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Soft Rot Disease Management of Imported Potato Designed for Cultivation During Early Summery Season in Egypt.

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ABSTRACT

The objectives of this study were to evaluate the role of four bioagents in the management potato soft rot disease, caused by *Erwinia carotovora* subsp. *carotovora*, either in field production or during storage. In the *in vitro* tests, *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride* antagonists have restricted the growth of *E. c.*subsp. *carotovora* by formation inhibition zone in agar assays. Maximum inhibition zones diameter was found with *B. subtilis*, followed by *P. fluorescens, T. harzianum* and *T. viride*, being 33.6, 30.0, 28.5 and 16.0 mm, respectively. In the *in vivo* tests, using the method of "slices of potato" bacterial and fungal biocontrol agents protected potato slices from the development of soft rot and reduced the amount of tissue maceration. In field experimente, tuber pieces coating before sowing with compost amended with *B. subtilis* followed by foliage spraying with *B. subtilis* suspension at 46, 60 and 80 days after planting, protected the potato tuber against soft rot disease, where the percentage of soft rot incidence were 4.5 and 4.0%, comparing with the incidence of 23.5 and 25.2 % in untreated plants, during the first and the second seasons, respectively. The above treatment increased the lengths of stem (plant height), the number of leaves and tuber yield. The above treatment also reducing the soft rot decay in stored daughter potato tubers for 12 weeks at room temprature. It was followed by each of *P. fluorescens* and *T. harzianum*, while *T. viride* was the less one. It could be suggested from the present study that, the pretreatment of potato tuber pieces with the identified biocontrol-agents followed by foliage spraying can retard the incidence of soft rot disease during crop cycle, improvement potato growth, increase the yield and protect stored daughter potato tubers from soft rot decay during storage.

Keywords: Potato plants, soft rot disease, biocontrol agents, field production and storage conditions.

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INTRODUCTION

Potato (*Solanum tuberosum* L.) is a worldwide cultivated tuber-bearing plant which is the fourth main food crop in the world after rice, maize and wheat, in terms of both area cultivated and total production [1, 2, 3]. In Egypt, potato crop has an important position among all vegetable crops, where about 20% of total area devoted for vegetable production is cultivated with potato. In addition, the total cultivation of potatoes reached 197,250 feddans which produce 2,039,350 tons of tubers with an average yield of 10.34 tons/feddan [4].

Bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora* (Jone) Dye is the major disease pathogens affecting potato seed tuber pieces after cultivation, during vegetative growth and on potato tubers during storage [5, 6, 7]. Approximately 22% of potatoes are lost per year due to viral, bacterial, fungal, and pest attack to potato tuber and potato plant, incurring an annual loss of over 65 million tones and bacterial soft rot alone accounts for 30-50% of this huge loss [7]. The effect of the disease is more pronounced in the countries where appropriate storage facilities are lacking or inadequate. In Egypt, *E.c.* subsp. *carotovora* is a well known pathogen of potato and was detected in many other Mediterranean countries [8, 9]. Tuber soft rot is initiated at lenticels, the stolon end and ⁄ or in wounds under wet conditions. The first symptom is the development of a water-soaked area on the tuber around a lenticel or the eye. If you cut through one of these lesions you'll find the tissue is extremely soft. The lesions rapidly expand and grow together and the tuber is destroyed. Frequently, all that remains of diseased tubers is the skin. These symptoms are found most frequently in packaged potatoes or in storage, but soft rot can occur in the field before harvest. In the field, the early decay of seed tuber pieces can result in nonemergency or blanking [10, 11, 6, 7]. When the rotted mother tubers could emerged, infection of the stems can occur [12]. Disease incidence of soft rot can be reduced by antibacterial treatments of seed tubers in field application [13]. Most compounds used contain antibiotics (mainly streptomycin and its derivatives). However, although treatments with antibiotics showed promise, larger-scale field studies are no longer allowed because of the risks of introducing resistance to bacterial pathogens of humans or animals. Biological control of plant pathogenic bacteria could be an alternative to chemical and physical control and breeding for resistance. Biocontrol strategies comprise the use of antagonists affecting pathogen populations directly, or via antibiosis, competition for nutrients or induction of plant systemic resistance [14, 15]. The strategy for biological control of plant diseases involves the use of antagonistic microorganisms before or after the infection takes place. Currently, the bacteria *Bacillus subtilis* and *Pseudomonas* spp. and the fungi *Gliocladium virens* and *Trichoderma* spp. are the organisms mostly used for biological control strategies [16, 17, 18, 19, 20]. Earlier studies reported that antagonistic fungi and rhizospheric bacteria have significant antagonistic activity against plant pathogenic bacteria including soft rot *Erwinia* genera [8, 21, 22, 23, 24]. Long *et al*. [21] reported that the genus Bacillus and fluorescent pseudomonads have antagonistic activity against various plant pathogenic bacteria including soft rot bacterium *E. carotovora* subsp. *carotovora* (Ecc) *in vitro*. Rahman *et al*. [23] found that only two isolates *Bacillus* E-45 and E-65 significantly inhibited the *in vitro* growth of *E. carotovora* subsp. *carotovora* Ecc P-138. The ability of these isolates to suppress the growth of various phytopathogenic bacteria makes them potential biocontrol agents. Many researchers previously exploited *B. subtilis*, *P. fluorescens* and *T. harzianum* to control soft rot bacteria in various plants [25, 22, 8]. The objective of this work aimed to study the role of four bioagents in management potato soft rot disease in field production and decreasing the softening tubers during storage.

MATERIALS AND METHODS

Source of *Erwinia carotovora* subsp. *carotovora* and potato tubers

The pathogen *Erwinia carotovora* subsp. *carotovora*, used throughout this study had been isolated from rotted potato tuber showing typical soft rot symptoms. The bacterial pathogen was isolated [26] using Nutrient glucose (2%) agar medium (3 g beef extract; 5 g peptone; 20 g glucose; 15 g agar and 1000 mL distilled water. pH, 7. 2). The isolated bacteria was identified in the Laboratory of Bacteriology, Plant Pathology Dept., National Research Centre, Giza, Egypt, according to pathological, morphological, cultural, physiological and biochemical characters [27] and confirmed by PCR using the specific primers. *E. carotovora* subsp. *carotovora* inocula were obtained by grown in Nutrient-broth medium (3 g beef extract; 5 g peptone; 20 g glucose; and 1000 ml distilled water. PH, 7. 2). Bacterial culture were incubated at 30^{o}C for 48 h. Then, the bacterial peletes were harvested and adjusted to cell density of about 10^{8} CFU/mL. Meanwhile, potato tubers

(cv. Nicola) obtained from Dept., of Vegetables Crop Research, Agricultural Research Centre, Giza, Egypt used in this study.

Biocontrol agents

The biocontrol agents, *viz*. *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma harzianum* and *T. viride*, were kindly obtained from Plant Pathology Dept., National Research Centre, Giza, Egypt. Inocula of each *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride* were prepared individually and the suspension was adjusted to 10^6 propagules / mL for fungal isolate and to an approximately 10^8 colony forming unit CFU/mL for bacterial isolates [28, 29].

Compost

Compost used as a carrier to biocontrol agents were purchased from the Egyptian company for solid waste utilization. Chemical analysis of the tested compost (Table, 1) was kindly carried out at the Nutrition Dept., (NRC) according to Ma & Zauzage [30] and Isaac and Johnson [31]. Compost was autoclaved for 30 min at 121 ^{o}C and 1.5 air pressure. Then, the bioagents suspensions (10^6 propagules / mL for fungal and 10^8 CFU / mL for bacterial biocontrol agents) were added individually to compost at the dose of 10 mL suspension / 100 g compost. Inoculated compost placed in under shadow was immediately used for tuber coating.

Table 1: Chemical analysis of the compost used as a carrier to biocontrol agents.

Total Nitrogen (%)	1.53
Total Phosphorus (%)	0.29
Total Potassium (%)	1.13
Total Iron (ppm)	1368
Total Zinc (ppm)	70.0
Total Copper (ppm)	12.0
Total organic matter (%)	31.0
Organic Carbon (%)	33.4
Carbon / Nitrogen ratio	12:1
pH (1:100)	8.1
EC	1:100
Humidity (%)	8.54

Antibiosis activity test

Antibiosis activity of the bacterial isolates, *viz*. *P. fluorescens* and *B. subtilis*, against the bacterial soft rot pathogen *E. carotovora* subsp. *carotovora* was tested *in vitro* using the method described by Faquihi *et al*. [20] with some modifications. Briefly, the bacterial suspension of *E. carotovora* subsp. *carotovora* with a cell density of about 10^8 CFU/mL was spread out on a petri dish containing 20mL of yeast peptone dextrose agar (YPDA) medium with four replicates of each bacterium. Excess of suspension was eliminated and the agar plate was dried for 15 min. Once dry, the disks (0.5 cm diameter) with the cream of antagonistic bacterial strains obtained from 72 h- old culture were deposited on the middle and the dishes were incubated at 30°C for 48h. The antibacterial activity was assayed by observing inhibitory zones in the background of petri dish after incubation period. When an inhibition zone appeared, its diameter was measured to evaluate the antibacterial activity of the probable antagonistic bacteria [32]. Each assay was performed in triplicate. Degree of antagonism shown was determined by measuring the average diameter of clear zone of inhibition. On the other hand, antibiosis activity of the fungal isolate *T. harzianum* and *T. viride* was tested *in vitro* using disc diffusion method [32]. A disc of 5 mm in diameter from *T. harzianum* and *T. viride* obtained from 7-days old culture was placed on the surface of oat meal agar OMA plates seeded with potato soft rot pathogen. The plates were incubated at 28^{o}C for 48 h. The inhibition zone around the discs indicated the antagonistic interaction and average diameter of clear zone were measured.

Slices of potato test

The antagonistic activity of the bacterial and fungal isolates *viz*. *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride* against the bacterial soft rot pathogen *E. carotovora* subsp. *carotovora* was tested *in vivo* using

the method of "slices of potato" used by Lapwood and Cans [33] and modified by Faquihi *et al*. [20]. Entire potato tubers (cv. Nicola) were weighted and soaked in a sodium hypochlorite solution (10%) for 10 min and rinsed with sterile distilled water. Then the tubers were aseptically cut into slices (2 cm), in each slice well of 1 cm diameter and 1 cm deep is carried in the middle using a sterilized cork borer (1-cm-diam.). The potato slices were put in Petri dishes containing sterilized filter paper impregnated with 2 mL of sterile distilled water. Thereafter, 100 µl of antagonistic suspension (10^6 propagules / mL for fungal isolate and approximately 10^8 CFU/mL for bacterial isolates) was injected in the well, and the test was incubated at 30°C for 24h. Afterwhile 24 h, 100 µL of pathogen suspension (10^6CFU/mL) was added to each treatment. Four repeats for each treatment are chosen with a positive control (soft rot pathogen only). All the treatments were incubated at 30°C for 24 h. Disease severity was estimated using the percentage of weight losses after rotting tissues were removal [34]. Percentage of disease reduction (PDR) was also calculated [19] as the following formula:

$$PDR = \frac{Ack - Atr}{Ack} \times 100$$

where Ack and Atr represent the severity of the disease in control and treated specimens, respectively.

Field experiment

Field experiments were carried out during two successive seasons at Omar Makram Village, El-Tahrir county, El-Behera governorate, to evaluate the suppressive effect of tested bacterial and fungal isolates *viz*. *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride* used as tuber piece coating and its integrated treatments with foliar spraying on soft rot incidence (%) in field production and during storage conditions.

All treatments as well as non-treated control were replicated three times in a randomized complete block design and each consisted of plots each three lines in width and 3.6 m length. Each line include 12 pits and one seed piece was sown in each pit. Seed tuber (cv. Necola) were cut longitudinally using sterilized knife into pieces with 2-3 sprout per piece. The potato seed pieces have been disinfected before use by deceiving in a solution of sodium hypochlorite solution (10%) for 10 min and rinsing twice with sterile distilled water. Disinfected potato seed pieces was air dried for 24 h under shadow place. Then, seed tuber pieces were coated before planting with compost amended with each of *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride* individually, and then they were planted in loamy clay well-drained soil to a depth of 10 cm. After 40, 60 and 80 days of planting, integrated treatments with foliar spraying of antagonistic suspension (10^6 propagules / mL for fungal isolate and approximately 10^8 CFU/mL for bacterial isolates) were done. Ten treatments were realized:

1. Tuber pieces coating with compost amended with *B. subtilis*.
2. Tuber pieces coating with compost amended with *P. fluorescens*.
3. Tuber pieces coating with compost amended with *T.harzianum*.
4. Tuber pieces coating with compost amended with *T.viride*.
5. Control (Tuber pieces coating with compost only).
6. Tuber pieces coating with compost amended with *B. subtilis* followed by foliage spraying with *B. subtilis* suspension at 46, 60 and 80 days after planting.
7. Tuber pieces coating with compost amended with *P. fluorescens* followed by foliage spraying with *P. fluorescens* suspension at 46, 60 and 80 days after planting.
8. Tuber pieces coating with compost amended with *T.harzianum* followed by foliage spraying with *T.harzianum* suspension at 46, 60 and 80 days after planting.
9. Tuber pieces coating with compost amended with *T.viride* followed by foliage spraying with *T.viride* suspension at 46, 60 and 80 days after planting.
10. Control (Tuber pieces coating with compost only followed by foliage spraying with sterilized water only).

In addition, irrigation and nutrients such as phosphorus, nitrogen and potassium were added to ensure adequate plants nutrition during mid-growth and tuberization as recommended [35]. Data on soft rot incidence was recorded during the crop cycle in 21-February to 21-May, as the percentage of lack of emergence plus potato plants showing aerial stem rot [8].

Vegetative growth and yield characters

A random sample of nine plants were taken 80 days after planting from each treatment to determent the average of stem length (plant height) and average number of leaves per plant [36]. Effect of biocontrol agents application on potato tuber yield under field conditions was studied. Therfore, potato tuber were harvested after 120 days of planting. Tuber yield per each treatment was recorded and the average of the tuber yield (metric ton / hectare) was calculated for each treatment.

Storage experiment

Samples of harvested potato tubers (apperently healthy from soft rot symptoms) were collected from each field application treatments and stored separately for 3 months at room temprature under natural infection conditions. Stored potato tubers were examined for the presence of soft rot symptoms through the storage period. Percentage of softening tubers, for each treatment, was calculated at the end of storage period. Percentage of disease reduction (PDR) was calculated according to **Hajhamed *et al*. [19]** as the following formula:

$$PDR = \frac{Ack - Atr}{Ack} \times 100$$

where Ack and Atr represent the incidence of the softening in control and treated tubers, respectively.

Statistical analysis

Statistical analyses of all the previously designed experiments were carried out according to (ANOVA) procedures reported by Snedecor and Cochran [37]. Treatment means were compared by the least significant difference test "LSD" at 5% level of probability.

RESULTS

Laboratory experiment

***In vitro* inhibition of *E. carotovora* subsp. *carotovora* growth**

In the *in vitro* tests, *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride* antagonists have restricted the growth of the soft rot pathogen of potato, *E. carotovora* subsp. *carotovora*. The diameter of inhibition zones ranged from 16.0 to 33.6 mm. Maximum inhibition zones diameter was found with *B. subtilis*, followed by *P. fluorescens, T. harzianum* and *T. viride*, being 33.6, 30.0, 28.5 and 16.0 mm, respectively (Table 2).

Table 2: Antibiosis activity of different biocontrol agents against *E. carotovora* subsp. *carotovora* as inhibition zone shown in agar plate.

Antagonism	Antibiosis activity
	Inhibition zone (mm)
Bacillus subtilis	33.6 a
Pseudomonas fluorescens	30.0 b
Trichoderma harzianum	28.5 c
Trichoderma viride	16.0 d

Means designated with the same letter in the same column in each season are not significantly different at 0.05 level of probability.

Potato slices test

The treatment *in vivo* with the bacterial and fungal biocontrol agents has shown a protection against the development of soft rot and reduced the amount of tissue maceration caused by *E. carotovora* subsp. *carotovora* in wounds of potato tuber slices (Table 3). For the antagonistic bacteria (*P. fluorescens* and *B. subtilis*) the protection reached 45 and 50.0%, whereas for antagonistic fungi (*T. harzianum* and *T. viride*) the

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protection reached 10 and 30.0%, compared to the control, which showed a spongy texture and 100% rotted tissue (Table 3).

Table 3: *In vivo* suppressive effect of different biocontrol agents against tuber soft-rot pathogen using the method of "slices of potato" under artificial infection conditions.

Treatment condition*	Tuber soft-rot severity (%) and reduction (%)	
	Soft-rot severity (%)**	Reduction (%)
Tuber coating with *B. subtilis*	50.0 d***	50.0
Tuber coating with *P. fluorescens*	55.0 d	45.0
Tuber coating with *T.harzianum*	70.0 c	30.0
Tuber coating with *T. viride*	90.0 b	10.0
Control	100 a	-

*Tuber coating was carried out by individual biocontrol agent and then inoculated with *Erwinia carotivora* var *carottivora*. ** Disease severity was estimated using the percentage of weight losses after rotting tissues were removal. *** Means designated with the same letter in the same column in each season are not significantly different at 0.05 level of probability.

Field experiment

Control of soft rot incidence during the crop cycle

In our field experimental conditions, the assay of biological control of *E. carotovora* subsp. *carotovora*, showed that the treatment by biocontrol agents inhibited the development of the soft rot and the incidence of disease was reduced by the range of 6.4 to 80.9 % and 16.7 to 84.1% during the first and the second seasons, respectively (Table 4).

Table 4: Effect of tuber pieces coating either alone or in combination with foliar spraying with different biocontrol agents on potato soft-rot incidence under field conditions.

Treatment condition*	Potato soft-rot	
	Incidence** (%)	Reduction (%)
First season		
Tuber pieces coating with *B. subtilis*	8.0 d***	65.9
Tuber pieces coating with *P. fluorescens*	14.0 c	40.4
Tuber pieces coating with *T.harzianum*	20.0 b	14.8
Tuber pieces coating with *T. viride*	22.0 a	6.4
Tuber pieces coating followed by foliage spraying with *B. subtilis*	4.5 e	80.9
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	7.5 e	68.1
Tuber pieces coating followed by foliage spraying with *T.harzianum*	13.0 c	44.7
Tuber pieces coating followed by foliage spraying with *T. viride*	15.0 b	36.2
Control	23.5 a	-
Second season		
Tuber pieces coating with *B. subtilis*	7.0 d	72.2
Tuber pieces coating with *P. fluorescens*	13.2 c	47.6
Tuber pieces coating with *T.harzianum*	17.3 b	31.3
Tuber pieces coating with *T. viride*	21.0 a	16.7
Tuber pieces coating followed by foliage spraying with *B. subtilis*	4.0 d	84.1
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	7.2 c	71.4
Tuber pieces coating followed by foliage spraying with *T.harzianum*	12.3 b	51.2
Tuber pieces coating followed by foliage spraying with *T. viride*	13.0 b	48.4
Control	25.2 a	-

* Tuber coating was carried out by individual biocontrol agent and sowing directly, foliage spraying was carried out by individual biocontrol agent at 40, 60 and 80 days after sowing, and the combined treatment were also done. **Data on soft rot incidence was recorded during the crop cycle in 21-February to 21-May, as the percentage of lack of emergence plus potato plants showing aerial stem rot. ***Means designated with the same letter in the same column in each season are not significantly different at 0.05 level of probability.

It was obseved that, integrated treatments showed stronger effecivity than single treatment by each of biocontrol agents in reducing the incidence of soft rot. The field application of tuber pieces coating before sowing with compost amended with *B. subtilis* followed by foliage spraying with *B. subtilis* suspension at 46, 60 and 80 days after planting, protected the potato tuber against soft rot disease, where the percentage of soft rot were 4.5 and 4.0%, compared with the incidence of 23.5 and 25.2 % in untreated plants, during the first and the second seasons, respectively (Table 4). The treatment *B. subtilis* was followed by the integrated treatment of each *P. fluorescens* and *T. harzianum*. Data presented in Table 4 also showed that the field application of *T. viride* either a single or integrated treatments were less effective in reducing the incidence of soft rot during the crop cycle (Table 4).

Control of soft rot incidence during storage conditions

After 3 months of storage at room temprature and under natural infection conditions, the percentage of potato decay, causing by soft rot disease, was in the range of 10.5 to 20.0 % with *B. subtilis*, 13.0 to 24.5 % with *P. fluorescens*, 20.5 to 30.0% with *T. harzianum* and 30.0 to 31.5% with *T. viride* comparing with 32.0 and 35.0 % in the un-treated plants control (Table, 5). It was obseved that, integrated treatments under field condtions showed stronger effecivity than single treatment by each of biocontrol agents in reducing the incidence of soft rot decay in stored daughter potato tubers. It is obvious that the field application of tuber pieces coating before sowing with compost amended with *B. subtilis* followed by foliage spraying with *B. subtilis* suspension at 46, 60 and 80 days after planting, were the most effective in reducing the soft rot decay in stored potato tubers. Results demonstrated that stored daughter potato tubers can be stored for 12 weeks with less percentage of softening tubers, when seed pieces were treated before sowing with bioagents of *B. subtilis* and/or *P. fluorescens* and integrated with the same bioagents foliage spraying. It was followed by the integrated treatment of *T. harzianum*. Data presented in Table 5 also showed that the field application of *T. viride* either in single or in integrated treatments were less effective in protection daughter potato tubers free from soft rot decay in stored potato tubers.

Table 5: Effect of tuber pieces coating either alone or in combination with foliar spraying with different biocontrol agents on potato soft-rot incidence during storage at room temprature for three months under natural infection conditions.

Treatment condition*	Tuber soft-rot during storage	
	Incidence (%)	Reduction (%)
First season		
Tuber pieces coating with *B. subtilis*	20.0 c**	37.5
Tuber pieces coating with *P. fluorescens*	24.5 b	23.4
Tuber pieces coating with *T.harzianum*	30.0 a	6.3
Tuber pieces coating with *T. viride*	31.0 a	3.1
Tuber pieces coating followed by foliage spraying with *B. subtilis*	12.0 d	62.5
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	15.0 d	53.1
Tuber pieces coating followed by foliage spraying with *T.harzianum*	25.0 b	21.9
Tuber pieces coating followed by foliage spraying with *T. viride*	30.0 a	6.3
Control	32.0 a	-
Second season		
Tuber pieces coating with *B. subtilis*	19.5 e	44.3
Tuber pieces coating with *P. fluorescens*	23.0 d	34.3
Tuber pieces coating with *T.harzianum*	28.5 c	18.6
Tuber pieces coating with *T. viride*	31.5 b	10.0
Tuber pieces coating followed by foliage spraying with *B. subtilis*	10.5 g	70.0
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	13.0 f	62.9
Tuber pieces coating followed by foliage spraying with *T.harzianum*	20.5 de	41.4
Tuber pieces coating followed by foliage spraying with *T. viride*	31.5 b	10.0
Control	35.0 a	-

* Tuber coating was carried out by individual biocontrol agent and sowing directly, foliage spraying was carried out by individual biocontrol agent at 40, 60 and 80 days after sowing, and the combined treatment were also done. **Means designated with the same letter in the same column in each season are not significantly different at 0.05 level of probability.

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Effect of biocontrol agents application on vegetative growth under field conditions

Data in Table (6) show that, under field conditions, applying the biocontrol agent treatments as seed pieces coating either individually or in integration with foliage spraying, increased both the lengths of stem (plant height) and the number of leaves in treated plants compared to untreated plants.

Table 6: Effect of tuber pieces coating either alone or in combination with foliar spraying with different biocontrol agents on some vegetative characters of potato plants grown under field conditions.

Treatment condition*	Vegetative characters	
	Stem height (cm)	No. leaves/stem
First season		
Tuber pieces coating with *B. subtilis*	51.0 c**	26.0 d
Tuber pieces coating with *P. fluorescens*	50.0 c	26.0 d
Tuber pieces coating with *T.harzianum*	50.0 c	25.5 d
Tuber pieces coating with *T. viride*	48.5 d	24.0 e
Tuber pieces coating followed by foliage spraying with *B. subtilis*	57.0 a	31.0 a
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	53.0 bc	28.0 c
Tuber pieces coating followed by foliage spraying with *T.harzianum*	55.0 b	30.0 b
Tuber pieces coating followed by foliage spraying with *T. viride*	50.5 c	26.0 d
Control	43.0 e	22.0 f
Second season		
Tuber pieces coating with *B. subtilis*	25.5 bc	28.0 b
Tuber pieces coating with *P. fluorescens*	49.1 cd	26.0 bc
Tuber pieces coating with *T.harzianum*	50.3 c	24.3 d
Tuber pieces coating with *T. viride*	47.2 d	21.0 e
Tuber pieces coating followed by foliage spraying with *B. subtilis*	62.0 a	35.0 a
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	51.2 bc	29.2 bc
Tuber pieces coating followed by foliage spraying with *T.harzianum*	49.5 b	31.5 b
Tuber pieces coating followed by foliage spraying with *T. viride*	50.3 c	21.2 e
Control	40.0 e	20.2 e

* Tuber coating was carried out by individual biocontrol agent and sowing directly, foliage spraying was carried out by individual biocontrol agent at 40, 60 and 80 days after sowing, and the combined treatment were also done. **Means designated with the same letter in the same column in each season are not significantly different at 0.05 level of probability.

It is clear that integrated treatments with each of biocontrol agent highly improved potato growth parameters than single treatment. Potato tuber pieces coating before sowing with compost amended with *B. subtilis* followed by foliage spraying with *B. subtilis* suspension at 46, 60 and 80 days after planting, gave the highest potato parameters, where the value of stem lengths (plant height) and the value of leaves number were (57.0 cm & 31.0) and (62.0cm & 35.0), compared with (43.0cm & 22.0) and (40.0cm & 20.2) in untreated plants, during the first and the second seasons, respectively (Table 6). It was followed by the integrated treatment of each of *P. fluorescens* and *T. harzianum*. Data also showed that the field application of *T. viride* either a single or an integrated treatments were less effective in improving potato plant parameters.

Effect of biocontrol agents application on potato tubers yield under field conditions

Potato tubers yield per feddan was tried with the same observation of vegetative growth parameters (Table, 7). It is clear that integrated treatments with each of biocontrol agent gave the highest potato tuber per feddan than single treatment.The higher increase in potato yield per feddan was obtained with *B. subtilis* (ranged from 33.6 to 49.6%) and *P. fluorescens* (ranged from 30.5 to 41.9%), during the two growing seasons (Table 7). The moderate increase in potato tuber yield with obtained with *T. harzianum* (ranged from 28.6 to 45.6%), while *T. viride* (ranged from 26.5 to 38.2%) showed the less one.

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Table 7: Effect of tuber pieces coating either alone or in combination with foliar spraying with different biocontrol agents on tuber yield of potato plants grown under field conditions.

Treatment condition*	Tuber yield (metric ton/ hectare)	
	Yield	Increase (%)
First season		
Tuber pieces coating with *B. subtilis*	26.9 b**	33.6
Tuber pieces coating with *P. fluorescens*	25.7 c	30.5
Tuber pieces coating with *T.harzianum*	25.0 c	28.6
Tuber pieces coating with *T. viride*	24.3 c	26.5
Tuber pieces coating followed by foliage spraying with *B. subtilis*	29.8 a	40.0
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	28.6 a	37.5
Tuber pieces coating followed by foliage spraying with *T.harzianum*	27.9 b	35.9
Tuber pieces coating followed by foliage spraying with *T. viride*	25.7 c	30.6
Control	17.9 d	-
Second season		
Tuber pieces coating with *B. subtilis*	28.6 b	43.3
Tuber pieces coating with *P. fluorescens*	25.2 c	35.8
Tuber pieces coating with *T.harzianum*	23.8 c	32.0
Tuber pieces coating with *T. viride*	24.5 c	33.9
Tuber pieces coating followed by foliage spraying with *B. subtilis*	32.1 a	49.6
Tuber pieces coating followed by foliage spraying with *P. fluorescens*	27.9 bc	41.9
Tuber pieces coating followed by foliage spraying with *T.harzianum*	29.8 b	45.6
Tuber pieces coating followed by foliage spraying with *T. viride*	26.2 bc	38.2
Control	16.2 d	-

* Tuber coating was carried out by individual biocontrol agent and sowing directly, foliage spraying was carried out by individual biocontrol agent at 40, 60 and 80 days after sowing, and the combined treatment were also done. * *Means designated with the same letter in the same column in each season are not significantly different at 0.05 level of probability.

DISCUSSION

To evaluate the activity of *P. fluorescens*, *B. subtilis*, *T. harzianum* and *T. viride* against *E. c.* subsp. *carotovora*, three experiments were carried out; the antibiosis test, slices of potato test, field and storage experiments. *In vitro* screening for antibiosis is frequently used to select prospective antagonists [38] which may be used for biocontrol essays in greenhouse as well in field. In the present study, maximum inhibition zones diameter against *E. carotovora* subsp. *carotovora* was found with *B. subtilis*, followed by *P. fluorescens, T. harzianum* and *T. viride*, being 33.6, 30.0, 28.5 and 16.0 mm, respectively. The results of the present study demonstrated also that the pretreatment of potato tubers slices with antagonistic biocontrol agents successfully prevented the initial infection and reduce soft rot disease of potato and multiplication of soft rot bacteria. Obtaned data are in agreement with those reported by [8, 21, 22, 23, 24]. Results in the present study indicated that the investigated antagonists significantly reduced potato soft rot disease incidence (%) during both crop cycle and under storage conditions, compared to untreated controls. Also, investigated antagonists causes an improvement to potato growth during crop cycle and increased the tuber yield at harvest. However, among the antagonists, *B. subtilis* followed by *P. fluorescens* showed high effect against *E. c.* subsp. *carotovora*. *T. harzianum* showed moderate effect while *T. viride* the less one. It was also obseved that, integrated treatments showed stronger effecive than single treatment by each of biocontrol agents in reducing the incidence of soft rot. This is due to an increase of inoculum of antagonists which inhibit the population of the pathogen bacteria during either crop cycle and under storage conditions. Obtaned data are accordence with those obtaned by [25, 39, 22, 8, 23, 24]. Sharga and Lyon [25] found that, gram-positive *B. subtilis* BS 107, was used as a biocontrol agent against soft-rot- and blackleg-causing bacteria. The strain BS 107 was active in overlay assays against not only human pathogenic or opportunistic but also against plant pathogenic *E. carotovora* subsp. *carotovora*. Cladera-Olivera *et al*. [40] reported a bacteriocin-like substance produced by *Bacillus* spp. P40 that was bactericidal to *E. carotovora* subsp. *carotovora*. This substance interacted with cell membrane lipids, provoking lysis of bacterial cells. It was also effective in protecting potato tubers against soft rot under standard storage conditions. Abdel-Ghafar and Abdel-Sayed [17] also stated that potato tubers treated with bioagents before planting in soil infested with *E. carotovora*, reduced soft rot severity in daughter potato tubers and increased the number and weight of tubers. Kastelein *et al*. [41]

reported that *Psuedomonas* spp. have shown to be potential candidates for biological control of soft rot disease. They are able to survive in the potato rhizosphere and in soil [42] and produce a variety of secondary antibacterial metabolites including mainly siderophores, antibiotics and surfactants [43]. On the othe hand, bacterial biocontrol agents enhanced the potato growth parameters by plant growth-promoting (PGPB) effects. PGPB affect the metabolism of the plants by providing substances that are usually in short supply. These bacteria are capable of fixing atmospheric nitrogen or solubilizing phosphorus and iron and producing plant hormones such as auxins, gibberellins, cytokinins, indole acetic acid (IAA) and ethylene [44]. *Trichoderma* spp. influence plant growth through numerous mechanisms, which mainly include enhancing the solubilization of soil nutrients [45], increasing root length and root hairs to explore larger spaces of soil to absorb nutrients [46] and improving the production of plant stimulatory compounds, such as growth hormones, *i.e.* indole acetic acid, cytokinin, gibberellins, and zeatin [47]. Thus, from the present study, the pretreatment of potato tuber pieces with the identified biocontrol-agents followed by foliage spraying can retard the incidence of soft rot disease during crop cycle, improvement potato growth, increase the yield and protect stored daughter potato tubers from soft rot decay during storage.

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