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* (Schlechend.: 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; Prasanth 2017).

Induced systemic defense reaction, using PGPR, in plants is one important means for management plant
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 10⁷ 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 10⁹ 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] with recommended dose 50 ml/l00 l (Syngenta Crop Protection AG. Basel, Switzerland).

Efficacy of tested bio-agents culture filtrates on spore germination of Podosphaera 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 × 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 × 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 × 5 × 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 (10⁵ conidiam⁻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 Pandy et al. (1989).

\[
\text{AUDPC} = D \left\{ \frac{1}{2}(Y_1 + Y_k) + (Y_2 + Y_3 + \ldots + Y_k - 1) \right\}
\]

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%), 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 Podosphaera xanthii conidial germination, 24 h after treatment and incubation at 28 ± 1 °C

| Treatments         | Concentration       | Germination (%) | Efficiency (%) |
|--------------------|---------------------|-----------------|---------------|
| T. harzianum       | 10^9 spore ml⁻¹     | 14.23b          | 76.06         |
| T. viride          | 10^9 spore ml⁻¹     | 10.62bc         | 82.13         |
| B. subtilis        | 10^9 CFU            | 5.25bc          | 91.17         |
| P. polymyxa        | 10^9 CFU            | 6.85bc          | 88.47         |
| S. marcescens      | 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 ≤ 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 Elsisi (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, Drexia 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. chitinophorus, P. polymyxa, T. harzianum, and T. viride. These results are in accordance with Elsisi (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 eradicative treatments reduced the powdery mildew disease incidence and severity.

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

| Treatments         | Days after application | Efficiency (%) | AUDPC       |
|--------------------|------------------------|----------------|------------|
|                    | Zero time   5 days 10 days 15 days 20 days |               |            |
| T. harzianum       | 13.38b      21.48b 29.13b 36.45b 50.76b | 39.72          | 595.61b    |
| T. viride          | 11.96bc     19.77b 27.65bc 34.41bc 45.45b | 44.07          | 552.63bc   |
| B. subtilis        | 8.46ef      11.15de 16.14ef 22.33de 29.14c | 65.38          | 342.06d    |
| P. polymyxa        | 9.13de      13.46cd 19.53de 23.57de 31.93c | 61.00          | 385.38d    |
| S. marcescens      | 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 ≤ 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 maximum leaf area (361.3 and 352.3 cm², respectively) than the control plants (208.0 cm²).

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), Garcia-Gutierrez et al. (2013), El-Sharkawy et al. (2014), Tanaka et al. (2017), and Elsisi (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 (cm²) |
|--------------|----------------------------|---------------------|-----------------|
| T. harzianum | 30.75d                     | 22.9cd              | 269.0e          |
| T. viride    | 35.30cd                    | 27.5c               | 324.7cd         |
| B. subtilis  | 43.90b                     | 36.4a               | 352.3ab         |
| P. polymyxa  | 41.40bc                    | 35.0ab              | 347.3bc         |
| S. marcescens| 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 ≤ 0.05) according to Duncan’s multiple range test.
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