuber slices method

Results showed that the cultural filtrates of *B. subtilis*, *B. pumilus*, *B. megaterium*, *P. fluorescens*, *T. harzianum*, *T. viride* and *T. virens* could reduce the incidence of soft rot disease on potato slices when inoculated with pathogenic strains, viz. Pcc_3, Pcc_4 and Pcc_5, at the same time of inoculation and 2 h before or after inoculation (Table 4). Results showed that *B. subtilis* when applied at 2 h before the bacterial pathogen inoculation (strains Pcc_4 and $Pcc_{5)}$ produced highly active rot symptoms on

Table 3 Inhibitory activity of the crude cultural filtrates of fungal agents against the bacterial growth of *Pectobacterium carotovorum* subsp. *carotovorum* strains in vitro tests

P.c. subsp. *carotovorum*	Inhibition zone (cm) of bacterial growth			
	Control	Trichoderma harzianum	Trichoderma viride	Trichoderma virens
Potato strains				
Pcc_1	0.00	0.88	0.00	1.08
Pcc_2	0.00	1.01	0.00	0.90
Pcc_3	0.00	0.71	0.75	0.73
Pcc_4	0.00	0.87	0.70	0.83
Sweet potato strain				
Pcc_5	0.00	0.73	0.75	0.78
Cucumber strains				
Pcc_6	0.00	0.00	0.00	0.00
Pcc_7	0.00	0.73	0.78	0.70
Pcc_8	0.00	1.08	0.81	1.10
Carrot strains				
Pcc_9	0.00	0.00	0.00	0.70
Pcc_{10}	0.00	0.00	0.00	0.00
Pcc_{11}	0.00	0.93	0.88	0.95
Eggplants strains				
Pcc_{12}	0.00	0.00	0.70	0.00
Pcc_{13}	0.00	0.70	0.00	0.00
Chili fruits strains				
Pcc_{14}	0.00	0.70	0.85	0.80
Pcc_{15}	0.00	0.66	0.93	0.78
Total mean	0.60	0.48	0.62	

$L.S.D._{0.05}$ Strains (I) = 0.04 Bioagent (B) = 0.08I x B = 0.15

inoculated potato slices, while Pcc_3 strain produced small active rot symptom. *Bacillus subtilis* when applied at the same time or 2 h after the pathogen inoculation strains Pcc_3, Pcc_4 and Pcc_5 could produce highly active rot symptoms. Results revealed that when *B. pumilus* applied at 2 h before the pathogen inoculation, no soft rot symptoms were occurred on inoculated potato slices with Pcc_3 and Pcc_4 strains, while highly active rot symptoms were produced by strain Pcc_5. *Bacillus pumilus* when applied at the same time of soft rot pathogen inoculation, strains Pcc_3 and Pcc_5 could produce highly active rot symptoms, while no soft rot symptoms were recorded with Pcc_4 strain. Strains Pcc_4 and Pcc_5 produced highly active rot symptoms, while no soft rot symptoms were recorded with strain Pcc_3, when *B. pumilus* applied after 2 h the pathogen inoculation. Results showed that no rotting symptoms were recorded by Pcc_3 and Pcc_4 on inoculated potato slices when *B. megaterium* applied 2 h before or at the same time of pathogen inoculation, while strain Pcc_5 produced highly active rot symptoms. *Bacillus megaterium* when applied after 2 h of pathogen inoculation; strains Pcc_3 and Pcc_5 could produce highly active rot symptoms, while no soft rotting was recorded with Pcc_4. *Pseudomonas fluorescens*, when applied 2 h before pathogen inoculation, could prevent the soft rot symptoms with all applied soft rot strains as well as when it applied at the same time of pathogen inoculation, except Pcc_5 strain which could produce highly active rot symptoms. *Pseudomonas fluorescens* when applied after 2 h of pathogen inoculation; no soft rotting, symptoms were recorded with strains Pcc_3, Pcc_4 and Pcc_5, respectively (Table 4).

Results revealed that *T. harzianum* when applied 2 h before pathogen inoculation, no soft rotting was recorded with Pcc_4 and Pcc_5 strains on inoculated potato slices, while Pcc_3 could produce highly active rot symptoms. *Trichoderma harzianum* also when applied at the same time or 2 h after pathogen inoculation, Pcc_3 and Pcc_4 could not produce any soft rot symptoms, while Pcc_5 could produce highly active rot symptoms. *Trichoderma viride* when applied 2 h before pathogen inoculation strains Pcc_3 and Pcc_5 could produce highly active rot symptoms, while no rotting was recorded with strain Pcc_4. Results showed that no soften symptoms were recorded using *T. viride* when applied at the same time or 2 h after the pathogen inoculation with tested soft rot bacteria, except Pcc_3 and Pcc_5 which could produce highly active rot symptom, respectively. *Trichoderma virens* when applied 2 h before the pathogen inoculation with strains Pcc_3 or Pcc_4 could produce highly active rot symptoms, while no soft rotting was recorded with strain Pcc_5. *T. virens* when applied at the same time or 2 h after of the pathogen inoculation where no soft rotting was recorded, except Pcc_3 and Pcc_5 which could produce highly and small active rot symptom at above periods, respectively (Table 4).

b. Whole potato tubers method

Results revealed that the cultural filtrates of *B. subtilis*, *B. pumilus*, *B. megaterium*, *P. fluorescens*, *T. harzianum*, *T. viride* and *T. virens* could differently protect the whole potato tubers against soft rot disease incidence, under artificially infection in storage, as shown in Table 5. The cultural filtrates of bacterial agents and fungal agents could reduce the soft rot disease incidence in the ranges of 4.5–12.5% and 3.2–21.9%, compared to the ranges of 5.5–83.0% in the untreated control during three months of storage, respectively. Results revealed that *B. pumilus* could protect potato tubers against the soft rot disease during storage period. On the other hand, *B. subtilis* could protect the potato tubers until the 9th week, and then, the soft rot disease incidence reached to 6.0% at the 10th week, followed by 12.2% at the two last weeks of storage. Application of *B. megaterium* could protect

Table 4 Inhibitory activity of the crude cultural filtrates of biocontrol agents against *Pectobacterium carotovorum* subsp. *Carotovorum* strains, when artificially inoculated on potato tuber slices, in vivo tests

Biocontrol agents	Soft rot symptoms								
	2 h. before inoculation			At the same time			2 h. after inoculation		
	Pcc_3	Pcc_4	Pcc_5	Pcc_3	Pcc_4	Pcc_5	Pcc_3	Pcc_4	Pcc_5
Bacterial agents									
Bacillus subtilis	++*	+++	+++	+++	+++	+++	+++	+++	+++
Bacillus pumilus	-	-	+++	+++	-	+++	-	+++	+++
Bacillus megaterium	-	-	+++	-	-	+++	+++	-	+++
Pseudomonas fluorescens	-	-	-	-	-	+++	-	++	+++
Fungal agents									
Trichoderma harzianum	+++	-	-	-	-	+++	-	-	+++
Trichoderma viride	+++	-	+++	+++	-	-	-	-	+++
Trichoderma virens	+++	+++	-	+++	-	-	-	-	++
Pathogen only									
P. c. subsp. *carotovorum* only	+++	+++	+++	+++	+++	+++	+++	+++	+++
Untreated control									
Water only	–	–	–	–	–	–	–	–	-

*-No rotting

+ Restricted rot < 1 cm

++ Small active rot 1-2 cm

+++ Highly active rot > 2 cm

Table 5 Inhibitory activity of biocontrol agents against incidence of bacterial soft rot disease on potato tubers when artificially inoculated in vivo tests

Biocontrol agents	Soft rot incidence (weight losses%) at weeks											
	1	2	3	4	5	6	7	8	9	10	11	12
Bacterial agents												
Bacillus subtilis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	12.2	12.2
Bacillus pumilus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bacillus megaterium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.1	5.1	12.5	12.5
Pseudomonas fluorescens	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5	4.5
Fungal agents												
Trichoderma harzianum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	21.9
Trichoderma viride	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	3.2	3.2	3.2	3.2
Trichoderma virens	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	3.2	3.2	3.2	3.2
Pathogen only												
P. c. subsp. carotovorum only	5.5	16.4	43.7	50.7	83.0	83.0	83.0	83.0	83.0	83.0	83.0	83.0

L.S.D. 0.05 Bioagent (B) = 1.4 weeks (W) = 1.7 B × W = 4.7

potato tubers to the 8th week, and the disease incidence ranged from 5.1 to 12.5% at the four last weeks of storage. *Pseudomonas fluorescens* could protect the potato tubers until the 10th week, and then, the disease incidence was 4.5% at the two last weeks of storage (Table 4). Application of *T. harzianum* could protect the potato tubers until the 10th week of storage, and then, the disease incidence ranged from 8.0 to 21.9% at the two last weeks of storage, respectively. Both *T. viride* and *T. virens* could protect the potato tubers until the 7th week of storage and then the disease incidence was 3.2% at the four weeks of storage (Table 4).

Discussion

The bacterial soft rot disease, caused by *P. carotovorum* subsp. *carotovorum*, causes severe losses in many vegetables in fields, storages and transit, where the losses ranged from 15 to 30% in harvested vegetables and reached 60%

in stored potatoes (Abd-El-Khair 2004). Application of chemical bactericides for controlling bacterial plant diseases is a common practice as well as they cause hazardous effects to human, animals and environment. Application of biological control agents may replace the chemicals and considered to be more effective and environmentally safe (Abd-El-Khair, and Seif El-Nasr 2012; Beric et al. 2012; Abdel-Gaied et al. 2020). Therefore, in this work we applied the cultural filtrates of *B. subtilis*, *B. pumilus*, *B. megaterium*, *P. fluorescens*, *T. harzianum*, *T. viride* and *T. virens* for testing their antagonistic activity against *P. carotovorum* subsp. *carotovorum* (15 strains) which isolated from some vegetables showing typical soft rot symptoms, viz. potato tubers, sweet potato roots, cucumber fruits, carrot roots, eggplant fruits and chili fruits (Mikhail et al. 2019).

Our results in vitro showed that the tested biocontrol agents had variable antagonistic effects against *P. carotovorum* subsp. *carotovorum* isolates, where *B. pumilus* had more antagonistic effect against soft rot bacteria, than *B. subtilis*, *B. megaterium* and *P. fluorescens*. On the other hand, *T.virens* showed more antagonistic effect, than *T. harzianum, T. viride*. Similar results revealed that *B. subtilis* (Rashid et al. 2013) and *P. fluorescens* (Sandipan et al. 2015; El-Hendawy and Abo-Elyousr 2016) had the stronger antagonistic activity against *E. carotovora* subsp. *carotovora* in vitro tests. *Bacillus subtilis* showed the maximum inhibition zones diameter, followed by *P. fluorescens*, *T. harzianum* and *T. viride* against *E. carotovora* subsp. *carotovora* in vitro tests as recorded by Makhlouf and Abdeen (2014) and El-Naggar et al. (2016).

Our results of in vivo tests showed that the cultural filtrate of *P. fluorescens* was more effective in reducing the soft rot disease incidence on soft rot pathogen-inoculated potato slices, when applied 2 h before or at the same time of pathogen inoculation, followed by *B. megaterium* and *B. pumilus*, while cultural filtrate of *B. subtilis* was not effective. On the other hand, *T. virens* was more effective in reducing the disease incidence, when applied at same time or 2 h after of pathogen inoculation, than *T. viride* and *T. harzianum.* It is cleared that the bacterial biocontrol agents may a preventive effect, while *Trichoderma* maybe have a curative effect against soft rot pathogen. These results are in agreement with those recorded by Xu and Gross (1986). They reported that the *fluorescent Pseudomonas* may play role in suppressing the *Erwinia* soft rot by producing the siderophores or antibiotics. Hajhamed et al. (2007) also revealed that *P. fluorescens* and *B.subtilis*, when applied after, before or at the same time of inoculation, could control soft rot disease in potato. *B.subtilis* was the best biocontrol agent against soft rot bacterium after 48 h of incubation in vitro tests (Rashid et al. 2013). El-Naggar et al. (2016) reported that *B. subtilis*, *P. fluorescens*, *T. harzianum* and *T. viride* protected the potato slices against the soft rot development and reduced the amount of tissue maceration in vivo tests.

Storage results showed that the tested biocontrol agents could protect the of potato whole tubers against soft rot disease. *Bacillus pumilus* showed the highest protective, followed by *P. fluorescens*, *B. subtilis* and *B. megaterium*. *Trichoderma harzianum* revealed a higher antagonistic effect, than *T. viride* and *T. virens*. These results are in agreement with those recorded by Rahman et al. (2012). They mentioned that *Bacillus* species showed the stronger antagonistic effect against *E. carotovora* subsp. *carotovora* in vitro tests, where it could reduce the soft rot infection until 22-week storage potatoes. *B. subtilis*; *P. fluorescens* and *T. viride* combined with chitosans (CS) had the stronger antagonistic activity against the growth of *E. carotovora* subsp. *carotovora* in vitro and in storage. The stronger antagonistic activity against *E. carotovora* subsp. *carotovora* was obtained by CS5% treatment with *T. viride*, *P. fluorescens* and *B. subtilis*, respectively. All treatments reduced the soft rot infection until 20-week storage (Makhlouf and Abdeen 2014). El-Hendawy and Abo-Elyousr (2016) reported that *B. subtilis* or *P.fluorescens* could suppress the growth of *Pectobacterium atrosepticum* growth individually or in combination in vitro, under greenhouse and field conditions, where the combination had a beneficial effect.

Conclusion

It can be concluded that fungal biocontrol agents, *i.e., T. harzianum*, *T. viride* and *T.virens*, and bacterial biocontrol agents, *i.e. B. subtilis*, *B. pumilus*, *B. megaterium* and *P. fluorescens* could control the most of *P.carotovorum* subsp. *carotovorum* isolates causing the soft rot disease varied in vitro and in vivo tests. Results revealed that the tested biocontrol agents were varied in their antagonistic activity against bacterial soft rot isolates in vitro tests, where the biocontrol cultural filtrates could not reduce the bacterial growth of some pathogenic isolates. In vivo tests, *T. viride* and *T. virens* were highly effective in reducing soft rot symptoms on inoculated potato tuber slices, when applied at the same time or 2 h before pathogen inoculation, while *B. megaterium* and *T. harzianum* were high effective when applied at the same time or 2 h after pathogen inoculation. In whole potato tubers technique, *B.pumilus* highly protected the stored potato tuber, than *P. fluoresces*, *T. harzianum*, *B. subtilis*, *T. viride*, *T. virens* and *B. megaterium*, respectively. It is clear that the application of *Trichoderma* spp. or bacterial species could play an important role in controlling bacterial soft rot disease in vegetables.

Abbreviations
T.: *Trichoderma*; *B.*: Bacillus; *P.*: *Pseudomonas* and *Pectobacterium*.

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Authors' contributions
TGA prepared the materials and carried out the experiment in the open field; MSM performed supervision and reviewing the manuscript; AIA was involved in data analysis and visualization; HIS wrote the manuscript; HAE was involved in suggesting the problem and helped in writing the manuscript. All authors revised, read and approved the final manuscript.

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The tested biocontrol agents, plant species and bacterial soft rot pathogens are available in Egyptian environment and were identified in the laboratory.

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The authors declare that they have no competing interests.

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