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ORIGINAL PAPER

Efficacy of Some Biocontrol Agents Against *Streptomyces scabiei* the Causative of Common Scab Disease in Potatoes

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ABSTRACT

The common scab disease caused by *Streptomyces scabiei* is an important disease infecting potato (*Solanum tuberosum*). In this study, twelve *S. scabiei* isolates were obtained from different governorates in Egypt. Efficacy of treatments with certain bioagents (*Pseudomonas fluorescence*, *Bacillus subtilis*, *Trichoderma harzianum*, *Trichoderma viride*) and chemicals (Capitan, Spectropan) to control common scab disease was evaluated. The results showed that treatment with Spectropan in infection time at a concentration of 2 mg/mL exhibited the highest disease reduction percentage, 61.13 and 65.49%, followed by *Trichoderma viride* by 52.63 and 69.8% for 2018 and 2019 seasons, respectively. Treatment of potato) Lady Rosetta cultivar) with *Pseudomonas fluorescence* and *Bacillus subtilis* resulted in a significant increase in the total phenols content and peroxidase and polyphenoloxidase activities during inoculation time and after inoculation time of Lady Rosetta cultivar with *S. scabiei*. However, the lowest activities of peroxidase and polyphenoloxidase were recorded due to treatment with Spectropan in inoculation time or after inoculation time. The obtained results revealed that the application of certain bioagents is an efficient method to control common scab in potatoes.

Keywords: Potato, *Solanum tuberosum*, Common scab, *Streptomyces scabiei*, *Bacillus subtilis*, *Pseudomonas fluorescence*, *Trichoderma harzianum*.

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INTRODUCTION

Potato (*Solanum tuberosum*) is a major vegetable crop, that considering also a source of complex carbohydrates, dietary fibers, vitamin C, B6 and contains many minerals like calcium, potassium, iron, manganese, copper, zinc, (Bastin, 1997). Potato scab is caused by many species of *Streptomyces*, e.g. *Streptomyces scabiei* the casual pathogen of common scab, *S. acidiscabies* the causal pathogen of netted scab and *S. turgidiscabies* is the bacterial species of russet scab (Kers *et. al*., 2005; Hosny *et al.*, 2016 and Abd El-Hafez and Abd El-Rahman 2019). Truper and De'clari (1997) actually used *Streptomyces scabiei* (ex Thaxter) Truper and De'clari, instead of *Streptomyces scabies* (Lambert and Loria 1989) due to a grammatical rule outlined in Rule 12c of the international code of bacterial nomenclature.

Biological control agents for plant diseases are being investigated as alternatives to synthetic pesticides due to their perceived enhanced degree of safety, less environmental implications and ability to reduce disease while being less damaging than conventional fungicides (Brimner and Boland, 2003). Lately, it has been established that biological control has shown to be an effective technique for combating plant infections (Kabeil *et al.*, 2008).

In vitro and *in vivo* tests were also carried out to find out the possible application of the antagonistic bacteria, *Pseudomonas fluorescence* and *Bacillus subtilis* and fungi belonging to *Trichoderma* spp. to determine their potentialities as biological agents for controlling common scab disease (Giyanto *et al.*, 2007; St-Onge *et al.*, 2011). Many ways to control the common scab disease such as treatments of potato tubers by chemicals (Davis *et al.*, 1976), soil pH changing (Pavlista, 1992) modifying irrigation (Adams and Lapwood, 1978), and strategies for rotation (Lu *et al.*,

2011).This work aimed to isolate, identify the common potato scab pathogen and to study the effect of three bioagents to control the potato scab pathogen under both *in vivo* and *in vitro* conditions.

MATERIALS AND METHODS

Source of the tested isolates of *Streptomyces scabiei*:

Samples of potato tubers with common scab disease symptoms were collected from several potato grown regions in Behira, Elminia, Sohag, Aswan and New Valley governorates.

Isolation, identification and pathogenicity of the causal bacterial isolates:

Infected potato tubers with well-developed deep and superficial scab symptoms were used in isolation trials. Cultural, morphological and biochemical assays were used to identify the pathogenic bacteria that had been isolated. On Yeast Malt Extract Agar (YMEA) media, colony form and conidial colour, substrate mycelium and chain morphology, melanin generation on tyrosine agar medium, and bacterial growth were all examined. Gram staining, catalase testing, starch and casein hydrolysis, growth on NaCl, growth at various temperatures and carbohydrate fermentation on various sugar sources were all documented as mentioned by (Bergey, 1994) using Bergeys Manual of Systematic Bacteriology for Methods and as mentioned by Whitman, & UBO, (2015) using in Bergey's manual of systematics of archaea and bacteria.

Tubers were completely cleansed in sterile water and surface sterilised with a 1 percent sodium hypochlorite solution after grown for 40 day. Bacterial isolates were cultivated for 14 days on OMA oat meal agar medium at 28°C. The germs were then extracted using a sterilised needle and thoroughly blended in sterile distilled water. Tubers were immersed in the bacterial suspension (108 CFU/mL) for 15 minutes before being placed in a closed sterilised beaker with cotton and filter paper wetted with sterile distilled water to provide high humidity, and cultured at 28°C for 7 days (Mckenna et al., 2001). *Streptomyces* spp. pathogenicity measured accordind Hao et al. (2009) index.

The tested bioagents:

The bioagents, *Pseudomonas fluorescence, Bacillus subtilis, Trichoderma harzianum, T. viride, Penicillium chrysogenum, P. purpurogenum, Saccharomyces cerevisiae* and *Gliocladium roseum* were isolated from potato rhizosphere and identified at Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt.

***In vitro* studies:**

Effect of the tested bioagents on the growth of *Streptomyces scabiei* isolate No. (8) in comparison with the standard reference of antibiotic Spectropan and biocides was studied. The standard antibiotic spectropan concentrations were prepared separately as prescribed doses. In a conical flask, 200 mL of NA medium were combined with 10 mL of 106 CFU/mL pathogen previously prepared. After mixing, the material was placed into plates and allowed to solidify before being bored with a cork borer (5 mm in diameter). The biocides were prepared in distilled water and their amounts estimated, produced and put to nutrient agar plates. To assess the biocide's capacity to suppress the pathogen, 50 µL from each treatment were placed to the well of the plate. As a control, 50 µL of sterile distilled water were used. Each treatment had three replicates and the effect was measured as the diameter of the inhibitory zone (mm) after five days of incubation at 28°C. (Kabeil *et al*., 2008).

Biological control of potato scab disease under greenhouse conditions:

In this treatment, potato tubers Lady Rosetta cultivar were washed with distilled water and dried then placed in a suspension of *S. scabiei* (isolate No. 8) for 20 minutes. The suspension was allowed to grow in NA medium for till reach 10^6 CFU per mL. Three tubers were immersed in the bacterial suspension for 20 minutes, then removed and left to dry.

Tubers treated with *S. scabiei* were placed in a suspension of the previous bioagents with 10 mL of 10^6 CFU for 20 minutes. For the control treatment, potato tubers were immersed only in water. Potato tubers with bioagents were planted in pots 30 cm diameter, five replicates were carried out for each treatment and were left to grow for three months. At the end of this period, the weight of potato tubers, fresh and dry weights of whole plants were recorded (Giyanto *et al.*, 2007).

***In vivo* studies:**

The most promising concentrations and treatments were chosen based on in vitro research to follow up on their effects under green house conditions against common scab disease. The trials were carried out in Plant

Pathology Dept, Faculty of Agriculture and Natural Resources, Aswan University, Aswan, Egypt, during 2018 and 2019 growing seasons.

Healthy potato tubers, Lady Rosetta cultivar were soaked in each of the tested concentrations of the bioagent, standard antibiotic Spectropan 2 mg/mL and biocide Capitan 5 mg/mL for 10 minutes directly before planting, then allowed to air dried. Fifty mL of the *S. scabiei* isolate No. 8 (10^8 CFU/mL) were added around each tuber at the time of planting. Potato plants were given frequent watering and aftercare as they grew. Also, following other agricultural practices for potato production as recommended by program of Egyptian Ministry of Agriculture. Two methods were used to evaluate the effect of biological treatments, on the first, potato tubers were treated in the time of inoculation with the pathogen, it will be referred to as (At Inoculation time). While the second method of evaluation the effect of biological treatments was at 48 hours after inoculation with the pathogen, and it will be referred to as (After Inoculation time). The disease index was determined at 90 days after planting (Hosny *et al*. 2016). Percentage of disease reduction was evaluated according to Hussein *et al*. (2019). A total of sixteen plants were used from each treatment to determine the crop yield.

Effect of different treatments on some biochemical changes in potato plants at inoculation time and after inoculation time:

Plant leaves samples were taken 9 days after treatments, to determine the biochemical changes occurred in treated plants and untreated plants by both methods of inoculation (at Inoculation time and after inoculation time).

a-Total phenol contents (TPC)

Plant leaves were collected, bathed in liquid N2, homogenized in 80 percent methanol (one gramme plant material in 10 mL) and stored at -20 °C in the deep freeze. The homogenates were then centrifuged at 10,000 rpm for 30 minutes at 4°C, the pellet was discarded after adding ascorbic acid (0.1 g per 5 mL) and the homogenates were evaporated three times for five minutes in a rotary evaporator at 65 °C. For each treatment, three replicates were used (Hoevermann *et al*., 1973).

Folin–Ciocalteau reagent was used to determine the total phenolic content of the samples. Deionized water was used to dilute the Folin–Ciocalteau reagent by ten times. The diluted reagent (0.75 mL) was combined with 0.1 mL sample and allowed to sit for 5 minutes at room temperature. After that, 0.75 mL of sodium carbonate solution (2%) was added. The absorbance of the solution was measured using a 2D Spectrophotometer at OD 750 nm (Spectronic 20D, USA) after 15 minutes of incubation at room temperature. Arnow's reagent was replaced with water to make blank samples. Catechol was used to create the standard curve (1-10 mg). The mg catechol equivalent/g dry weight unit of total phenol contents (TPC) test was expressed (Sun *et al*., 2007).

b-Peroxidase activity (PO):

Activities of enzymes in potato plants were performed by cutting one-gram fresh weight leaves of potato plants treated with liquid N_2 then homogenized with 10 mL 0.1 M Na-acetate buffer pH 5.2. The supernatants were collected for assessing enzyme activity after centrifugation for 30 minutes at 10,000 rpm at 4°C. For each treatment, five replicates were utilized.

The activity of peroxidase was measured spectrophotometrically using guaiacol as a common peroxidase substrate. A 0.2 mL homogenate was incubated at 25 °C for 5 minutes with 0.1 mL of 0.1 M Na-acetate-buffer (pH 5.2), 0.2 mL 1 percent guaiacol, and 0.2 mL 1 percent H_2O_2 and detected at 436 nm (Maehly and Chance, 1954). The blank was a Na-acetate buffer. Changes in absorbance were used to calculate enzyme activity, which was then expressed as:

Peroxidase activity =

OD_{436} nm/mg protein (µmol min^{-1} mg^{-1} protein).

c-Polyphenoloxidase activity (PPO):

A 0.5 mL homogenate of potato plant leaves was homogenized with 2.0 mL 50 mM Sorensen (phosphate buffer) and 0.5 mL Brenzcatechol (Sigma Aldrich) and incubated at 37 °C for 2 hours before being measured at OD_{410} nm (Batra and Kuhn, 1975).

PPO activity =

OD_{410} nm/mg protein (µmol min^{-1} mg^{-1} protein)

Statistical analysis:

All the tests were put up in a complete randomized design. Statistical Analysis System, SAS Institute Inc., was used to do analysis of variance (ANOVA) on the data (SAS, 2012). At P 0.05 levels, the means were compared using the L.S.D. test (Gomez and Gomez 1984).

RESULTS:

Source of the tested isolates of *Streptomyces scabiei*

Naturally diseased potato tubers with typical symptoms of common scab disease were collected from various locations in the governorates of Behira, Elminia, New Valley, Sohag, and Aswan.

Isolation, identification and pathogenicity of the causal bacterial isolates

Twelve isolates from the most diseased samplels showing severe symptoms were obtained Table (1a), revealed that *S. scabiei* isolates had a creamy appearance and a reddish colour, and that all *S. scabiei* isolates grew well at pH levels of 6 and 8. All of the *S. scabiei* isolates tested, on the other hand, did not grow at pH 3 or 4. Carbon is obtained from glucose, fructose, maltose, mannitol and raffinose in isolates. In Table (1b), other results are clarified.

Isolated strains proved to be pathogenic and generate common scab in potato tubers, and the most severe strain of *Streptomyces scabiei* was chosen for further biocontrol investigations based on their morphological, cultural, and physiological properties. Accordingly, the obtained isolates were identified as *Streptomyces scabiei* (Bergey, 1994 and Whitman, & UBO, 2015).

Table (1a): Morphological characteristics of the isolated pathogenic isolates of *Streptomyces scabiei*.

Test	Isolate No.											
	1	2	3	4	5	6	7	8	9	10	11	12
Shape of cell	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral	spiral
Motility	-	-	-	-	-	-	-	-	-	-	-	-
Sporulation	+	+	+	+	+	+	+	+	+	+	+	+
Color of colonies	white	white	white	white	white	white	white	white	white	white	white	white
Gram staining	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+
Esculin Hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Growth 4 °C	-	-	-	-	-	-	-	-	-	-	-	-
Growth 40 °C	-	-	-	-	-	-	-	-	-	-	-	-
H^S production	-	-	-	-	-	-	-	-	-	-	-	-
Levan production	-	-	-	-	-	-	-	-	-	-	-	-
Methyl-red test (MR)	-	-	-	-	-	-	-	-	-	-	-	-
Phenyl alanine deminase	-	-	-	-	-	-	-	-	-	-	-	-
Casein hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-		-	-	-	-	-	-	-

+ = positive reaction; - = negative reaction

Table (1b): Physiological characteristics of the isolated pathogenic isolates of *Streptomyces scabiei*.

Test		Isolate No.											
		1	2	3	4	5	6	7	8	9	10	11	12
Glucose	Acid	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-
Fructose	Acid	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	Acid	-	-	-	-	-	-	-	-	-	-	-	-
	Gas	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	Acid	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-
Maltose	Acid	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-
Manitol	Acid	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-
Dextrin	Acid	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-
Anhydrous dextrose	Acid	+	+	+	+	+	+	+	+	+	+	+	+
	Gas	-	-	-	-	-	-	-	-	-	-	-	-

+ = positive reaction; - = negative reaction

Biological control of the disease under greenhouse conditions:

Bioagents isolates sources:

Bioagents were isolated from potato plants rhizosphere and were identified at the Plant Pathology Department, Faculty of Agriculture, Assiut University, Assiut, Egypt as: *Pseudomonas fluorescence, Trichoderma viride, Trichoderma harzianum* and *Bacillus subtilis*.

Effect of bioagents and biocide captain on the growth of *S. scabiei*:

Data presented in Table (2) indicate that Capitan (5 mg/mL) gave the highest reduction of the pathogen growth, followed by the standard antibiotic Spectropan (2 mg/mL) followed by *Penicillium chrysogenum, P. fluorescence, T. viride, T. harzianum, Saccharomyces cervisiea* and *B. subtilis.* The lowest inhibition was due to *P. purpurogenum* and *Gliocladium roseum*.

Biological control of potato scab disease under greenhouse conditions at and after inoculation time:

Data presented in Table (3) show the effect of treating infested potato tubers (Lady Rosette cv) by four bioagents, Capitan and Spectropan as standard antibiotic at time of inoculation on infection by potato scab expressed as disease severity (%) and percentage of disease reduction. Data revealed that treated tubers with Spectropan at concentration 2 mg/mL exhibited the highest disease reduction percentage by 61.13, and 65.49 % in 2018 and 2019 seasons, respectively. This was followed *by T. viride*, 52.63 and 69.8 % in 2018 and 2019 seasons, respectively. While, Capitan (5 mg/mL) exhibited the lowest disease reduction percentage, being 30.36 and 25.88 % for both 2018 and 2019 seasons.

Table (2): Effect of certain bioagents, antibiotic and fungicide on growth of *S. scabiei in vitro*

Treatments	Inhibition zone (mm)
Pseudomonas fluorescence	37.33
Bacillus subtilis	11.00
Trichoderma harzianum	31.33
Trichoderma viride	32.00
Saccharomyces cerevisiae	25.67
Penicillium chrysogenum	44.00
Penicillium purpurogenum	0.00
Gliocladium roseum	0.00
Capitan 5 mg/mL	80.00
Spectropan 2 mg/mL	74.00
Control	0.00
LSD 0.05	5.12

Table (3): Effect of treatments in infection time on common scab severity under greenhouse conditions in inoculation time

Treatments	Disease severity %		Reduction %	
	2018	2019	2018	2019
Pseudomonas fluorescence	39.67	38.66	51.82	54.51
Bacillus subtilis	38.67	34.00	53.04	60.00
Trichoderma harzianum	57.00	29.66	30.77	65.10
Trichoderma viride	39.00	25.66	52.63	69.80
Capitan 5 mg/mL	57.33	63.00	30.36	25.88
Spectropan 2 mg/mL	32.00	29.33	61.13	65.49
Control 1 (infected)	82.33	85	0.00	0.00
Control 2 (noninfected)	0.00	0.00	100	100
L.S.D 0.05	3.6	4.1	-	-

Similarly, different treatments caused great reduction in disease severity by common scab when applied after inoculation time (Table, 4). Data revealed that treated plants with Capitan and Spectropan at concentration 5 and 2 mg/mL, exhibited the highest disease reduction percentage by 40.89, and 39.22; 40.08, 33.33 % for 2018 and 2019 seasons, respectively, followed by *P. fluorescence*, 38.06, and 31.37 % for 2018 and 2019 season, respectively. While, *T. harzianum* exhibited the lowest disease reduction percentage, being 16.6 and 20.78 % for both seasons, respectively.

Effect of treatments on some potato growth characteristics:

At inoculation time:

Data in Table (5) represent the effect of treatment on some potato growth parameters. Data revealed that the highest fresh weight for Lady Rosetta cultivar was due to treatment with *T. harzianum*, being 230.00 and 246.67 g/plant for 2018 and 2019 seasons, respectively. While, treatment with Capitan (5 mg/mL) showed the lowest fresh weight in both seasons. Moreover, the highest dry weights were obtained when the tubers were treated by Spectropan (2 mg/mL).

The analogous values were 80.00 and 86.67 g for the two studied seasons respectively. Treatment by *B. subtilis* resulted in the lowest dry weight, being 66.67 and 86.67 g, for 2018 and 2019 seasons, respectively. The maximum tuber yield was observed due to the treatment of potato cultivar with *T. viride*, being 500.00, and 530.00 g, for both seasons. While, the lowest yield production (290.00 and 326.67 g) was recorded for the Spectropan treatment, for both seasons, respectively.

Table (4): Effect of treatments on common scab severity under greenhouse conditions after inoculation time

Treatments	Disease severity %		Reduction%	
	2018	2019	2018	2019
Pseudomonas fluorescence	51.00	58.33	38.06	31.37
Bacillus subtilis	60.00	58.33	27.13	31.37
Trichoderma harzianum	68.67	67.33	16.60	20.78
Trichoderma viride	62.00	66.00	24.70	22.35
Capitan 5 mg/mL	48.67	51.67	40.89	39.22
Spectropan 2 mg/mL	49.33	56.67	40.08	33.33
Control 1 (infected)	82.33	85.00	0.00	0.00
Control 2 (noninfected)	0.00	0.00	100	100
L.S.D 0.05	5.3	3.9	-	-

Table (5): Effect of treatments at inoculation time on some growth parameters of potato tuber's under greenhouse conditions

Treatments	Fresh weight (g)/plant		Dry weight (g)/plant		Yield (g)/plant	
	2018	2019	2018	2019	2018	2019
Pseudomonas fluorescence	180.00	256.67	68.33	83.33	283.33	376.67
Bacillus subtilis	221.67	243.33	66.67	86.67	305.00	323.33
Trichoderma harzianum	230.00	246.67	81.67	78.33	425.00	503.33
Trichoderma viride	231.67	233.33	76.67	85.00	500.00	530.00
Capitan 5 mg/mL	185.00	180.00	83.33	83.33	343.33	330.00
Spectropan 2 mg/mL	236.67	216.67	80.00	86.67	290.00	326.67
Control 1 (inoculated)	178.33	186.67	66.00	73.33	286.67	305.00
Control 2 (uninoculated)	206.67	270.00	83.33	95.00	393.33	456.67
L.S.D 0.05	14.60	11.20	6.70	3.40	18.80	19.10

After inoculation time:

Effect of treatments of potato tubers after inoculation time on growth parameters of potato plants under greenhouse conditions:

Data in Table (6), represent the effect of bioagents on some potato parameters after inoculation time. The data revealed that the highest fresh weight for Lady Rosetta cultivar was obtained for the treatment with *B. subtilus*. The results were 226.67 and 236.67 g for 2018 and 2019 seasons, respectively. While, the treatment with Capitan (5 mg/mL), showed the lowest fresh weight of the cultivar on both seasons by 196.67 and 186.67 g. Moreover, the highest dry weight was obtained for the treatment by Spectropan (2 mg/mL), it was 83.33 g for the both seasons. Treatment by Capitan resulted in lowest dry weight as 70.67 and 76.67 g, for 2018 and 2019 seasons, respectively. The maximum yeild production was observed for the treatment of potato cultivar with *B. subtilus* by 406.67, and 513.33 g, for both seasons. While, the lowest yield production (300.33 and 310.00 g) was recorded for the Spectropan treatment, for both seasons.

Effect of bio-treatments of potato tubers on some biochemical changes in plants at and after inoculation time:

Results presented in Table (7) indicate that *P. fluorescence* and *Bacillus subtilis* gave the highest total phenol contents when potato tubers

were treated at or post inoculation time with *S. scabiei* in both seasons. While, *T. harzianum* showed the lowest total phenol contents when potato tubers were treated at or post inoculation time in both seasons. Concerning peroxidase activity, results in Table (8) indicate that *P. fluorescence*, showed the highest amount of peroxidase in both seasons. While, the lowest activity of peroxidase was recorded for the treatment with Spectropan at inoculation time. Moreover, treatment of tubers with Capitan post inoculation time recorded the lowest peroxidase activity in case of inoculation the tubers with the pathogen.

Table (6): Effect of treatments after inoculation time on growth parameters of potato tubers under greenhouse conditions

Treatments	Fresh weight (g)		Dry weight (g)		Yield (g)	
	2018	2019	2018	2019	2018	2019
Pseudomonas fluorescence	186.67	220.00	70.00	88.33	380.00	456.67
Bacillus subtilis	226.67	236.67	68.33	81.67	406.67	513.33
Trichoderma harzianum	233.33	196.67	80.00	70.00	436.00	393.33
Trichoderma viride	236.67	213.33	76.67	80.00	356.67	446.00
Capitan 5 mg/mL	196.67	186.67	70.00	76.67	300.33	310.00
Spectropan 2 mg/mL	216.67	173.33	83.33	83.33	306.00	315.67
Control 1 (inoculated)	173.33	176.67	76.67	70.00	298.00	296.67
Control 2 (un-inoculated)	213.33	230.00	90.00	95.00	296.67	340.00
L.S.D 0.05	16.1	9.5	8.8	7.1	18.8	16.2

Table (7): Effect of bio-treatments on total phenol contents under greenhouse conditions in 2018 and 2019 growing seasons

Treatments	Total phenol contents (mg/g dry weight)			
	at inoculation time		after inoculation time	
	2018	2019	2018	2019
Pseudomonas fluorescence	2.40	1.87	3.03	1.17
Bacillus subtilis	1.90	1.73	2.13	1.97
Trichoderma harzianum	1.13	1.13	0.77	0.80
Trichoderma viride	0.73	2.03	0.93	0.77
Capitan 5 mg/mL	1.27	0.63	1.40	2.07
Spectropan 2 mg/mL	0.90	1.37	1.03	1.20
Control 1 (infected)	1.30	0.73	1.17	1.83
Control 2 (noninfected)	0.60	0.50	0.57	0.63
L.S.D 0.05	0.34	0.21	0.45	0.30

Table (8): Effect of bio-treatments on peroxidase activity in 2018 and 2019 growing seasons

Treatments	Peroxidase activity (µmol min^{-1} mg^{-1} protein)			
	at inoculation time		after inoculation time	
	2018	2019	2018	2019
Pseudomonas fluorescence	0.97	0.93	0.95	0.92
Bacillus subtilis	0.77	0.83	0.84	0.92
Trichoderma harzianum	0.57	0.58	0.50	0.47
Trichoderma viride	0.86	0.93	0.40	0.29
Capitan 5 mg/mL	0.65	0.50	0.20	0.19
Spectropan 2 mg/mL	0.43	0.43	0.40	0.20
Control 1 (infected)	0.45	0.48	0.46	0.44
Control 2 (noninfected)	0.30	0.30	0.27	0.27
L.S.D 0.05	0.13	0.09	0.089	0.18

Results in Table (9) indicate that *P. fluorescence* and *Bacillus subtilis* gave the highest activity of polyphenoloxidase when potato tubers were treated at or post inoculation time with *S. scabiei* in both seasons. While, Spectropan showed the lowest activity of polyphenoloxidase when the tubers of potato were treated at or post inoculation time in both seasons.

Table (9): Effect of treatments at and post inoculation time on polyphenoloxidase activity in 2018 and 2019 growing seasons

Treatments	Polyphenoloxidase activity (µmol min^{-1} mg^{-1} protein)			
	in inoculation time		after inoculation time	
	2018	2019	2018	2019
Pseudomonas fluorescence	0.123	0.224	0.233	0.157
Bacillus subtilis	0.129	0.137	0.147	0.190
Trichoderma harzianum	0.073	0.045	0.106	0.103
Trichoderma viride	0.100	0.176	0.087	0.069
Capitan 5 mg/mL	0.029	0.024	0.034	0.027
Spectropan 2 mg/mL	0.017	0.026	0.016	0.020
Infected control	0.074	0.064	0.059	0.070
Control 1 (infected)	0.013	0.013	0.017	0.017
Control 2 (noninfected)	0.038	0.022	0.040	0.018

DISCUSSION

In this study, certain biocides, antibiotics and bioagents have been used to inhibit the growth of *Streptomyces scabiei* the causative of potato common scab disease *in vitro*. as well as in controlling of the disease under greenhouse conditions. Results indicated that Capitan had a clear inhibitory effect on bacteria growth in plates. The obtained results are in agreement with those reported by Liu *et al.* (2019). Our results indicated that Spectropan has the ability to inhibit common scab pathogen both *in vitro* and *in vivo*. These findings are consistently with those reported by Gharate *et al.* (2016). Results of studying the effect of the antagonistic bioagents such as *P. fluorescence, B. subtilis T. harzianum, T. viride, S. cerevisiea, P. chrysogenum, P. purpurogenum and G. roseum* against *S. scabiei* isolate No. 8 *in vitro* indicated that there were significant variations in inhibition values. The highest inhibition was recorded by *P. chrysogenum, P. fluorescence, T. viride* and *S. cerevisiae*. The obtained results are in agreement with those recorded by Shoda (2000); Sturz *et al*., (2004); Giyanto *et al*., (2007).

Results concerning the effect of certain promising antibiotics, biocides and bioagents obtained from *in vitro* studies on common scab severity on Lady Rosetta potato cultivar grown under greenhouse conditions showed that potato tubers with Spectropan at concentration 2 mg/mL exhibited the highest disease reduction percentage. In previous study by Kobayashi *et al.* (2015), reported that application of Spectropan for treatment of common scab disease in potato had been reduced disease severity.

Different treatments with bioagents and biocides applied after inoculation time on Lady Rosetta cultivar produced different yield quantities. Treatment with *B. subtilis* after inoculation time gave the highest yield of potato tubers, followed by *T. viride* and *T. harzianum*, respectively. Our results revealed that the highest fresh weight for Lady Rosetta cultivar was obtained due to treatment with *B. subtilus*. The highest dry weight was obtained due to the treatment by Spectropan. While, Spectropan and infected control gave the lowest fresh weight similar to those obtained by St-Onge *et al*. (2011) who pointed out that common scab disease reduces crop value and, in extreme cases, yield production. These findings are in contrast to the results reported by Lambert and Loria (1989) and Couillerot *et al.*, (2009) who pointed out that while common scab disease does not reduce yields, it degrades the appearance and quality of tubers, which is particularly significant when growing potatoes for food nourishment. Bernard *et al.* (2014) reported that using commercial biocontrol agents of *Trichoderma lignorum* and *T. viride*, common scab was successfully reduced, with positive impacts on plant growth, development, and yield. Larkin (2008) mentioned that the use of certain biocontrol agents, including *T. harzianum* and *T. virens* can reduce common

scab of potato disease in the greenhouse and field. These findings match those provided by Larkin (2008) who mentioned that the use of certain biocontrol agents, including *B. subtilis* can reduce common scab disease of potato in the greenhouse and field.

In this experiment, total phenol contents, enzymes production, peroxidase (PO) and polyphenoloxidase (PPO) enzymes by *S. scabiei* isolate No. (8) were determined. Our results indicated that *P. fluorescence* and *Bacillus subtilis* gave the highest total phenol. While, *T. harzianum* showed the lowest total phenol contents when the potato cultivar was treated after or at inoculation time. *P. fluorescence*, represented the highest amount of peroxidase. These results also are in accordance with those mentioned by Ben-Shalom *et al*. (2003); Passardi *et al*. (2005) and Asselbergh *et al*. (2007).

Treatment potato tubers with each of *P. fluorescence* and *Bacillus subtilis* at and post inoculation time gave the highest activity of polyphenoloxidase with *S. scabiei*. While, Spectropan showed the lowest activity of polyphenoloxidase when potato tubers were treated after or at inoculation time in both seasons. Results are match with studies of Dong and Cohen, (2002; Mahmoud *et al*. (2004) and Seleim, *et al*. (2014). Specrtopan showed the lowest activity of the enzyme when the potato tubers were treated after inoculation time (Nuutila *et al*., 2003; Prakash *et al*., 2007; and Lu *et al*., 2011). These enzymes can either form a firm wall or generate ROS to make it more flexible; they can defend against microbial invasion by raising physical boundaries or defend with a high production of ROS, as seen by an elevation in polyphenoloxidase (PPO) activity in potato plants. These findings corroborated those published by Mayer, (2006 and Abo-Elyousr *et al*. (2008).

CONCLUSION

In Egypt, potato common scab disease is one of the principle causes of a decline in the quality of locally consumed and exported potatoes, prompting researchers to look for alternatives to harmful chemicals. The findings showed that treating potato with fungal strain *Trichoderma viride*, bacterial strains *Pseudomonas fluorescence* and *Bacillus subtilis* resulted in a significant reduction in the severity of common scab disease, indicating that the use of specific bioagents is an effective way to control common scab in potatoes.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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