The Future of Horticulture

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Evaluating the Efficacy of Some Biological Control Applications in Controlling Powdery Mildew Disease of Cucumber Plants in Greenhouses

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Future Science Association

Available online free at
www.futurejournals.org

Print ISSN: 2692-5826

Online ISSN: 2692-5834

DOI:
10.37229/fsa.fjh.2024.11.10

Received: 15 September 2024
Accepted: 30 October 2024
Published: 10 November 2024

Publisher's Note: FA stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: The purpose of this investigation was to compare the effectiveness of the tested biological control treatments, *i.e.*, *Trichoderma album*, *T. harzianum*, *T. hamatum* and *T. viride*; biopesticides, such as Blight Stop (*T. harzianum*), which is used at the rate of 1 L/100 liters of water; and additionally natural compounds, such as micronized sulfur fungicide, which is utilized at a rate of 250 gm/100 liters of water in decreasing germination and severity of *Podosphaera fuliginea* powdery mildew disease on cucumber cv. Hybrid 20 to the control under *in vitro* and greenhouses. The treatment with Micronic sulfur fungicide reduced inhibitory germination percentages and disease severity the most (92.69 and 87.30%), followed by the *T. harzianum* isolate (86.96 and 81.71%) as compared to the control treatment. The application of cucumber plants with *T. harzianum* at a rate of 1:100 exhibited the greatest decrease in the inhibition of disease severity and the greatest increase in yield, biochemical analysis, including total chlorophyll, total phenolic content, peroxidase (PO), and polyphenol oxidase (PPO) enzyme activities throughout the growing season 2024 under greenhouse condition, compared to untreated plants. On the other hand, *T. hamatum* demonstrated the least response from treatments. There was no observable, major variations between the other treatments and the control.

Key words: Cucumber, *Podosphaera fuliginea*, Biological control and Micronic sulfur.

1. Introduction

The cucumber (*Cucumis sativus* L.), a member of the Cucurbitaceae family, is one of the most significant vegetable crops because of its economic value for both domestic and international consumption. Cucumber is produced in greenhouses or open fields to prolong its growing season and shield it from pests, illnesses, and unfavorable weather (**Mahdy *et al.*, 2006**). *Podosphaera fuliginea* (Schltdl.) U. **Braun & S. Takam**., (2000) is the cause of powdery mildew, a worldwide disease that damages all of the aerial parts of cucumber plants in both greenhouse and field settings and significantly reduces output (**Yang *et al*. 2008; El-Shoraky *et al.*, 2018**).

The majority of methods for controlling powdery mildew depend on chemical fungicides, which are very hazardous to water and the food chain, endangering human health while destroying biodiversity and contaminating the environment (**Kamel *et al*., 2017**). In recent years, there has been a serious need to develop alternate techniques to chemical fungicides that are ecologically friendly and not harmful to peoples, animals, and advantageous microbes, such as the use of various biological agents, natural substances (**O'Brien, 2017**), and plant extracts in cucumber powdery mildew disease management programs (**Ketta *et al*., 2017**).

The antagonistic impact of Trichoderma species against a variety of plant diseases makes them promising and effective bio-control agents (**Abdel-Kader *et al*., 2012**). Additionally, Pseudomonas and Bacillus species were employed as detrimental bacteria to control a number of plant diseases (**El-Shoraky, Fathia *et al.*, 2018**). Effective biological management techniques, such as *Trichoderma* spp., *Pseudomonas fluorescens*, and *Bacillus subtilis*, decreased cucurbits powdery mildew in greenhouse settings (**El-Ghanam *et al.*, 2018**).

Plants treated with *Bacillus* sp. may develop systemic resistance. It has the ability to increase the activity of two enzymes involved in plant-induced systemic resistance (ISR): peroxidase (PO) and polyphenol oxidase (PPO). It can also raise the tannins' and flavonoids' total phenol concentration (TPC) (**Elsisi, 2019**).

Trichoderma harzianum, *T. viride*, *Bacillus subtilis*, *Paenibacillus polymyxa*, and *Serratia marcescens* culture filtrates, along with the fungicide score (Difenoconazole), significantly decreased *Podosphaera xanthii* conidial germination *in vitro* and the severity of the disease in *vivo* on cucumbers. In addition, compared to the untreated plants, the treated cucumber plants with bio-agents exhibited an important rise in growth characteristics, yield parameters, activity of defense-related enzymes (**Sarhan *et al.*, 2020 and Ahmed *et al.*, 2023**).

The application of cucumber plants with both *Bacillus. licheniformis* and *B. aerius* bacteria reduced the severity of powdery mildew disease and conidial germination in greenhouse settings. Furthermore, fresh, dry weight, total phenols, polyphenol oxidase, and peroxidase were all significantly increased in treatment plants after being treated with these bacterial strains (**Abo-Elyousr *et al.*, 2022**). This research goals to develop alternatives to pesticides, due to their high toxicity in the food chain. Additionally, economically destructive fungi have begun to modify their genetic makeup in order to escape being harmed by pesticides. As a result, biological control is one of the safest and most successful ways to combat powdery mildew disease on cucumbers in addition continuing to produce safe food in high quality and quantities (**Ahmed *et al*., 2023**).

2. Methods

2.1. Pathogen Identification for Cucumber Powdery Mildew

Cucumber leaf powdery mildew samples were gathered from Alexandria Desert Road, Wadi El Natrun, Beheira Governorate, Egypt and stored in paper bags. The bags were securely fastened, marked, and transferred to the Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC) in Giza, Egypt. The excessive elimination of epidermis method involved applying colorless adhesive tape to the infected leaf spots, pasting it above the areas of mold, pressing it with fingers, and then removing it from the diseased leaf, which had conidiophores on an enameled epidermis that carried the fungus's conidiospore chains. The cuticle-containing conidiophore strip is inverted onto a lactophenol-stained microscope slide and counterstained (**Moreira *et al*., 2014**). The causal pathogen was identified by its physical traits, such as the location of mycelia on leaf tissue, the presence of dimorphic conidia, the branching of the conidiophore, and the size and form of the conidia (**Braun and Takamatsu, 2000**).

2.2. Antagonistic microorganisms

Trichoderma album, T. hamatum, T. harzianum, and *T. viride* are four preserved isolates of *Trichoderma* spp. by **Ahmed (2018)** that were graciously provided from CLOA, ARC, Giza, Egypt, and employed at a rate of 1 liter/100 liters of water/fed.

2.3. Preparation of biocontrol agent inoculum

In the present investigation, suspensions that contain the biocontrol agents' tested propagules—antagonistic fungi *Trichoderma album, T. harzianum, T. hamatum*, and *T. viride*—were grown on liquid Gliotoxin Fermentation (GF) medium at 25±2ºC for 10 days in complete darkness (**Ahmed *et al.*, 2023**). Each culture was blended separately in an electrical mixer for two minutes before being employed as a concentration-based suspension of 30×10^6 spores/ml with a 1:100 dilutions.

2.4. Blight stop biocide

The suggested biofungicide, which contains $30\text{x}10^6$ spores/ml of *T. harzianum*, is applied at a rate of 1 lit/100 lit water/fed. In order to compare it with the studied treatments, it was received from CLOA.

2.5. Micronic sulfur

In order to evaluate the examined treatments against the recommended fungicide, Micronic sulfur, at a dosage of 250 grams per 100 liters of water. Produced by Kz, Egypt, micronized Soreil/Samark contains 70% weight sulfur.

2.6. Evaluation of Specific Bioagents for Conidiospore Germination Prevention

The following bioagents were investigated for their ability to prevent conidiospore germination. Viable *P. fuliginea* conidial spores were obtained by gently shaking immature sporulating lesions with a glass rod (**El-Morsi *et al.*, 2012**). Glass slides held the freshly harvested spores. The evaluated bioagents' cell suspension was added to the slides after they had been thinly coated with 2% water agar. The control slides were identical, with the exception that they were placed on glass rods in sterile Petri dishes with completely wet filter papers and incubated at 25 °C for 24 hours in a light-filled condition after being covered with an agar-free cell suspension. Germination was assessed by looking at the spores at 40x magnification; spores that produced a germ tube as long as the breadth were deemed to have germinated. One hundred spores were used to calculate the spore germination percentage. Each treatment was analyzed in three duplicates.

The following formula was used to determine the percentages of germination in microscopy on a slide:

$$\text{Conidial germination \%} = \frac{\text{No. of conidial spore germinated}}{\text{No. of total conidial}} \times 100$$

$$Efficacy = \frac{\text{No. of conidial spore germinated in } (Control - Treatment)}{\text{No. of conidial spore germinated in } Control} \times 100$$

2.7. Experimental greenhouses

Research investigations were conducted in a plastic greenhouse (40 m × 9 m) with a completely randomized block design at El Adleya Association, 4th Zone, point No. 16, Belbies, El Sharkia Governorate, Egypt, during the 1St March 2024 season. Autoclaved commercial potting medium (peat moss and vermiculite) was used to fill rectangular plastic trays measuring 5 × 5 × 7 cm. Cucumber seeds cv. Hybrid 20 from Bayer Co. were planted in trays under greenhouse conditions (25±5°C and 75-90% R.H.) and covered with 3 cm thicknesses of sterile sand. *Trichoderma album, T. harzianum, T. hamatum, T. viride*, Blight Stop, and Micronic Sulfur were conducted, each with three duplicates and the recommended dosages listed above. There were twelve plants in each replication. After three weeks, the seedlings were moved to the two sides of the ridge, with a 50 cm distance between each seedling in the row. In terms of fertilization, during the soil preparation process, mature compost was implemented at a rate of 1.2 kg/m^2 two weeks prior to the transplanting date (**Ahmed *et al*., 2021**). This location had access to Nile water through a drip irrigation system. Powdery mildew infestation on cucumbers has historically been significant in greenhouses in this area.

In cucumber plants of hybrid variety 20, foliar spraying was done when the first five true expanding leaves showed signs of a natural powdery mildew infection at the rate of 1 L/100 liters/ water of various biological controls and 250 gm/100-liter water of the fungicide micronized sulfur were employed. Two weeks later, the spraying was done again. For comparison, the control treatment was sprayed with water only to investigate the influence of the number of sprays on the disease's severity.

2.8. Determine the disease severity

The disease severity was measured 15 days after inoculation. **Morishita *et al*. (2003)** divided evolution into five groups using the following scale: 0 indicates no visible infection, 1 indicates a 1–5% infection, 2 indicates a 6–25% infection, 3 indicates a 26–50% infection, and 4 indicates that fungal colonies cover more than 50% of the leaf area. Fifteen days following inoculation, the last disease assessment was carried out.

$$Disease\ severity\ \% = \frac{\Sigma\ (\mathrm{n\ xv})}{5\mathrm{N}} \times 100$$

In this equation, n represents the number of infected leaves in each category, v represents their numerical values, and N represents the overall number of infected leaves.

$$Disease\ reduction\ \% = \frac{\mathrm{Control}\ - \mathrm{Treatment}}{Control} \times 100$$

2.9. Assessment of yield characteristics

Cucumber fruits of marketable size were harvested every two or three days for seven days in order to examine yield characteristics such number of fruits/plant, mean weight fruit (g), weight of fruits/plant (kg), and mean product/greenhouse (ton).

2.10. Biochemical changes of cucumber leaves

Chlorophyll measurement

A portable leaf chlorophyll meter (Minolta SPAD-502, Japan) was used to measure the amount of chlorophyll (greenery) in fully grown cucumber leaves in accordance with **Torres-Netto *et al*. (2005)**.

Determining the Total Phenol Level

The Folin-Ciocateu technique, updated by **Singleton *et al.* (I999)**, was utilized for estimating the overall phenolic amount of extracts.

Assessment of the activity of antioxidant enzymes

Samples of cucumber leaves were gathered a week following the last treatment. Using the **Thimmaiah (1999)** procedure for polyphenol oxidase (PPO) and the **Bollage (1996)** technique for peroxidase (PO), the biochemical activities were measured with unit/mg protein.

2.11. Statistical Analysis

The gathered data was analyzed using the one-way ANOVA, as explained by **Gomez and Gomez (1984)**. Treatment averages were compared using the least significant difference (LSD) at a 5% probability level.

3. Results and Discussions

3.1. Impact of the culture filtrate of the studied bioagents on Cucumber *Podosphaera fuliginea* conidial germination

The information in Table (1) shows that, *Trichoderma album*, *T. harzianum*, *T. hamatum*, *T. viride*, Blight Stop, and Micronic Sulfur all considerably reduce the *in vitro* germination of *Podosphaera fuliginea* compared to the control treatment. The most successful fungicide treatment was Micronic Sulfur, followed by *T. harzianum* and Blight Stop. These treatments resulted in 5.67%, 9.86%, and 10.12% germination percentages, respectively, with inhibition percentages of 92.69%, 87.29%, and 86.96% compared to the control. In contrast to the control treatment, *T. viride* exhibits the least efficacy when compared to other treatments.

The findings are consistent with those reported by many investigators (**Ebrahim and Helmy, 2017)**. They demonstrated the effectiveness of the substances under investigation in managing powdery mildew diseases.

The inhibition of fungal conidia germination may be the reason for Fungicides and RICs reduce disease severity and prevent infection (**Raju *et al.*, 2017**).

The pronounced antifungal activity of The lytic enzymes produced by *Trichoderma* spp., including chitinases, peroxidases, polyphenoloxidases, glucan 1-3 B-glucosidases, and proteases, destroyed the cell wall pathogen (**Ahmed, 2018**). These results are in line with those of **Elsisi (2019)** who showed that the bioagents may prohibit powdery mildew conidial spores from germinating, possibly because of hydrolytic enzymes. This decline may be the consequence of the investigated bioagents' potential as PGPR, that is widely employed to manage plant diseases (**Sarhan *et al*., 2020**).

This antifungal effect implied the use of antibiotics and/or other direct suppressive substances, such as siderophores, hydrogen cyanide, or hydrolytic enzymes (**Ahmed *et al*.,2021**).

Table (1). Effects of different biocontrol agents and the fungicide Micronic Sulfur on the germination of *Podosphaera fuliginea* after 24 hours incubation at 28±1°C

Treatments	Spore germination %	Inhibition%
Trichoderma album	12.24	84.22
T. hamatum	14.36	81.49
T. harzianum	9.86	87.29
T. viride	16.43	78.82
Blight Stop	10.12	86.96
Micronic Sulfur	5.67	92.69
Control (Untreated)	77.59	0.00
LSD at 1%	1.02	--

3.2. The impact on cucumber cv. Hybrid 20 of various biological controls and natural chemical treatments

Disease severity

The findings in Table (2) shows that all tested biological control treatments (*Trichoderma Album*, *T. harzianum*, *T. viride*, Blight stop (*T. harzianum*), and natural compounds (Micronic sulfur) greatly decreased the severity of powdery mildew disease on cucumber cv. Hybrid 20 in comparison to the control. In terms of reducing disease severity, Micronic sulfur fungicide achieved the greatest reduction

(87.30%), followed by *T. harzianum* isolate (81.71%). *T. viride*, on the contrary, demonstrated the least decrease (76.50%) in managing the disease.

Chemical elicitors can cause systemic acquired resistance and induced systemic resistance, which prepares the immune system to defend against pathogens before infection (**Hafez *et al*., 2008**). *Trichoderma* spp. have direct effects on plant pathogenic fungi by competing for space, food, and oxygen, as well as their capacity for fungal mycoparasitism and antibiotic production (**Hafez *et al*., 2018** and **Sarhan *et al.*, 2020**). The *in vivo* results can be interpreted in light of the chemical effects of antioxidants, that are known to improve plant physiology, metabolism, and induce systemic resistance (ISR), as well as the influence of biotic factors that create growth regulators (**Ahmed *et al*., 2021 and Frem *et al.*, 2022**).

Table (2). The impact of various treatments on cucumber powdery mildew severity in greenhouse settings 2024

Treatments	Disease severity %	Reduction in disease severity %
Trichoderma album	13.55	72.02
T. hamatum	16.44	66.05
T. harzianum	8.86	81.71
T. viride	19.09	60.58
Blight Stop	11.38	76.50
Micronic Sulfur	6.15	87.30
Control (Untreated)	48.43	0.00
LSD at 5%	1.22	--

Yield parameters

The information data in Table (3) shows that using biological control and natural compounds in accordance with organic agriculture law significantly increased yield components such as number of fruits/plant, fruit weight (g), weight of fruit/plant (Kg), and yield/greenhouse (Ton) compared to non-treated cucumber cv. Hybrid 20 in greenhouse conditions.

In terms of production qualities, the biological treatments performed better than the fungicide, even though the sulfur pesticide was most effective in lowering the spore germination rate and the severity of infection caused by *Podosphaera fuliginea*, which causes powdery mildew.

T. harzianum as a biocide, showed the greatest significant increase in yield parameters, with numbers of fruits/plant, fruit weight (g), weight of fruit/plant (Kg), and yield/greenhouse (Ton) increasing by 45.7, 82.2, 3.76, and 3.01 respectively. *T. album* isolate came in second, with 44.2, 81.5, 3.60, and 2.88 respectively, when compared to untreated plants. In contrast, during the experiment, the *T. viride* treatment had the least impact when compared to the other treatments rather than untreated plants during investigation. Other ways that these PGPR impact plants include the synthesis of plant growth regulators, such as gibberellins, cytokinin, and indoleacetic acid, which decreased the disease severity of powdery mildew, promote plant development and boost fruit yield (**El-Naggar *et al.* (2012); El-Sharkaway *et al.* (2014); Reddy *et al.* (2014) and Singh *et al*. (2018).** The results obtained are consistent with **Ahmed (2018) and Elsisi (2019)**, who reported that the biocontrol agents increased the yield components while reducing the frequency and the severity of powdery mildew infections.

These results are in line with those of **Sarhan *et al*., 2020** and **Ahmed *et al.* (2021)**, who noted that the formation of growth regulators and resistant inducers by biocontrol agents contributes to an improvement in yield productivity and quality (**Abo-Elyousr *et al*., 2022 and Ahmed *et al*., 2023**).

Table (3). The impact of various treatments on cucumber yield components in greenhouse settings 2024

Treatments	No. of fruit/plant	Mean weight fruit (g)	Weight of fruit/ plant (kg)	Mean product/ greenhouse (Ton)
Trichoderma album	42.8	81.1	3.47	2.78
T. hamatum	39.4	80.3	3.16	2.53
T. harzianum	45.7	82.2	3.76	3.01
T. viride	37.5	79.4	2.98	2.38
Blight Stop	44.2	81.5	3.60	2.88
Micronic Sulfur	41.3	80.7	3.33	2.67
Control (Untreated)	19.9	76.8	1.53	1.22
LSD at 5%	1.10	2.43	0.32	0.22

3.3. Total phenol, cholophyll, and enzyme activity in response to the development of *Podosphaera fuliginea* disease and the resistance characteristics of cucumber plants

Resulted data Table (4), shows that applying any of biocontrol agents or fungicide treatments during the investigation in greenhouses 2024 significantly increased enzyme activities in cucumber plants, like Peroxidase (PO), Polyphenol Oxidase (PPO), total phenols (mg/100 g Fw) and chloropyll (SPAD), compared to untreated plants. *T. harzianum* isolate has been proved to be the most successful approach in this regard, raising plant enzyme activity by 187.2 and 118.1 units/mg protein/min for polyphenol oxidase (PPO) and peroxidase (PO), respectively. Additionally, *T. harzianum* was considerably improved the total phenols (67.8 mg/100g FW) and chlorophyll (45.2 SPAD), respectively, compared to others. The Blight Stop was ranked second. *T. viride* isolation spray was the least effective treatment compared to other treatments.

These findings suggest that endophytic factors help cucumber plants grow through raising defense-related PO enzymes. Many studies have found that increased PO activity correlates with plant resistance to fungal, bacterial, and viral diseases. These results are congruent with those published by (**Esmaeili *et al*., 2017**). PPO activity is probably important for plant disease resistance because it can oxidize phenolic chemicals to quinines, which are often more poisonous to microbes than the original phenolics (**Prasannath, 2017 and Taranto *et al*., 2017**).

Plant resistance against fungal plant infections and disease resistance are intimately linked to the activation of phenolic chemicals **(Wang *et al*., 2018)**. These findings showed that all treatments maintained the health and optimal growth of cucumber plants, which may have been highlighted by the control treatment's excessively low chemical content. Many researches have been conducted that have proven by **El-Shoraky *et al.* (2018)** that the final result through the agreement to control white powdery mildew and natural compounds, which are an alternative to specialized pesticides, is very effective in controlling late white powdery mildew.

The total phenol content of treated cucumber plants was higher than that of infected and healthy control plants. Because phenolic compounds are toxic to pathogens, their accumulation has been connected to preventing the growth of pathogens at the infection site **(De Silva *et al*., 2019)**.

Table (4). The efficacy of several treatments on peroxidase (PO), polyphenoloxidase (PPO) activity, chlorophyll and total phenol

Treatments	Peroxidase activity (PO) mg/ml	Polyphenoloxidase activity (PPO) (mg/ml)	Total phenols (mg/100 g FW)	Chlorophyll (SPAD)
Trichoderma album	180.3	113.8	60.4	44.3
T. hamatum	175.9	108.6	52.6	42.5
T. harzianum	187.2	118.1	67.8	45.2
T. viride	171.4	103.7	49.9	41.4
Blight Stop	183.1	115.2	63.2	44.8
Micronic Sulfur	178.8	111.4	58.7	43.7
Control (Untreated)	52.7	46.3	22.5	20.1
LSD at 5%	1.65	1.11	0.83	0.67

Numerous studies by **Elsisi (2019) and Sarhan *et al.* (2020)** have demonstrated a direct correlation between the reduction in the severity of the powdery mildew disease in cucumber and squash and the induced resistance in plants. This resistance causes phenolic compounds to accumulate and increase, as well as the creation of defense-related enzymes PO and PPO in additionally to TPC in squash plants grown in greenhouses (**Frem *et al.*, 2022**). It does this by chemically modifying the nutrients of plants, which leads to the preparation of the systemic plant from the amount of chlorophyll, phenols and plant activities in plants.

Additionally, it generates secure, toxic-free and environmentally friendly production **Ahmed *et al.*, (2021)**. A shift in the cytoplasmic pH of plant cells caused by an increase in phenolic acid content may also promote resistance, preventing pathogen growth (**Ahmed *et al.*, 2023**). In the current investigation, treatment with bacterial bioagents led to an increase in the buildup of phenolic chemicals in reaction to an infection by a disease **(Shnaider *et al*., 2023).**

4. Conclusion

In order to reduce the toxicity of chemicals throughout the food chain and yield enough high-quality and quantity food, this study intends to assess the effectiveness of various biocontrol agents, including *Trichoderma album*, *T. harzianum, T. hamatum* and *T. viride*; biopesticides, such as Blight stop (*T. harzianum*), which is used at the rate of 1 L/100 liters of water; and additionally natural compounds, such as micronized sulfur fungicide, which is used at the rate of 250 gm/100 liters of water, in controlling powdery mildew disease of cucumber hybrid 20 under greenhouse conditions in 2024. All treatments were efficient in reducing powdery mildew disease caused by *Podosphaera fuliginea* while enhancing productivity, yield parameters, quality, chemical components, and enzyme activity of cucumber hybrid 20 when compared to untreated plants. Cucumber plants treated with *T. harzianum* at a rate of 1:100 exhibited the greatest reduce in the inhibition of fungal conidia germination and disease severity. They also showed the greatest increase in yield and biochemical analysis, including total chlorophyll, total phenolic content, peroxidase (PO), and polyphenol oxidase (PPO) activities during the growing season 2024 under greenhouses, compared to untreated plants.

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