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Field application of bio-control agents and aqueous plant extracts for controlling bacterial soft rot and enhancement yield quality of *Solanum tuberosum* L. cv. Diamond

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Abstract

Background: Application of chemical bactericides, for controlling soft rot bacteria, causes environmental pollution and toxic hazards to human. In addition, it is ineffective, expensive, and limited. Therefore, application of bio-control agents, plant extracts, or safe chemicals may play an important role as safe alternative approaches for controlling phyto-pathogenic bacteria.

Objective: This work is aimed to apply bio-control agents (*Bacillus subtilis*, *Bacillus pumilus*, *Trichoderma harzianum*, and *Trichoderma virens*), aqueous plant extracts (lantana flowers and leaves, lemongrass leaves, and olive cake), and citric acid as pre-sowing treatment for controlling bacterial soft rot pathogen and study their ability for improving yield and quality of potato tubers in field and storage. All treatments were applied as soil treatment and/or foliar spray, except citric acid applied as foliar spray only.

Results: The cultural filtrates of bio-control agents of *B. subtilis*, *B. pumilus*, *T. harzianum*, and *T. virens*; aqueous plant extracts of lantana, lemongrass, and olive cake; and citric acid could protect daughter potato tubers against bacterial soft rot disease in field application, except lemongrass (as soil treatment). The bio-control agents highly increased the activities of peroxidase, polyphenol oxidase, and chitinase enzymes, than other treatments. The bio-control agents also improved the shoot parameters, viz, shoot length, number of shoots/pit, and number of leaves/pit and yield parameters, viz, tuber weight/pit, tubers number/pit, and total tubers weight/pit, compared to citric acid and plant extracts, respectively. The treatments as foliar spray have good results for protecting the potato tubers through storage, than soil treatment. The treatments highly enhanced the stored potato tubers quality, viz, dry matter, reducing sugars, total carbohydrates, specific gravity, and starch content.

Conclusions: The tested treatments could protect the potato tubers in field and/or storage against soften development. The treatments could improve the plant growth and yield parameters in field as well as enhanced the stored potato tubers quality and increase the stored time. It is clear that the treatments can be applied as pre-sowing treatment for controlling *Erwinia* soft rot bacteria.

Keywords: Field application, Bacteria soft rot, Pre-sowing treatments, Storage, Safe treatments

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Background

Erwinia carotovora subsp. *carotovora*, the causal agent of soft rot disease, is one of the worldwide destructive bacterial pathogen in vegetables including potato (*Solanum tuberosum* L.). The disease may occur on potato tubers in the field, in transit, and in storage causing economic damage to tuber yield reaching up to 2, 10, and 60%, respectively. Soft rot bacterium can live as epiphyte and endophyte on plant debris or as saprophyte in soil. Then, the bacterium could penetrate the plant host through wounds or natural openings (Makhlouf-Abbeer and Abdeen, 2014; Viswanath et al. 2018). The bacterium can release enzymes of cellulase, protease, pectate lyase, polygalacturanase, and pectin-methylesterase which degrade the plant cell wall. The bacterium has the widest host range of many economically important vegetables including eggplant, cabbage, carrot, Chinese cabbage, cucumber, garlic cloves, onion bulbs, pepper, potato tubers, radish roots, squash, sweet potato, and tomato (Bhat et al. 2012). Application of chemical bactericides for controlling soft rot bacteria is ineffective and their usefulness for disinfecting of potato tubers is limited. In addition, it is ineffective and expensive and had harmful effect to environment and human health (Van der Wolf and de Boer 2007). Therefore, application of bio-control agents, aqueous plant extracts, or safe chemicals may play an important role as safe alternative approaches for controlling soft rot pathogen.

Field application of *Trichoderma harzianum* and *Bacillus subtilis* could prevent the development of soft rot disease in daughter potato tubers, when applied as pre-sowing treatments. The treatments also increased the plant growth parameters of potato (Abd-El-Khair and Haggag 2007). The strongest antagonistic activity against *E. carotovora* subsp. *carotovora* was obtained by application of *Trichoderma viride*, *Pseudomonas fluorescens*, and *B. subtilis* combined with chitosans, respectively, where all treatments also could reduce the soft rot development until 20-week storage (Makhlouf and Abdeen 2014). Application of aqueous plant extract of neem leaves or seeds (Bdliya and Dahiru, 2006) as well as mahogany bark (Bdliya and Abraham 2010) significantly reduced the incidence and severity of soft rot disease in potato tubers under artificially inoculation conditions. The Jute leaf extract significantly protected the treated potatoes until 22 weeks against soft rot development in storage (Rahman et al. 2012). Aqueous plant extracts of lemongrass, garlic, aloe vera, and neem had the greatest inhibitory effects against *Erwinia* sp. (Simeon and Abubaker 2014). The cold water leaf extract of neem has the most inhibitive against *E. carotovora* using agar diffusion method (Ijato 2016). Immersion of potato tubers in aqueous solutions (1%) of each citric, acetic, ascorbic, or malonic acid alone significantly reduced the soft rot

symptoms (Bartz and Kelman 1986). Acetic acid or boric acid showed the bactericidal activity against *P. carotovorum* subsp. *carotovorum* in vitro tests. All treatments significantly decreased the infection rate or weight loss. Boric acid was the most effective in controlling the soft rot disease of potato in storage, than acetic acid (Rahman et al. 2017).

This work is aimed to study the bactericide effects of four of bio-control agents (*Bacillus subtilis*, *Bacillus pumilus*, *Trichoderma harzianum*, and *Trichoderma virens*), three of Egyptian plant species [lantana flowers and leaves (*Lantana camara* L.), lemongrass leaves (*Cymbopogon citrates* L), and olive cake (*Olea europaea* L.)], and citric acid, against soft rot pathogen. All treatments were applied at pre-sowing as soil treatment or foliar spray, except citric acid was applied as foliar spray only. The ability of applied treatments for protecting daughter potato tubers and improving the growth and potato tuber yield in field as well as reducing soft rot incidence in stored potato tubers and enhancing their quality was studied.

Methods

Antibacterial treatments

Two of rhizobacteria, i.e., *B. subtilis* and *B. pumilus*, and two *Trichoderma* spp., i.e., *T. harzianum* and *T. virens*, were obtained from Plant Pathology Dept., National Research Centre, Egypt; three of Egyptian plant species, viz, lantana, lemongrass, and olive cake, were applied as soil treatment and/or foliar spray, while citric acid as safe chemical was applied as foliar spray treatment only.

Preparation of cultural filtrates of bio-control agents

For preparation of cultural filtrates of bacterial bio-control agents, nutrient glucose (2%) broth [peptone 5 g, beef extract 3 g, glucose 20 g, distilled water 1 L, pH 7.2] was prepared and sterilized in conical flasks. A medium flask was separately inoculated with 1 ml of 48-h-old culture of each *B. subtilis* and *B. pumilus*. Then, the inoculated flasks were incubated at 30 ± 2 °C for 48 h. The antagonistic bacterial cells were centrifuged at 4500 rpm for 15 min, and then, the supernatant was filtered through filter paper (Whatman no.1). The cultural filtrates were sterilized by filtration through sterile 0.45- μ m membrane filter (cellulose nitrate, Whatman). Then, the crude supernatant of each bacterial antagonist was kept at -20 °C until used (Tshikalange et al. 2005).

For preparation of cultural filtrates of *Trichoderma* spp., Martin medium [glucose 10 g, peptone 5 g, KH_2PO_4 1 g, MgSO_4 0.5 g, Rose Bengal 30 μ g, streptomycin 0.03 g, distilled water 1 L] was prepared and sterilized in conical flasks. The medium flask was separately inoculated with 1-cm-diameter disc of 1-week-old culture of each *Trichoderma* spp. The inoculated flasks

were incubated at 30 ± 2 °C for 1 week. Then, the *Trichoderma* spp. propagules were filtered through filter paper (Whatman no. 1). The cultural filtrates were sterilized by filtration through sterile 0.45- μ m membrane filter (cellulose nitrate, Whatman), and then, the crude supernatant of each *Trichoderma* spp. stored as mentioned before (Tshikalange et al. 2005).

Preparation of aqueous plant extracts

Cold water extraction method was applied for plant extract preparation. The dried parts of each plant species were firstly grinded into powder using a hand grinder, and then, about 20 g of each plant material was separately soaked in 100-ml sterilized distilled water in 250-ml conical flask overnight in a refrigerator. Then, the aqueous plant extract was firstly filtered through double-layered muslin cloth twice in conical flasks, and then, each plant extract was sterilized by filtration through sterile 0.45- μ m membrane filter (cellulose nitrate, Whatman). All aqueous plant extracts were stored as mentioned before until used (Tshikalange et al. 2005).

Field experiments

Field experiment, in a randomized complete design, was conducted at El-Kanater, Kalubiyah Governorate, Egypt, during the summer growing season (February–May, 2017) for testing of different materials. All treatments were applied at pre-sowing in field. The field experiment was subjected to under naturally infection with *E. carotovora* subsp. *carotovora*. Field experiment (66 plots) consisted of small plots 4 m² (2 × 2 m) in an area with three replicates (plots) was applied for each treatment as well as the controls. Each plot consisted of four lines, where each line included six pits (24 pits/plot). In soil treatments, *B. subtilis*, *B. pumilus*, *T. harzianum*, and *T. virens* were applied at 50-ml antagonist filtrate in sowing hole, while aqueous plant extract of each lantana, lemongrass, and olive cake were applied at the rate of 50 ml in sowing hole. In foliar spray, the same above treatments were applied at 3–4 leaf growth stage of plant at rate of 50-ml antagonist filtrate or 50-ml plant extract per 1 m² of area. Citric acid was applied at 50 ml of solution 10% per 1 m² of area. For controls, PDB or NB media were applied. One tuber-piece cv. Diamond containing one or more sprouts was sown in each pit. Irrigation and fertilization were carried out as recommended. Effect of the tested materials was recorded on:

Soft rot incidence

The incidence of soft rot was recorded as percentages of soften daughter potato tubers' weight to whole tubers' weight in each treatment at harvest time according to

the modified formula described by Abd-El-Khair and Haggag (2007).

$$\text{Soften incidence\%} = \frac{\text{Soften potato tuber weight}}{\text{Whole of potato tubers weight}} \times 100$$

Enzymatic plant activity

Six potato plants were randomly chosen from each treatment as well as controls after 2 months of sowing. Potato leaves were kept in ice box and then transferred to the laboratory for enzymatic activity studies. Leaf tissue (5 g) was homogenized with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM B-mercaptoethanol at the rate of 1/3 W/V. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was applied to determine enzyme activities (Tuzun et al. 1989).

Peroxidase (POD) Peroxidase activity was determined by incubation of 0.1 of plant extract with 4 ml of guaiacol for 15 min at 25 °C. The guaiacol solution consisted of 3 ml of 0.05 M potassium phosphate (pH 5.7), 0.5 ml of guaiacol 2%, and 0.5 ml of H₂O₂ 0.3%. Peroxidase activity was expressed as the increase in absorbance at 470 nm (Abeles et al. 1971).

Polyphenol oxidase (PPO) The activity of polyphenol oxidase was determined using the method described by Matta and Dimond (1963). The reaction mixture contained 0.1 ml of plant extract, 0.5 ml of 0.3 M potassium phosphate (pH 6.5), and 0.1 ml of 3(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) brought to a final volume of 3.0 ml with distilled water. The activity of polyphenol oxidase was expressed as the increase in absorbance at 475 nm (Morsy 2005).

Chitinase (Ch) Colloidal chitin was prepared from chitin powder (Sigma Co.) according to the method described by Ried and Ogryd-Ziak (1981). About 20 g of chitin powder suspended in 250 ml of phosphoric acid (85%) was stored at 4 °C for 24 h, and then, they were blended in 2 l of distilled water using an awning blender. The suspension was centrifuged. This washing procedure was repeated twice. The colloidal chitin suspension was adjusted to pH7 with 1 N NaOH and re-centrifuged. The pelleted colloidal chitin was stored at 4 °C until used. One milliliter of colloidal chitin was added to 3 ml of 0.05 M citrate phosphate buffer (pH 6.6) in test tubes. One milliliter of plant extract was added and mixed by shaking. Tubes were incubated in water bath at 37 °C for 60 min, then cooled and centrifuged before assaying. Reducing sugars were determined in 1 ml of the supernatant by adding 1 ml of dinitro-salicylic acid

(DAS) solution. After boiling to 5 min, the optical density was determined at 540 nm (Monreal and Reese 1969).

Plant growth and yield parameters

Six potato plants were randomly chosen from each treatment as well as controls after 60 days of sowing. Effect of the aforementioned materials on the averages of plant growth parameters, i.e., shoot length/pit, number of shoots/pit, and number of leaves/pit, was recorded. At harvest time, the averages of yield parameters, i.e., tuber weight/pit, tuber number/pit, and the total tuber weight/pit, were recorded.

Storage experiment

After harvest, potato tuber yield of six potato pits were separately collected from field experiment, where potato tubers with no visible soft rot symptoms were chosen. Then, the potato tubers of each treatment were separately stored in plastic net. Three plastic nets were employed as replicates for each treatment as well as the controls. The tubers' weight in each plastic net was recorded in the beginning of experiment. The potato tubers were stored for 3 months at room temperature.

Effect on soft rot incidence

Stored potato tubers were weekly assayed for development of soft rot incidence according to the modified formula described by Abd-El-Khair and Haggag (2007).

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

Effect on quality of stored potato tubers

Dry matter content Dry matter content of stored potato tubers was determined at 65 °C for 72 h using the standard methods as illustrated by AOAC (1990).

Reducing sugars Total reducing sugars of stored potato tubers were extracted by ethanol (80%), then water replaced ethanol, and lead acetate was clarified. The excess of the lead acetate was precipitated by sodium oxalate. Reducing total sugars were determined in the clarified solution as described by Shaffer and Hartman (1921). The non-reducing sugars were calculated from the difference between the percentage of reducing sugars and total sugars.

Determination of total carbohydrates Total carbohydrates of stored potato tubers were determined according to the method described by Dubois et al. (1956). About 5 ml of sulfuric acid (67%) was added to 0.1 g of the dry matter of potato tuber in a test tube. One hour

later, the volume was completed to 100 ml with distilled water and the solution was filtered. One milliliter of the filtrate was pipetted into a test tube, and then, 1 ml of aqueous phenol solution (5%) was added to the solution, followed by 5 ml of concentrated sulfuric acid from a fast delivering pipette. Measurements of the color intensity were taken by using a spectrophotometer at 490 nm, and the content was calculated from the standard curve of pure glucose.

Specific gravity Tuber specific gravity of stored potato tubers of stored potato tubers was calculated from the sample weight in air and water.

Starch content The specific gravity of stored potato tubers was used to calculate the starch amount as methods described by Butron (1948).

Statistical analysis

Data were subjected to analysis of variance using Computer Statistical Package (CO-STATE) User Manual Version 3.03, Barkley Co., USA. The means were compared at 5% level of probability by Duncan's new multiple range test (Snedecor and Cochran 1980).

Results

Field experiment

Effect of soft rot incidence

Results revealed that the cultural filtrates of bio-control agents, viz, *B. subtilis*, *B. pumilus*, *T. harzianum*, or *T. virens*, and aqueous extracts of lantana, lemongrass, or olive cake (as soil treatments or foliar spray treatments) as well as citric acid (as foliar spray treatment) could protect the daughter tubers against naturally soft rot infection, where no soft rot symptoms were recorded at harvest time, except NB medium only and lemongrass (as soil treatment) which show the percentages of soft rot incidence about 20.4 and 17.9%, respectively.

Effects on plant enzymatic activity

In soil treatment, the cultural filtrates of bio-control agents could increase the activity of POD, PPO, and Ch enzymes in the ranges of 214–222%, 292–1047%, and 342–633%, where rhizo-bacteria highly increased the activity of tested enzymes, than *Trichoderma* spp., respectively. Aqueous plant extracts could increase the activity of POD, PPO, and Ch enzymes in the ranges of 151–152%, 239–292%, and 267–317%, respectively. Lemongrass, olive cake, and lantana highly increased the POD activity, while lantana, lemongrass, and olive cake highly increased the PPO and Ch enzymes, respectively (Table 1).

In foliar spray, the cultural filtrates of bio-control agents increased the activity of POD, PPO, and Ch enzymes in the ranges of 89–116%, 255–453%, and 379–

Table 1 Effect of bio-control agents, aqueous plant extracts, and citric acid as soil treatment and/or foliar spray on plant enzyme activity of peroxidase, polyphenol oxidase, and chitinase in field application

Treatments	Plant enzymatic activity											
	Soil treatment						Foliar spray					
	Peroxidase		Polyphenol oxidase		Chitinase		Peroxidase		Polyphenol oxidase		Chitinase	
	OD	Incr.%	OD	Incr.%	OD	Incr.%	OD	Incr.%	OD	Incr.%	OD	Incr.%
<i>Bacillus subtilis</i>	2.711a*	222	0.413a	1047	0.088a	633	2.300abc	89	0.210a	453	0.067cde	379
<i>Bacillus pumilus</i>	2.675 a	218	0.164a	356	0.078ab	550	2.583ab	112	0.195a	413	0.103bc	636
<i>Trichoderma harzianum</i>	2.638 a	214	0.147bc	308	0.077ab	542	2.634a	116	0.187a	392	0.070 cd	400
<i>Trichoderma virens</i>	2.665 a	217	0.141bc	292	0.053abc	342	2.609a	114	0.135bcd	255	0.106bc	657
Lantana	2.051 b	144	0.141bc	292	0.050bc	317	2.106bcd	73	0.128 cd	237	0.017ef	21
Lemongrass	2.117 b	152	0.122bc	239	0.048bc	300	1.934cde	59	0.165abc	334	0.133b	850
Olive cake	2.107 b	151	0.126bc	250	0.044c	267	2.053cde	69	0.183ab	382	0.064cdef	357
Citric acid	NT						2.197abc	80	0.202a	432	0.221a	1479
PDB medium only	1.494 c	78	0.106bc	194	0.043 cd	258	1.635def	34	0.054e	42	0.026def	86
NB medium only	1.665 c	98	0.107bc	197	0.035 cd	192	1.609a	32	0.086de	126	0.030def	114
Control	0.840 d	-	0.036 c	-	0.012 d	-	1.218f	-	0.038e	-	0.014f	-

NT not test

*Means in each column followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

657%. *Trichoderma* spp. highly increased the POD activity than rhizo-bacteria, while rhizo-bacteria highly increased the activity of PPO and Ch enzymes, than *Trichoderma* spp., respectively. Aqueous plant extracts increased the activity of POD, PPO, and Ch enzymes in the ranges of 59–73%, 237–382%, and 21–580%, respectively. Lantana, lemongrass, and olive cake highly increased the POD enzyme; olive cake, lemongrass, and lantana increased the activity of PPO enzyme, while olive cake, lemongrass, and lantana highly increased Ch enzyme, respectively (Table 1).

Plant growth parameters

In soil treatment, the cultural filtrates of bio-control agents increased the shoot length (SL) in the ranges of 50.3–54.1%, where *Trichoderma* spp. highly increased SL than rhizo-bacteria, respectively. The cultural filtrates of bio-control agents increased the shoot number (SN) in the ranges of 100.0–200.0%, where *T. virens* had highly increased the SN, than *B. pumilus*, *B. subtilis*, and *T. harzianum*, respectively. The same treatments increased the leaf number (LN) in the ranges of 247.9–287.4%, where *T. virens* had highly increased, than *B. pumilus*, *T. harzianum*, and *B. subtilis*, respectively. Aqueous plant extracts increased the growth parameters, e.g., SL, SN, and LN, in the ranges of 35.2–52.2%, 60.0–140.0%, and 105.2–201.0%, respectively. Lantana highly increased all shoot parameters, followed by olive cake and lemongrass, respectively (Table 2).

In foliar spray, the cultural filtrates of bio-control agents increased the SL in the ranges of 58.7–69.4%, where rhizo-bacteria highly increased the shoot length,

than *Trichoderma* spp., respectively. The SN of potato was increased in the ranges of 16.3–62.8%, while LN increased in the ranges of 76.2–126.7% when applied of cultural filtrates of bio-control agents. *B. pumilus* had highly increased the two growth parameters, than other agents. Applications of aqueous plant extracts increased SL, SN, and LN in the ranges of 62.5–75.0%, 42.2–44.6%, and 108.9–120.8% with aqueous plant extracts. Olive cake highly increased SL and LN, while lantana highly increased SN, respectively. The above plant growth parameters were 41.2, 66.7, and 78.2% with citric acid when applied as foliar spray only, respectively (Table 2).

Yield parameters

In soil treatment, the average of potato tuber weight increased in the ranges of 17.2–65.1% with antagonistic, where *Trichoderma* spp. highly increased tuber weight, than rhizo-bacteria, respectively. Aqueous plant extracts increased the tuber weight averages in the ranges of 10.0–26.9%, where lemongrass had highly increase tuber weight, followed by lantana and olive cake. The potato tuber number per pit was increased in the ranges of 17.2–65.1% with bio-control agents, where *Trichoderma* spp. showed highly increased potato number, than rhizo-bacteria. The tuber number ranged from 27.9 to 67.4% with aqueous plant extracts, where lemongrass, followed by lantana and olive cake highly increased the potato number, respectively. The total tuber yield per pit ranged from 79.7 to 93.2% with bio-control agents, compared to 43.4–88.2% with aqueous extracts. *B. subtilis*, followed by *T. harzianum*, *T. virens*, and *B. pumilus*, while aqueous extracts of lantana, followed by

Table 2 Effect of bio-control agents, aqueous plant extracts, and citric acid as soil treatments and/or foliar spray on shoot parameters of potato plants in field application

Treatments	Soil treatment						Foliar spray					
	Shoot length		Shoot no./pit		Leaves no./pit		Shoot length		Shoot no./pit		Leaves no./pit	
	cm	Incr.%	No.	Incr.%	No.	Incr. %	cm	Incr.%	No.	Incr.%	No.	Incr.%
<i>Bacillus subtilis</i>	47.8a*	50.3	2.6ab	160.0	66.8a	247.9	53.8a	68.1	2.0abcd	62.8	39.8a	97.0
<i>Bacillus pumilus</i>	48.2a	51.6	2.8ab	180.0	73.4a	282.3	54.2a	69.4	2.6a	20.9	45.8a	126.7
<i>Trichoderma harzianum</i>	49.0a	54.1	2.0b	100.0	72.8a	279.2	50.8ab	58.7	2.4ab	16.3	38.8ab	92.1
<i>Trichoderma virens</i>	48.4a	52.2	3.0a	200.0	73.8a	287.4	51.6ab	61.2	2.2abc	16.3	35.6abc	76.2
Lantana	48.4a	52.2	2.4ab	140.0	57.8ab	201.0	52.0a	62.5	1.6bcd	67.4	42.2a	108.9
Lemongrass	43.0ab	35.2	1.6bc	60.0	39.4b	105.2	53.6a	67.5	2.4ab	27.9	42.8a	111.9
Olive cake	46.6a	46.5	2.2ab	120.0	46.0b	139.6	56.0a	75.0	1.4 cd	27.9	44.6a	120.8
Citric acid	NT						45.2b	41.2	2.0abcd	66.7	36.0abc	78.2
PDB medium only	35.6bc	11.9	1.3bc	30.0	24.8bc	26.3	36.8c	15.0	1.3 cd	8.3	27.4 cd	45.6
NB medium only	35.6bc	11.9	1.4bc	40.0	26.4bc	37.5	35.8c	11.9	1.3 cd	8.3	28.4bcd	40.6
Control	31.8c	-	1.0c	-	19.2c	-	32.0c	-	1.2d	-	20.2d	-

NT not test

*Means in each column followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

lemongrass and olive cakes highly increased the yield of potato tubers, respectively (Table 3).

In foliar spray, the averages of potato tuber weight/pit were in the ranges of 71.4–104.0% when applied the cultural filtrates of antagonistic, and it was in the ranges of 11.2–41.6% with aqueous plant extracts application, compared to 39.9% with citric acid, respectively. The cultural filtrates of antagonistic highly increased tuber weight average, than others. The potato tuber number per pit was increased in the ranges of 23.8–54.8% with cultural filtrates of bio-control agents, while it was ranged from 26.2 to 59.5% with

aqueous plant extracts, compared to 59.5% with citric acid, respectively. *T. harzianum* and lemongrass highly increased the potato tuber number parameters, than others. The total tuber yield per pit ranged from 126.8 to 178.6% with cultural filtrates of bio-control agents, where *T. virens* and *B. subtilis* highly increased the total tubers yield, than *B. pumilus* and *T. harzianum*, respectively. The yield per plant was in the ranges of 50.8–117.3% with aqueous plant extracts, compared to 125.1% citric acid, respectively. Olive cake highly increased the yield, than lemongrass and lantana, respectively (Table 3).

Table 3 Effect of bio-control agents, aqueous plant extracts, and citric acid as soil treatment and/or foliar spray on yield parameters of potato plants in field application

Treatments	Soil treatment						Foliar spray					
	Tuber weight/pit		Tuber no./pit		total yield /pit		Tuber weight/pit		Tuber no./pit		Total yield /pit	
	g	Incr.%	No.	Incr.%	g	Incr.%	g	Incr.%	No.	Incr.%	g	Incr.%
<i>Bacillus subtilis</i>	83.7bc*	17.2	7.0ab	17.2	590.5a	93.2	146.3a	104.0	5.3bcd	26.2	753.1ab	154.9
<i>Bacillus pumilus</i>	101.1ab	40.4	5.2c	40.4	549.3a	79.7	138.7ab	93.4	5.2bcd	23.8	709.1bc	140.0
<i>Trichoderma harzianum</i>	119.5a	65.1	5.0c	65.1	581.2a	90.1	122.9b	71.4	5.7abc	35.7	669.9bc	126.8
<i>Trichoderma virens</i>	118.0a	63.9	5.0c	63.9	557.2a	82.3	130.5ab	82.0	6.5a	54.8	822.9a	178.6
Lantana	83.6bc	16.7	7.2a	16.7	575.3a	88.2	84.7 cd	18.1	5.3bcd	26.2	445.4de	50.8
Lemongrass	91.4bc	26.9	5.5bc	26.9	506.1ab	65.6	79.7d	11.2	6.7a	59.5	529.4d	79.2
Olive cake	79.2bc	10.0	5.5bc	10.0	438.4abc	43.4	101.5c	41.6	6.3ab	50.0	641.9c	117.3
Citric acid	NT						100.3c	39.9	6.7a	59.5	667.6bc	125.1
PDB medium only	74.6c	2.6	4.5c	2.6	335.8bc	9.8	74.8d	4.3	4.6 cd	9.5	344.3ef	16.6
NB medium only	80.8bc	8.8	4.5c	8.8	363.5bc	18.9	75.0d	4.6	4.7a	11.9	352.6ef	19.4
Control	72.0c	-	4.3c	-	305.7c	-	71.7d	-	4.2d	-	295.4f	-

NT not test

*Means in each column followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

Storage experiment

Effect on soft rot incidence

In soil treatment, the cultural filtrates of *B. subtilis* and *B. pumilus* could protect the stored potato tubers against soft rot development for the 1st month of storage, and then, the soften incidence reached up to 9.3 and 28.3% at the 12th week, respectively. On the other hands, the cultural filtrates of *T. harzianum* and *T. virens* could protect the stored potato tubers for the 1st and 2nd weeks, and then, the soften incidence reached up to 27.2 and 31.8% at the 12th week, respectively. Aqueous extracts of lantana and lemongrass protected potato tubers for the 4th week, while olive cake could protect tuber for the 1st week, where soften incidence reached up to 21.1, 40.2, and 21.5% at the 12th week, respectively (Table 4).

In foliar spray, the cultural filtrates of *B. subtilis* and *B. pumilus* protected the stored potato tubers for the 1st and 11th weeks, where the soften incidence reached up to 30.5 and 17.0% at the 12th week, respectively. The cultural filtrates of *T. virens* could protect the potato tubers until the 2nd week, while the cultural filtrates of *T. harzianum* protected the potato tubers for the 3rd week, and then, soften incidence reached up to 25.4 and 18.8% at the 12th week. The aqueous extract of lantana protected the potato tubers until the 6th week, and the disease soften incidence was 18.1% at the 12th week of storage. Lemongrass and olive cake aqueous extract protected the stored potato tubers until the 4th week, where disease incidence was 8.8 and 28.1% at the 12th week, respectively. Citric acid protected the stored potato tubers until the 4th week of storage, and then, the soft rot incidence was 14.3% at the 12th week of storage (Table 5).

Effect on quality of stored potato tubers

In soil treatment, the percentages of dry matter were in the ranges of 20.8–23.6% with cultural filtrates of bio-

control agents, while it was in the ranges of 19.7–25.1% in stored potato tubers treated aqueous plant extracts, compared to 18.5% in untreated control, respectively. The reducing sugars in the ranges of 46.4–50.3 mg/100 g was significantly increased with cultural filtrates of bio-control agents, while it was in the ranges of 41.2–44.5 mg/100 g, compared to 51.5 mg/100 g in untreated control, respectively. The total carbohydrates in stored potato tubers were in the ranges of 38.6–43.0% with cultural filtrates of bio-control agents, while in the ranges 43.3–49.7% with aqueous plant extracts, compared to 35.0%, respectively. The bio-agents increased the specific gravity which was in the ranges of 1.04–1.08 g/cm³ in stored potato tubers with cultural filtrates of bio-control agents, while it was in the ranges were in the ranges of 1.02–1.08 g/cm³ with the aqueous plant extracts, compared to 1.02 g/cm³ in untreated control, respectively. The starch content was in the ranges of 41.8–49.4% in storage potato tubers with cultural filtrates of bio-control agents, and it ranged from 48.2 to 49.7% with aqueous plant extracts, compared to 45.9% in untreated control, respectively (Table 6).

In foliar spray, the dry matter of stored potato tubers was in the ranges of 20.4–22.8% with cultural filtrates of bio-control agents, while it was in the ranges of 19.7–26.0% with aqueous extracts increased, compared to 20.6% with citric acid and 18.5% in untreated control, respectively. The reducing sugars of stored potato tubers were in the ranges of 50.7 mg/100 g with cultural filtrates of bio-control agents, while it was in the ranges of 42.0–44.5 mg/100 g with aqueous plant extracts, compared to 40.4 mg/100 g with citric acid and 51.5 mg/100 g, in untreated control, respectively. The total carbohydrates in stored potato tubers were in the ranges of 36.6–49.7% with cultural filtrates of bio-control agents, while it was in the ranges of 44.3–47.4% with aqueous plant extracts,

Table 4 Effect of bio-control agents and aqueous plant extracts as soil treatment against bacterial soften potato tubers disease (as weight losses %) under storage conditions

Treatments	Softening tubers (weight losses%) at weeks											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
<i>Bacillus subtilis</i>	0c*	0d	0e	0e	9.3e	9.3e	9.3e	9.3e	9.3g	9.3i	9.3h	9.3h
<i>Bacillus pumilus</i>	0c	0d	0e	0f	9.4e	9.4e	9.4e	9.4e	28.3b	28.3c	28.3c	28.3d
<i>Trichoderma harzianum</i>	0c	6.5b	6.5d	6.5d	11.2bc	11.2bc	11.2bc	16.4b	27.2c	27.2d	27.2d	27.2e
<i>Trichoderma virens</i>	0c	0d	7.7c	7.7c	11.6b	11.6b	11.6b	17.1b	17.1e	17.1g	17.1f	31.8c
Lantana	0c	0d	0e	0e	10.8bc	10.8bc	10.8bc	11.5c	11.5f	11.5h	11.5g	21.1f
Lemongrass	0c	0d	0e	0e	11.3bc	11.3bc	11.3bc	28.1a	31.5a	31.5b	31.5b	40.2b
Olive cake	0c	6.9b	6.9cd	6.9cd	6.9f	6.9f	6.9f	12.1c	12.1f	12.1h	12.1g	21.5f
PDB medium only	3.3b	3.3c	9.8b	9.8b	9.8de	9.8de	9.8de	11.2cd	17.8e	21.6f	27.3d	27.3e
NB medium only	2.6b	2.6c	7.6c	7.6c	10.5cd	10.5cd	10.5cd	10.5d	22.3d	23.4e	25.2e	40.5b
Control	9.8a	9.8a	11.6a	14.6a	19.2a	22.5a	25.5a	28.8a	31.6a	35.3a	42.0a	48.8a

*Means in each column followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

Table 5 Effect of bio-control agents, plant extracts, and citric acid as foliar spray against bacterial soften potato tubers disease (as weight losses %) under storage conditions

Treatments	Softening tubers (weight losses%) at weeks											
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
<i>Bacillus subtilis</i>	0d*	4.7c	4.7c	4.7e	4.7 h	4.7i	4.7i	4.7j	4.7i	21.0d	21.0d	30.5c
<i>Bacillus pumilus</i>	0d	0d	0e	0e	0i	0j	0j	0 k	0j	0j	0j	17.0 h
<i>Trichoderma harzianum</i>	0d	0d	0e	6.9c	6.9f	6.9 h	6.9 h	6.9i	6.9 h	6.9i	6.9i	18.8f
<i>Trichoderma virens</i>	0d	0d	6.3b	6.3d	6.3g	9.5e	9.5e	9.5g	14.4f	18.1e	18.1e	25.4e
Lantana	0d	0d	0e	0f	0i	0j	9.3e	13.2e	18.1d	18.1e	18.1e	18.1g
Lemongrass	0d	0d	0e	0f	8.8d	8.8f	8.8f	8.8 h	8.8g	8.8 h	8.8 h	8.8j
Olive cake	0d	0d	0e	0f	12.6c	12.6d	15.1d	23.6c	23.6c	23.6c	23.6c	28.1d
Citric acid	0d	0d	0e	0f	8.1e	8.1g	8.1g	10.9f	14.3f	14.3g	14.3g	14.3i
PDB medium only	4.3c	4.3c	4.3d	14.3a	14.3b	14.3c	16.2c	16.2d	16.2e	16.2f	16.2f	18.4g
NB medium only	4.9b	4.9b	4.9c	11.2b	19.1a	31.9a	31.9a	36.5a	38.6a	38.6a	40.4b	40.4b
Control	9.8a	9.8a	11.6a	14.6a	19.2a	22.5b	25.5b	28.8b	31.6b	35.3b	42.0a	48.8a

*Means in each column followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

compared to 50.7% with citric acid and 35.0% in untreated control, respectively. The specific gravity was in range of 1.05–1.08 g/cm³ and 1.03–1.089 g/cm³ with cultural filtrates of bio-control agents and aqueous plant extracts, compared to 1.09 g/cm³ with citric acid and 1.02 g/cm³ in untreated control, respectively. The starch content in storage potato tubers was in the ranges of 42.3–50.8% and 50.0–50.8% with bio-control agents and aqueous plant

extract, compared to 51.1% with citric acid and 45.9% in untreated control, respectively (Table 6).

Discussion

The obtained results revealed that bio-control agents, viz, *B. subtilis*, *B. pumilus*, *T. harzianum*, *T. virens*; aqueous plant extracts, viz, lemongrass, lantana, and olive cake; and citric acid protected the daughter potato

Table 6 Effect of bio-control agents, aqueous plant extracts, and citric acid as soil treatment and/or foliar spray on quality parameters of potato tubers under storage conditions

Treatments	Soil treatments					Foliar spray				
	Dry matter (%)	Reducing sugars (mg/100 g)	Total carbohydrates (%)	Specific gravity (g/cm ³)	Starch content (%)	Dry matter (%)	Reducing sugars (mg/100 g)	Total carbohydrates (%)	Specific gravity (g/cm ³)	Starch content (%)
<i>Bacillus subtilis</i>	20.8 cd*	47.3de	43.0d	1.07ab	49.4a	21.8 cd	48.1b	49.7ab	1.06bc	42.3d
<i>Bacillus pumilus</i>	23.2b	46.4e	44.0 cd	1.08a	48.2ab	22.8c	46.6c	43.9c	1.08ab	50.8a
<i>Trichoderma harzianum</i>	23.6b	50.3abc	36.7ef	1.04d	43.5 cd	20.4de	50.7a	36.6ef	1.08ab	47.6Bc
<i>Trichoderma virens</i>	20.9 cd	49.6bc	38.6e	1.06bc	41.8de	20.6de	49.5b	37.7de	1.05 cd	45.6 cd
Lantana	23.6b	41.2g	49.7a	1.08a	49.7a	26.0a	42.0e	47.4b	1.08ab	50.0ab
Lemongrass	19.7de	42.8 fg	46.4bc	1.07ab	48.2ab	19.7ef	43.7d	44.3c	1.03de	50.8a
Olive cake	25.1a	44.5f	43.3d	1.02e	49.4a	21.7 cd	44.5d	45.0c	1.09a	50.0ab
Citric acid	NT					20.6de	40.4e	50.7a	1.09a	51.1a
PDB medium only	19.7de	51.4ab	35.6f	1.07ab	45.4c	19.4ef	50.1a	36.0ef	1.03de	48.5abc
NB medium only	21.9c	48.8 cd	48.7ab	1.05 cd	40.2e	24.5b	48.9b	39.6d	1.07abc	43.9d
Control	18.5e	51.5a	35.0f	1.02e	45.9bc	18.5f	51.5a	35.0f	1.02e	45.9 cd

NT not test

*Means in each column followed by different letter(s) are significantly different according to Duncan's multiple range test at $P \leq 0.05$

tubers against soft rot infection in field application, except lemongrass as soil treatment. The bio-agents also highly increased the activity of POD, PPO, and Ch enzymes, than citric acid and plant extracts. The protective activities of bio-agents may be related with their ability in increasing of tested enzyme activities, where these enzymes play an important role as defense mechanism against soil-borne pathogens. Application of biotic or chemical agents increased the defense of plants against diseases, where resistance induction enhanced the plants health status against diseases either by application of inducers through optimized soil management techniques or by foliar application (Zeller 2006; Song et al. 2013; Tamma et al. 2011). These results are in agreement with those recorded by Caruso et al. (2001) and Nawar and Kuti (2003). They reported that the bio-control agent accumulation of some enzymes, viz, Ch, POD, and PPO, plays an important role in plant defense mechanisms against pathogen infection, where there are positive relationships between enzymatic activity and resistance development in plants.

Trichoderma spp., as soil treatments, also increased the levels of Ch, POD, and PPO enzymes activity in treated bean plants (Abd-El-Khair and Khalifa, 2010). *B. subtilis* and *T. harzianum*, as seed-coating formulation, significantly increased the growth parameters as well as increased defense-related enzymes, i.e., PPO, POD, and superoxide dismutase (SOD) activities in treated tomato seedlings (Kumar et al. 2015). The systemic induction of defense-related genes expression and significant increase in antioxidant enzyme activities of SOD, POD, catalase (CAT), PPO, and phenylalanine ammonia-lyase (PAL) could play a pivotal role in disease resistance against *E. carotovora* subsp. *carotovora* when applied with *B. subtilis* in tomato as reported by Chandrasekaran and Chun (2016). The high levels of PAL, PPO, POD, and total phenols were obtained in a single application of *B. pumilus* and *B. amyloliquefaciens* against *P. carotovorum* subsp. *carotovorum* strains after 8 h of application in potato (Gerayeli et al. 2017). Aqueous plant extracts of basil, chilli, castor beans, chamomile, lantana, lemongrass peppermint, and onion seeds increased the activities of Ch, PPO, and POD enzymes in bean plants (Abd-El-Khair and El-Gamal-Nadia, 2011). Plant extracts of two-leaf plants rich in bioactive phenolic compounds, olive and carob, reduced the soft rot severity by increase the activity of PAL after 7.5 h of application on potato tuber slices (Ouanas et al. 2017). The spent green tea extracts (SGT) were able to kill *Pectobacterium* spp., where it led to the significant decrease in pectin lyase, polygalacturonase, and pectin methyl esterase activity in inoculated carrots (Joe et al., 2017).

In soil treatment, the cultural filtrates of bio-control agents improved the shoot parameters, viz, length,

number of shoots, and number of leaves/pit of potato plants, as well as increased the yield parameters of potato tubers, viz, tuber weight, tuber number, and total tuber weight/pit, when compared with aqueous plant extracts. The same trend was noticed with same treatments, followed by citric acid in foliar spray. These results are in agreement with those recorded by Malik et al. (2005). They reported that *T. harzianum*, as soil amendments, significantly increased the plant heights and weight. *B. subtilis* and *T. album* improved plant height, number of stems, and number of leaves/pit of treated potato. *B. subtilis* gave the best tuber yield as tuber treatments, while *T. album* was the best as a soil application with potatoes cv. Nicola (Abd-El-Khair and Seif El-Nasr, 2012).

Our results showed that the bio-control agents as well as aqueous plant extracts highly protected stored potato tuber in foliar spray, than soil treatment, where *B. pumilus*, *T. harzianum*, *B. subtilis*, and *T. virens*, followed by lantana, olive cake, and lemongrass, were the most effective for protecting stored potato tubers. The treatments also highly enhanced the potato tubers' quality, viz, dry matter, reducing sugars, total carbohydrates, specific gravity, and starch content. These results are in agreement with those recorded by Makhlof-Abeer and Abdeen (2014). They reported that *T. viride*, *P. fluorescens*, and *B. subtilis* combined with chitosans showed a stronger antagonistic activity against *E. carotovora* subsp. *carotovora*, respectively, and all treatments reduced the soft rot development until 20-week storage. The Jute leaf extracts significantly protected the treated potatoes against the soft rot development until 22 weeks under storage conditions (Rahman et al. 2012). Aqueous plant extracts of *Datura stramonium* and *Trigonella foenum graecum* (seeds) and *Azadirachta indica* (leaves) inhibited the growth of *E. carotovora* in vitro tests (Viswanath et al. 2018). Finally, these results are in agreement with the recent trend in shifting toward safer and more eco-friendly alternatives for controlling of postharvest decays, where the use of antagonistic micro-organisms is becoming popular throughout the world. *B. subtilis* and *T. harzianum* are being used (Sharma et al. 2009).

Conclusion

It can be concluded that bio-control agents of *Bacillus subtilis*, *Bacillus pumilus*, *Trichoderma harzianum*, and *Trichoderma virens* and aqueous plant extracts of lantana, lemongrass, and olive cake can be applied as pre-sowing for controlling bacterial soft rot disease caused by *Erwinia carotoora* subsp. *carotovora* in potato field. These treatments also improved the growth and yield parameters of potato plants. The treatments could protect the stored potato tubers against soften development and enhancement of the quality of potato tubers

Abbreviations

POD: Peroxidase; PPO: Polyphenol oxidase; Ch: Chitinase; DAS: Dinitro-salicylic acid; NB: Nutrient broth; SL: Shoot length; SN: Shoot number; LN: Leaves number; SOD: Superoxide dismutase; CAT: Catalase; PAL: Phenylalanine ammonia-lyase

Authors' contributions

Prepared the materials and carried out the experiment in the open field—TGA; supervision and reviewing the manuscript—MSM; data analysis and visualization—AlA; writing the manuscript—HIS; suggesting the problem and helped in writing the manuscript—HAE. All authors revised, read, and approved the final manuscript.

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Availability of data and materials

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

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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