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Biological control of cucumber powdery mildew (*Podosphaera xanthii*) (Castagne) under greenhouse conditions

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Abstract

Cucumber powdery mildew disease caused by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff severe disease-causing yield losses worldwide. This research study was conducted to evaluate the efficacy of the tested bio-agents, *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis*, *Paenibacillus polymyxa*, and *Serratia marcescens*, as well as the fungicide score (Difenoconazole), on cucumber infected with *P. xanthii*, *in vitro* and under greenhouse conditions. Results indicated that culture filtrate of the tested bio-agents and the fungicide (control) significantly reduced *P. xanthii* conidial germination *in vitro*; the reduction percentage ranged between 91.17 and 76.06%. Also, score recorded the highest reduction percentage (97.19%). All treatments significantly decreased the disease severity and area under disease progress curve (AUDPC) post spraying the bio-agents on cucumber plants under greenhouse conditions. Score followed by *B. subtilis* significantly decreased disease severity percentage (67.33 and 65.38%, respectively) and AUDPC (322.84 and 342.06) than the untreated control (988.13 AUDPC). Additionally, treated cucumber plants showed a significant increase in plant growth parameters (plant height, total chlorophyll, fresh, and dry weight) and yield parameters (fruit number/plant and fruit weight/plant) as well the activity of defense-related enzymes, i.e., peroxidase (PO) and polyphenol oxidase (PPO), and total phenols content (TPC) compared to the untreated plants.

Keywords: Biological control, Cucumber, Powdery mildew, *Podosphaera xanthii*, Enzymes activity, Plant growth parameters, Greenhouse

Background

Cucumber (*Cucumis sativus* L.) is one of the most widely grown greenhouse crops. Powdery mildew is caused by *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff (formerly *Sphaerotheca fuliginea* (Schlechtend.: Fr) Pollacci). It is a major foliar disease worldwide, reducing crop quality and yield (Rur et al. 2018).

Fungicides are one of the principal tools for managing cucumber powdery mildew (Lebeda et al. 2010). However, due to the harmful effects on the environment because of the use of fungicide (Ozkara et al. 2016),

biological control is an important alternative to reduce risk and diminish the hazardous effects of the fungicides application that achieved remarkable success in plant diseases and powdery mildew disease control (Tanaka et al. 2017; Rur et al. 2018).

Applications of plant growth-promoting rhizobacteria (PGPR) as biotic inducers have a potential in controlling plant diseases (O'Brien 2017). This positive performance of PGPR has a direct and indirect effects on plants, direct promotion by production of metabolites that improves the plant growth, and indirect growth effects by removal of the pathogens via the secondary metabolites production (Sarhan and Shehata 2014; Prasannath 2017).

Induced systemic defense reaction, using PGPR, in plants is one important means for management plant

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diseases as it can induce plant defense in the host plants in response to fungal infection including defense-related enzymes and pathogenesis-related proteins, indoleacetic acid (IAA), lignin synthesis, and phenolic compounds accumulation (Reddy et al. 2014; Prasannath 2017).

Defense-related enzyme PO and PPO are mentioned as the plant induced systemic resistance (ISR) that correlates with the disease control (El-Sharkaway et al. 2014; Prasannath 2017; Elsisi 2019). These enzymes result in the biosynthesis of plant metabolites such as phenolic compounds, flavonoids, tannins, and lignin (Prasannath 2017; Singh et al. 2018). These products can provide defense in plants against pathogenic attack (Hahlbrock and Scheel 1989). Many studies have indicated that the increase of defense-related enzymes activity due to greater accumulation of phenolics can offer protection against plant diseases (Hafez et al. 2018; Elsisi 2019).

Many investigators demonstrated the application of biological control against cucumber powdery mildew disease (El-Sharkaway et al. 2014; Rur et al. 2018; Punja et al. 2019). The applications of bio-agents were effective in controlling cucumber powdery mildew development through inhibition of the conidial germination and mycelial growth rate of the pathogen decreased percent of infected leaf area (El-Naggar et al. 2012; Rur et al. 2018; Punja et al. 2019).

The objectives of the present study were to evaluate the efficacy of some biotic inducers on inducing resistance to cucumber plants against powdery mildew under the greenhouse conditions and the reaction of host metabolic, i.e., defense-related enzymes and phenolic compounds and cucumber plants growth parameters and yield.

Materials and methods

Source of bio-agents

The tested fungal bio-agents, i.e., *Trichoderma harzianum* and *T. viride*, and tested bacterial bio-agents, i.e., *Bacillus subtilis*, *Paenibacillus polymyxa*, and *Serratia marcescens*, were obtained from the Microbiology Dept, Soil, Water and Environment Res. Inst, (SWERI), Agricultural Research Center, Giza, Egypt.

Preparation of tested bio-agents inocula

T. harzianum and *T. viride* strains were grown for 10 days on PDA medium and then separately. Their spore's suspensions were prepared and adjusted, using hemocytometer slide, to 10^7 spore ml^{-1} with sterilized water. *B. subtilis*, *P. polymyxa*, and *S. marcescens* strains were grown separately in flasks; 250 ml contained nutrient liquid medium for 3–4 days on an orbital shaker at 150 rpm. The suspension of each bacterial strain was adjusted at 10^9 cell ml^{-1} using a hemocytometer slide.

Fungicide score 25% EC

Common name: difenoconazole, chemical name: dimethyl [1-((2-(2-chloro-4-(4-chlorophenoxy) phenyl)-4-methyl-1,3-dioxolan-2-yl) methyl)-1H-1,2,4-triazole)], and recommended dose 50 ml/100l (Syngenta Crop Protection AG, Basel, Switzerland).

Efficacy of tested bio-agents culture filtrates on spore germination of *Podospaera xanthii*

Viable *P. xanthii* conidial spores were obtained by softly shaken by a glass rod from young sporulating lesions (Godwin et al. 1987). Newly collected spores were placed on glass slides previously cleaned by ethyl alcohol and air-dried as described by Nair and Ellingboe (1962). Slides were covered by thin layers of water agar 2%, amended by the filter-sterilized culture filtrate of the tested bio-agents. The slides, covered by agar-free culture filtrate, were used as a control then, laid over glass rods in sterilized Petri dishes, containing numerous filter papers fully water-moistened and incubated under continuous light at 25 °C for 24 h (Reifschneider et al. 1985). Spore produced a germ tube as long as the width considered being germinated. Spores were microscopically examined at $\times 40$ magnifications to determine germination. Percentages of spore germination were calculated for 100 spores (Menzies et al. 1991). Three replicates were examined for each treatment.

Greenhouse experiments

Experiments were carried out under plastic greenhouse conditions (40 m \times 9 m) in randomly complete block design during the seasons of 2019 at Dahshour, Giza Governorate, Egypt, using cucumber hybrid F1 Sinai1. Rectangular plastic trays (5 \times 5 \times 7 cm) were filled by autoclaved commercial potting medium (a mix of peat moss, vermiculite). Cucumber seeds were sown in trays and covered with layers (3 cm) of sterile sand. Seven treatments with 3 replicates per treatment were carried out. Each replicate contained 12 plants. Seedlings were transplanted after 3 weeks, on the 2 sides of the ridge, at a spacing of 50 cm between them within the row. The plants were distributed in 3 rows; each was 0.7 m wide and 2 m length. Plants were fertilized by recommended doses.

Artificial inoculation was conducted under greenhouse conditions by freshly collected conidia of *P. xanthii* suspension (10^5 conidia ml^{-1}) performed according to Kamel (2003). Three weeks old cucumber plants were sprayed by spore suspension of *P. xanthii*, about 15 ml of spores' suspension. Then, the inoculated cucumber plants were covered by plastic sheets for 24 h to keep high humidity levels (El-Sharkaway et al. 2014). In addition, plants were sprayed by the previously prepared fungal and bacterial bio-agents strains as well as the fungicide at the

previously mentioned dose. Control treatment was sprayed by sterilized tap water.

Disease assessment

Disease severity was estimated at 5 days intervals for 20 days post inoculation transplanting. Plants were examined periodically for symptoms appearance, and disease severity was measured, using the 0–11 scale as mentioned by Horsfall and Barrett (1945). Ten random plants were used for each replicate. The severity of powdery mildews was measured, using the following formula:

$$\text{Disease severity (\%)} = \frac{\sum (n \times v)}{11 N} \times 100$$

where n = number of infected leaves in each category; v = numerical values of each category; and N = total number of the infected leaves.

The mean (AUDPC) was calculated, using the formula of Pandey et al. (1989).

$$\text{AUDPC} = D [1/2(Y_1 + Y_k) + (Y_2 + Y_3 + \dots + Y_{k-1})]$$

where D = time interval; Y_1 = first disease severity; Y_k = last disease severity; and Y_2, Y_3, Y_{k-1} = intermediate disease severity.

Chlorophyll content measurements

Total chlorophyll contents were determined in the 5th apical fully expanded leaf, using the greenness measurements, portable leaf chlorophyll meter SPAD-501 (Minolta Corp) Yadava (1986).

Evaluation of growth and yield parameters

Growth and yield parameters were determined/plant. The average leave numbers/plant and leaf area was determined at 50 days in the experimental plants. Measurement of plant leaf area was carried out, using the CI-202 Portable Laser Leaf Area Meter CID (Bio-Science, Inc 1554 NE 3rd Avenue, Camas, WA, 98607, USA). Leaf area was measured as square centimeters. The average fruit numbers and fresh weight/plant were assessed by harvesting the fruits every 2 days, while the fruits at the marketable size, for 90 days after transplanting. Yield was expressed as fruits number and weight/plant.

Biochemical assays

Peroxidase (PO), polyphenol oxidase (PPO) activity, and total phenol content (TPC) were determined in tissues on bio-agents treated cucumber leaves as well as in the untreated control treatment.

Samples' collection

Samples were collected at 6 days post inoculation with the pathogen and then grounded with liquid nitrogen (L-N₂) as fine powder with a mortar and pestle. One gram of the grounded tissues was mixed with 1 ml of extraction buffer phosphate, pH 6.0 according to Bollage

et al. (1996). Samples were vortexed and centrifuged at 8000 rpm for 25 min under 4 °C. Then, clear supernatant (crude enzyme source) was kept at – 20 °C for further studies (Biles and Martyn 1993).

Determination of peroxidase

Activity of PO was determined according to Allam and Hollis (1972) spectrophotometrical method in absorbance at 430 nm/g fresh weigh/15 min. PO activity was expressed as enzyme unit/mg protein/min.

Determination of polyphenol oxidase

Activity of PPO was determined according Ishaaya (1971) spectrophotometrical method at an absorbency of 405 nm. PPO activity was expressed as enzyme unit/mg protein/min.

Determination of total phenols contents

One gram was extracted at 70 °C for 15 min by 10 ml of 80% methanol of cucumber leaves sample. TPC was determined using the method described by Zieslin and Ben-Zaken (1993) Folin-Ciocalteu reagent colorimetric analysis method. TPC amount was expressed as microgram GAE/g fresh weight.

Statistical analysis

The Wasp software (Web Agriculture Stat Package) was used to the ANOVA statistical analysis of the data. According to Duncan's multiple range tests, at $P \leq 0.05$, all measurements and comparisons mean values were determined (Gomez and Gomez 1984).

Results and discussion

Effect of tested bio-agents culture filtrate on conidial germination of *P. xanthii*

The efficacy of the tested bio-agents culture filtrate *T. harzianum*, *T. viride*, *B. subtilis*, *P. polymyxa*, and *S. marcescens* as well as the fungicide score (25% EC) was evaluated as percentage of *P. xanthii* conidial germination in vitro.

Results shown in Table 1 indicate that all culture filtrate of the tested bio-agents significantly inhibited the conidial germination of *P. xanthii*, in vitro. The highest reduction was attributed to the fungicide being (97.19%), followed by *B. subtilis* (91.17.0%), *P. polymyxa* (88.47%), and *S. marcescens* (85.46%) whereas, *T. viride* and *T. harzianum* recorded (82.13% and 76.06%) reduction, respectively.

In the present study, the strong inhibitory action against *P. xanthii* spore germination revealed a strong antifungal activity of the tested bio-agents. This antifungal activity suggested the production of antibiotic(s), and/or another direct inhibitory substances as hydrolytic enzymes, hydrogen cyanide, or siderophore (Sarhan and

Table 1 Effect of bioagent culture filtrate on *Podospaera xanthii* conidial germination, 24 h after treatment and incubation at 28 ± 1 °C

Treatments	Concentration	Germination (%)	Efficiency (%)
<i>T. harzianum</i>	10^7 spore ml ⁻¹	14.23b	76.06
<i>T. viride</i>	10^7 spore ml ⁻¹	10.62bc	82.13
<i>B. subtilis</i>	10^9 CFU	5.25bc	91.17
<i>P. polymyxa</i>	10^9 CFU	6.85bc	88.47
<i>S. marcescens</i>	10^9 CFU	8.64bc	85.46
Fungicide	0.25 ml l ⁻¹	1.67c	97.19
Control	-	59.43a	0.00

Values assigned to similar letters are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Shehata 2014; Rais et al. 2017; Prasannath 2017; Tanaka et al. 2017). Such results are in harmony with those previously obtained by García-Gutiérrez et al. (2013), El-Sharkaway et al. (2014), Tanaka et al. (2017), Hafez et al. (2018), and Elsis (2019) who showed the ability of the bio-agents to inhibit powdery mildew conidial spores germination.

Effect of tested bio-agents on controlling powdery mildew on cucumber plants

Results presented in Table 2 showed that all the tested bio-agents decreased area under disease progress curve (AUDPC) than the control. *B. subtilis* achieved the highest efficiency in reducing disease severity and decrease of AUDPC, being 65.38% efficiency and 342.06 AUDPC for powdery mildew, followed by *P. polymyxa*, and then *S. marcescens*, *T. harzianum*, and *T. viride* being 61.00, 52.44, 44.07, and 39.72% efficiency and 385.38, 469.93, 552.63, and 595.61 AUDPC, respectively, compared to the control (988.13 AUDPC). The fungicide recorded the highest efficiency (67.33%) and AUDPC being 322.84.

These results are in agreement with many previous studies, indicated the effective of bio-agents *Bacillus* spp. and *Trichoderma* spp. for controlling cucumber powdery

mildew disease (El-Naggar et al. 2012; Sawant et al. 2017; Tanaka et al. 2017; Punja et al. 2019). This reduction may be due to the potential of the tested bio-agents as PGPR, which is widely applied for controlling plant diseases (Sarhan and Shehata 2014; Prasad et al. 2017). Recently, numerous successful researches investigated several bio-agents such as *Serratia* spp., *Bacillus* spp., and *Trichoderma* spp. for protection against airborne pathogens particularly powdery mildew disease. El-Kot and Derbalah (2011) indicated that by producing antifungal compounds, *Trichoderma* spp. reduced the squash powdery mildew disease severity. El-Naggar et al. (2012) found that *T. viride* significantly reduced cucumber powdery mildew disease. El-Sharkaway et al. (2014) found that spraying cucumber plants with the bio-agents, *B. subtilis*, *Pseudomonas fluorescens*, *Derxia gummosa*, and *T. harzianum*, significantly reduced the severity of both cucumbers powdery and downy mildew diseases. Tanaka et al. (2017) demonstrated that production of the antibiotic prumycin was a major factor in biocontrol by *Bacillus amyloliquefaciens* against powdery mildew of cucumber. Hafez et al. (2018) found that a significant reduction in the squash powdery mildew severity, and (AUDPC) in infected plants treated with the bio-agents, i.e., *B. pumilus*, *B. megaterium*, *B. subtilis*, *B. chitosporus*, *P. polymyxa*, *T. harzianum*, and *T. viride*. These results are in accordance with Elsis (2019) who found that spraying squash plants with the bio-agents *B. subtilis*, *P. polymyxa*, *T. harzianum*, *T. viride*, *T. hamatum*, and *T. album* decreased the powdery mildew incidence and the severity under greenhouse conditions. Punja et al. (2019) demonstrated that the application of the biocontrol agent *B. subtilis* on greenhouse cucumber plants either preventative or eradicated treatments reduced the powdery mildew disease incidence and severity.

Table 2 Effect of bioagent treatments on powdery mildew (*Podospaera xanthii*) disease severity (DS %) and (AUDPC) of cucumber plants

Treatments	Diseases severity (DS %)					Efficiency (%)	AUDPC
	Days after application						
	Zero time	5 days	10 days	15 days	20 days		
<i>T. harzianum</i>	13.38b	21.48b	29.13b	36.45b	50.76b	39.72	595.61b
<i>T. viride</i>	11.96bc	19.77b	27.65bc	34.41bc	45.45b	44.07	552.63bc
<i>B. subtilis</i>	8.46ef	11.15de	16.14ef	22.33de	29.14c	65.38	342.06d
<i>P. polymyxa</i>	9.13de	13.46cd	19.53de	23.57de	31.93c	61.00	385.38d
<i>S. marcescens</i>	10.55cd	15.89c	23.53cd	28.98cd	40.64bc	52.44	469.93c
Fungicide	6.73f	9.57e	15.14f	21.24e	30.54c	67.33	322.84d
Control	28.21a	34.44a	48.36a	60.64a	80.16a	-	988.13a

Values assigned to similar letters are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Effect of tested bio-agents on chlorophyll content and growth parameters of cucumber plants

Data in Table 3 showed that chlorophyll content and growth parameters (leaf area and the number of leaves) significantly increased in cucumber plants treated with the tested bio-agents. Highest chlorophyll content was recorded in cucumber plants treated with the fungicide (44.90), followed by *B. subtilis*, *P. polymyxa*, *S. marcescens*, *T. viride*, and *T. harzianum* (43.90, 41.40, 38.85, 35.30, and 30.75, respectively) compared to the untreated control (25.30). The numbers of leaves/plant and leaf area significantly increased in the treated cucumber plants. The fungicide recorded the highest number of leaves/plant (37.0), followed by *B. subtilis*, *P. polymyxa*, *S. marcescens*, *T. viride*, and *T. harzianum* (36.4, 35.0, 32.7, 27.5, and 22.9, respectively) than the untreated control plants (21.3). As well, the fungicide and *B. subtilis* recorded the maximum leaf area (361.3 and 352.3 cm²), followed by *P. polymyxa*, *S. marcescens*, *T. viride*, and *T. harzianum* (347.3, 337.0, 324.7, 324.7, and 269.0 cm², respectively) than the control plants (208.0 cm²).

Obtained results are in harmony with many investigators who stated that the improvement of plant growth parameters regarded to the role of the PGPR that has an important role in the resistance to infection with diseases and consequently, improved growth parameters (Reddy et al. 2014; Singh et al. 2018). Treated mildewed cucumber or squash plants with the bio-agents, *Bacillus* spp., *Serratia* spp., and *Trichoderma* spp., significantly increased chlorophyll content and growth parameters/plant (El-Sharkaway et al. 2014; Hafez et al. 2018). Youssef et al. (2018) found that treated tomato leaves with the bioagent *Serratia proteamaculans* significantly increased chlorophyll content higher than the untreated control plants.

Effect of bio-agents on yield parameters of cucumber plants

Data in Table 4 revealed that yield parameters significantly increased in mildewed cucumber plants sprayed with the tested bio-agents. The fungicide recorded the highest fruit number/plant being (37.7 fruit/plant) with

an average increase of 59.07% more than the control plants (23.7 fruit/plant), followed by *B. subtilis* (35.7 fruit/plant), *P. polymyxa* (33.3 fruit/plant), *S. marcescens* (32.0 fruit/plant), *T. viride* (31.3 fruit/plant), and *T. harzianum* (30.7 fruit/plant), with an average increase of 50.63, 40.51, 35.02, 32.07, and 29.54% than the control, respectively. Similarly, fruits yield/plant markedly increased than the control. The fungicide and *B. subtilis* showed the highest fruits yield (3.65 and 3.50 kg/plant), with an average increase of 100.55 and 92.31% over the control, followed by *P. polymyxa* (3.20 kg/plant), *S. marcescens* (2.95 kg/plant), *T. viride* (2.75 kg/plant), and *T. harzianum* (2.45 kg/plant), with an average increase of 75.82, 62.09, 51.10, and 34.62%, respectively.

Additional mechanisms by which these PGPR affect plants involve the production of plant growth regulator (indoleacetic acid, gibberellins, and cytokinin) resulting in stimulation of plant growth and increases in fruits yield (Reddy et al. 2014 and Singh et al. 2018). Obtained results are in harmony with those reported by El-Naggar et al. (2012), García-Gutiérrez et al. (2013), El-Sharkaway et al. (2014), Tanaka et al. (2017), and Elsis (2019) who found that the biological treatments reduced powdery mildew disease and increased in crop yield.

Effect of some bio-agents on biochemical changes in mildewed cucumber plants

The tested bio-agents (*B. subtilis*, *P. polymyxa*, *S. marcescens*, *T. harzianum*, and *T. viride*) as biotic inducers increased activity of the PO and PPO defense-related enzymes as well as the TPC in cucumber leaves infected with powdery mildew disease (*P. xanthii*). Data in Table 5 showed that the highest activity of PO was induced by *B. subtilis* (228.3), followed by *P. polymyxa*, *S. marcescens*, *T. viride*, and *T. harzianum* recording (187.7, 180.0, 169.3, and 155.3), respectively. Meanwhile, the fungicide achieved the least effective one recording (149.7). Similarly, *B. subtilis* recorded the highest level of the activity of PPO (127.7), followed by *P. polymyxa*, *S. marcescens*, and the fungicide score (119.3, 111.7, and 96.3, respectively), whereas the least enzyme activity was recorded by *T.*

Table 3 Effect of bioagent treatments on chlorophyll content and growth parameters of powdery mildewed cucumber plants

Treatments	Chlorophyll content (SPAD)	No. leaves plant ⁻¹	Leaf area (cm ²)
<i>T. harzianum</i>	30.75d	22.9cd	269.0e
<i>T. viride</i>	35.30cd	27.5c	324.7cd
<i>B. subtilis</i>	43.90b	36.4a	352.3ab
<i>P. polymyxa</i>	41.40bc	35.0ab	347.3bc
<i>S. marcescens</i>	38.85c	32.7b	337.0bc
Fungicide	44.90a	37.0a	361.3a
Control	25.30e	21.3d	208.0f

Values assigned to similar letters are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

Table 4 Influence of bio-agents treatments on yield parameters of powdery mildewed cucumber plants

Treatments	No. fruits plant ⁻¹	Average increase over the control %	Fruit yield (kg plant ⁻¹)	Average increase over the control %
<i>T. harzianum</i>	30.7d	29.54	2.45c	34.62
<i>T. viride</i>	31.3cd	32.07	2.75bc	51.10
<i>B. subtilis</i>	35.7ab	50.63	3.50a	92.31
<i>P. polymyxa</i>	33.3bc	40.51	3.20ab	75.82
<i>S. marcescens</i>	32.0bcd	35.02	2.95bc	62.09
Fungicide	37.7a	59.07	3.65a	100.55
Control	23.7e	-	1.82d	

Values assigned to similar letters are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

viride and *T. harzianum*, being 84.3 and 83.3. Also, TPC significantly increased in all tested treatments, particularly *P. polymyxa* (68.7) than the control (24.7), followed by *B. subtilis* (58.3), *S. marcescens* (52.7), *T. viride* (49.7), and *T. harzianum* (47.3), while the fungicide (45.3) showed the least effect on TPC.

Using PGPR, such as *Serratia* spp., *Trichoderma* spp., and *Bacillus* spp., for inducing systemic defense reaction in plants is considered an important approach to suppress plant disease. Induced plant defense responses are directly linked with induction of defense-related enzymes PO and PPO and accumulation of phenolic compounds (Walters et al. 2005; Singh et al. 2018). Many investigators demonstrated the positive relationships between defense-related enzymes and phenolic compounds and resistance to powdery mildew disease. El-Naggar et al. (2012) and El-Sharkaway et al. (2014) indicated that the treatment with bio-agents significantly decreased the powdery mildew disease severity in cucumber plants and induced defense-related enzymes PO, PPO, and increased phenolic compounds. As well, Hafez et al. (2018) and Elsisy (2019) reported that resistance to powdery mildew disease was correlated to the increase of biochemical changes, i.e., defense-related enzymes PO and PPO, and TPC of squash plants as a result after

Table 5 Effect of some bio-agents treatments on biochemical changes of powdery mildewed cucumber plants after 6 days from inoculation

Treatments	Peroxidase (enzyme unit/mg protein/min)	Polyphenol oxidase (enzyme unit/mg protein/min)	Total phenols (μ g GAE/g fresh weight)
<i>T. harzianum</i>	155.3abc	83.3d	47.3bc
<i>T. viride</i>	169.3ab	84.3cd	49.7bc
<i>B. subtilis</i>	228.3a	127.7a	58.3ab
<i>P. polymyxa</i>	187.7ab	119.3ab	68.7a
<i>S. marcescens</i>	180.0ab	111.7abc	52.7bc
Fungicide	149.7bc	96.3bc	45.3c
Control	81.7c	52.7e	24.7d

Values assigned to similar letters are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test

spraying squash plants with the bio-agents under greenhouse conditions.

Conclusion

The present study indicated that the application of bio-agents, i.e., *Bacillus* spp., *Serratia marcescens*, and *Trichoderma* spp., significantly protected cucumber plants against powdery mildew disease, mainly through the induction of systemic resistance. However, the application of such bio-agents in the control of cucumber powdery mildew on the field scale provides a practical environmentally friendly disease management against cucumber disease and can be used through integrated disease management.

Abbreviations

AUDPC: Area under disease progress curve; DS: Diseases severity; EC: Emulsifiable concentrate; GAE: Gallic acid equivalent; PDA: Potato dextrose agar; PGPR: Plant growth-promoting rhizobacteria; PO: Peroxidase; PPO: Polyphenol oxidase; TPC: Total phenol content

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Authors' contributions

Ehab A. D. Sarhan: conception and design of the work; the acquisition, analysis, and interpretation of data; editing the manuscript. Corresponding author. Michael H. F. Abd-Elsyed: the acquisition, analysis, and interpretation of data. Azza M. Y. Ebrahiem: the acquisition, analysis, and interpretation of data. The authors read and approved the final manuscript.

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Availability of data and materials

All data and materials are available upon request.

Ethics approval and consent to participate

All authors agree (consent) to participate in this research article.

Consent for publication

All authors participated in the work and consent for publication.

Competing interests

The authors declare that they have no competing interests.

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