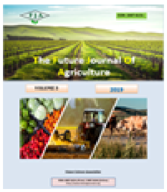
FSA

Available online free at www.futurejournals.org

The Future Journal of Agriculture

Print ISSN: 2687-8151 Online ISSN: 2687-8216

Future Science Association



Future J. Agric., 3 (2021) **52-63** **OPEN ACCES**

https://doi.org/10.37229/fsa.fja.2021.08.18

INFLUENCE OF BIOLOGICAL CONTROL ON SWEET PEPPER POWDERY MILDEW DISEASE AND ITS IMPACT ON GROWTH AND YIELD UNDER GREENHOUSE CONDITION

Ahmed, M.F.A[1,*]; Ahmed, M.S.M.[2] and Mervat G. Abd El-Aziz[3]

[1]Central Lab. of Organic Agriculture (CLOA), Agricultural Research Center (ARC), Giza, **Egypt.**
[2]Central Lab. for Agricultural Climate (CLAC), Agricultural Research Center (ARC), Giza, **Egypt.**
[3]Potato and Vegetatively Propagated Vegetables Department, Hort. Res. Inst., ARC, Giza, **Egypt.**

[*]**Corresponding author:** mohamed_faah@yahoo.com - https://orcid.org/0000-0002-0118-3422

Received: 5 July 2021 **Accepted:** 10 August 2021 **Published:** 18 August 2021

ABSTRACT: This investigation was conducted in greenhouses protected, Al-Haram, Ministry of Agriculture and Land Reclamation, Giza, Egypt during the two seasons 2019/20 and 2020/21 to evaluate some biological control agents *i.e. Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens*, as well as the biofungicide -Blight Stop and micronic sulfur treatments to control *Leveillula taurica*, the main causal of powdery mildew disease in sweet pepper plants cv. Godeon Hybrid F1. All previous treatments proved efficacy in reducing disease incidence and severity of *L. taurica in vitro* and greenhouses, as well as recorded an increase in productivity, fruit quality, chemical constituents and enzymatic activity of sweet pepper cv. Godeon Hybrid F1, compared to untreated plants. The treatment of pepper plants with Blight stop at the rate of 1 L:50 L water recorded the highest percentage of decrease in disease incidence and severity of pepper plants, and also recorded the highest increase in vegetative growth, yield, fruit quality such as "total soluble solids and ascorbic acid"; biochemical analysis such as total chlorophyll; total phenol content, peroxidase and polyphenoloxidase activities during the two growing seasons, followed by *T. harzianum* isolate in effectiveness. On the contrary, micronic sulfur treatment showed less effect compared to other treatments except untreated plants. The study was recommended the use of biocide Blight stop at a concentration of 1 L: 50 Lwater for sweet pepper cultivation to obtain the best growth, highest productivity and quality of pepper fruits with the lowest percentage of powdery mildew infection under greenhouse condition.

Key words: Sweet Pepper, *Leveillula taurica*, powdery mildew, Biocontrol agent, biocide and Micronic sulfur

INTRODUCTION

Sweet pepper (*Capsicum annuum* L.) has become a significant vegetable crop in Egypt and global agriculture due to its economic importance and high profit and nutritional worth for human health **(Rajput and Poruleker, 1998)**.

In Egypt, it is the second-largest area dedicated to growing vegetables in greenhouses. The overall area, yield, and production of sweet pepper grown in greenhouses were around 9.68 million m^2, 8.193 Kg/m^2, and 81330 ton, respectively **(FAO, 2021)**.

The fungus *Leveillula taurica* (Le'v.) G. Arnaud (anamorph: *Oidiopsis taurica* (Le'v.) Salmon) is the cause of powdery mildew of pepper due to the optimal temperature as well as relative humidity, one of the most common and destructive diseases of pepper crops. Both in greenhouses and in the field, this disease has the potential to cause significant damage **(Kim**

***el al*., 2009)**. The development of white mycelium on the abaxial leaf surfaces and the emergence of necrotic chlorotic lesions on the adaxial sides are two signs of powdery mildew **(Tsror *et al*., 2004)**. Defoliation, burnt fruit, and reduced photosynthetic activity result from the affected leaves falling off the plant.

The application of bio-control agents *i.e. B. subtilis*, *Trichoderma harzianum*, and arbuscular mycorrhizal fungus (AM) can prevent powdery mildew because they give plants nutrients that encourage growth promoting **(Kiss, 2003 and Kim *et al*., 2013)**.

In greenhouse conditions, *T. harzianum* T39 greatly reduced *Leveillula taurica* leaf colonisation on sweet pepper at temperatures between 15 and 25°C, although it only significantly reduced disease at temperatures around 20 to 25°C. Sulfur, on the other hand, decreased disease and leaf colonisation at all temperatures **(Brand *et al*., 2009)**.

According to **Sudha and Lakshmanan (2009)** and **Ownley *et al.* (2010)** *Leveillula taurica* (Lev.) Arn. is the main cause of powdery mildew disease on the chilli (*Capsicum annuum* L.), which consider one of the most destructive diseases that reduces production. *Trichoderma viride* Per., *T. harzianum* Rifai., and *Pseudomonas fluorescens* Migula's culture filtrates showed the greatest reduction in chilli powdery mildew disease among the antagonistic microorganisms screened and were also highly effective at preventing *L. taurica*'s conidial germination in greenhouse and field trials. In addition, the chilli leaves' activity for total phenol, peroxidase, polyphenol oxidase, phenylalanine ammonialyase, and protein was raised.

Trichoderma asperellum (Samuels, Lieckf. and Nirenberg) and *Metarhizium anisopliae* (Metschn.) as biocontrol agents were showed the highest effect in reducing the disease severity of pepper powdery mildew (*Leveillula taurica*) and showed the highest increasing in chitinolytic and b- 1,3-glucanase enzyme activities **(Lopez *et al*., 2019)**.

Regarding to the modes of action of *Trichoderma* spp. secretion toxins, proteases, antimicrobial peptides and other molecules which act as mycoparasitism and antibiosis, often in combination with extracellular cell wall-degrading enzymes (*e.g.* chitinases and glucanases), competition for nutrients and stimulation of plant resistance **(Harman *et al*., 2004; Mukherjee *et al*., 2012 and Vinale *et al*., 2014)**. Some *Trichoderma* spp. also produce secondary metabolites or differ antibiotics were determined the pathogens of powdery mildew and promote the growth of plants and adaptation of plants to environmental conditions **(Ahmed, 2018)**.

In order to control the sweet pepper powdery mildew disease and improve the productivity and quality of the pepper fruit crop as a healthy, safe food under greenhouse conditions, the goal of this study is to assess the influence of some biological compounds and micronic sulfur as the safe, non-toxic factors in the production chain.

MATERIAL AND METHODS

Antagonistic microorganisms

Different biocontrol agents, including *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens*, were graciously provided by the Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC), Giza, Egypt.

Commercial biofungicide (Blight stop)

The commercial biofungicide (*Trichoderma* spp. $30x10^6$) which used at the rate of 1 L./50 liter water and recommended by CLOA was used as a comparison with other treatments.

Micronic sulfur

In order to compare the tested treatments to the suggested Micronic sulfur at the rate of 250gm/100 liter water as fungicide. Micronized Soreil/Samark with 70% weight sulfur, manufactured by Kz, Egypt.

Sweet Pepper seeds

Sweet pepper seeds (cv. Godeon Hybrid F1), were purchased from Syngenta Company, Qaha, Qalyubia Gov., Egypt.

Preparations of the biocontrol agents

The antagonistic fungi *i.e. Trichoderma harzianum* and *T. viride* were grown for 10 days at 25±2ºC on a liquid Gliotoxin Fermentation (GF) medium whereas the antagonistic bacteria *i.e. Bacillus subtilis* and

Pseudomonas fluorescens were grown for 3 days at 25±2ºC on a liquid Nutrient Glucose (NG) medium under complete darkness conditions **(Ahmed, 2018)**.

All cultures were individually blended in an electrical blender for 2 min, used as suspension at concentration of ($30×10^6$ spores/ml) with a dilution 1 L:50 liter water which mixed with 5% Arabic gum and 5% potassium soap and wetting the leaves, using a sprayer to increase adhesive capacity and improve distribution of bioagent on the surface of treated plants.

In vitro assay

Collection of powdery mildew conidia

In the fall of 2019/2020 and 2020/2021, naturally infected leaf samples of sick sweet pepper (cv. Godeon Hybrid F1) exhibiting powdery mildew signs were gathered from greenhouses of Protected Agriculture in Al Haram, Ministry of Agriculture and Land Reclamation, Giza, Egypt. Morphological characteristics of the powdery mildew pathogen, *i.e.* the placement of mycelium on the host, the presence of dimorphic conidia, branching of conidiophore, size and shape of the conidia were recorded. Epidermal strips from the infected pepper leaves harbouring the fungal conidiophores and conidia were placed on glass slides, dyed with lactophenol cotton blue, covered with cover glass, and then viewed using light microscopy at 10, 20, and 40x to determine the causal pathogen. Slides were stored at 4ºC after returning them to the laboratory, and conidia were measured within 72 h. A minimum of 100 conidia was measured **(Correll *et al*., 1987)**.

Inoculum production of *Leveillula taurica*

Conidial suspensions of *L. taurica* were created using **Souza and Cafe-Filho (2003)** techniques. Conidia were harvested from the leaves by drenching the infected leaves in sterile distilled water. The conidial suspension was centrifuged twice at 4000 rpm for 30 minutes after being strained through two layers of cheese cloth. With sterile distilled water, the conidial concentration was adjusted to $5×10^4$ per ml for additional experiments.

Efficacy of different treatments on conidial germination of *Leveillula taurica*

According to the procedure outlined by **Correll *et al*., (1987)** the pathogenicity of the powdery mildew fungus was evaluated. The sweet pepper (cv. Godeon Hybrid F1) plants growing in the greenhouse where the fungus growth was obtained. Infected and sporulation host tissue was collected, kept refrigerated in a plastic bag and incubated for 24 to 48 hours at 30°C before being used as an inoculum source. Conidia of *L. taurica* were directly removed with the aid of a small paintbrush from extensively infected pepper plants and dropped onto glass slides together with drops of the various biocontrol agents that had been tried or micronic sulfur which preparation above. The slides were then placed in moist chambers prepared by placing two moist filter papers in the inner surfaces of a Petri plate. Conidia immersed in distilled water only served as control. For each treatment, three replications were produced. Under a light microscope, the percentage of germination was calculated using formula suggested by **(Kumar *et al*., 2006)** after the slides were incubated at 25 ±2°C for 24 hours.

$$\text{Reduction in spore germination \%} = \frac{\text{Control - Treatment of spore germination}}{\text{Control}} \times 100$$

Greenhouse evaluation of different treatments against *Leveillula taurica*

In this experiment, four isolates of biocontrol agents *i.e. Trichoderma harzianum, T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens*, as well as the biofungicide Blight stop and natural component micronic sulfur were evaluated to control powdery mildew of sweet pepper cv. Godeon Hybrid F1 under greenhouse condition of Protected Agriculture in Al Haram, Ministry of Agriculture and Land Reclamation, Giza, Egypt during 2019/20 and 2020/21 two successive seasons. Original high tunnel seven greenhouses (6x40m) were used during the investigation.

The area of each greenhouse plot was 240 m^2 comprised of 3 replicates, raised bed (40 m long ×1.5 m width), with about 50 cm apart and 2.8 m height. Each raised bed was planted with 160 seedlings of sweet pepper. Regarding to fertilization, mature compost was applied at the rate of 1.2 Kg/ m^2 two weeks before the transplanting date throughout the soil preparation **(Ahmed, 2013)**. Nile water was available in this area with a drip irrigation system. Greenhouses in this area have history of high infestation with powdery mildew on pepper. Sweet pepper (cv. Godeon Hybrid F1) seeds were sown in ordinary seed boxes filled with peat moss-vermiculate (1:1, w/w) for 40 days old under greenhouse conditions (25±5 ºC and 75-90% R.H.), then transplanted with three true leaves to greenhouses on 15th September 2019/2020 and 2020/2021, respectively. Plants were hooked to a wire to allow growth to 2 m.

Treatment of different bioagents or micronic sulfur mixed with 5% Arabic gum, and 5% potassium soap and used as foliar spray individually in each greenhouse were applied twice, the first time before the predicted time of flowering (60 days from transplanting) and the second was 15 days after flowering (90 days from transplanting) at the rate of 1 L/50 liter water of different biocontrol agents and 250gm/100 liter water of micronic sulfur. Greenhouse sprayed with water served as a control treatment.

Disease assessment

Disease incidence

The number of diseased plants relative to the total number of growing plants for each raised bed was used to calculate the percentage of the disease incidence, and the average of the disease incidence was then calculated **(Farag *et al.*, 2018)**.

Disease severity

After 30 days of inoculation, disease intensity was assessed by examining the leaves from each treatment at random, using a 0 – 4 scale, where 0=no disease; 1=1– 10% leaf area affected; 2=11 – 25% leaf area affected, 3=26 – 50% leaf area affected and 4≥50% leaf area affected **(Reuveni *et al.* 1998)**. The percentage disease index was calculated by using the formula:

$$\text{DSI \%} = \frac{\Sigma\,(n\,xv)}{ZN} \times 100$$

Where:

D.S.I= Disease severity index, n = Number of leaves in each category, v = Numerical value of each category, z = Numerical value of highest category and N = Total number of leaves in the sample.

$$\text{Efficacy of diseases \%} = \frac{\text{Control -Treatment}}{\text{Control}} \times 100$$

Vegetative growth characteristics

Samples of sweet pepper plants from each treatment were taken at the beginning of fruiting stage to estimate average plant height (cm), fresh and dry weights (g/plant) which determined for three plants pulled off randomly from each replicate and dried at 70ºC.

Chlorophyll estimation

The estimation of total chlorophyll was done as per methods of **Litchenthaler, 1987**.

Yield components and quality

The yield was computed as an average fruit weight kg/plant as well as the total yield ton/greenhouse. Red-ripening fruits were harvested from plants during the growing season. Nine samples of red-ripening sweet pepper fruits were taken from each experimental plot to determine fruit quality parameters *i.e.*, total soluble solids (TSS) using a Carlzeiss hand Refractometer and quantification of ascorbic acid (mg/100g FW) as described by **(A.O.A.C., 2005 and Offor *et al.*, 2015)**.

Biochemical changes in sweet pepper plants due to application of chemicals

In the greenhouse experiment, leaf samples from the various treatments were taken 1, 3, and 5 days after spraying and used for biochemical analysis. Five hundred mg of leaf samples from different treatments were ground with 5 ml of a 0.1 M sodium phosphate buffer with a pH of 7.0 in the enzyme extraction procedure. After centrifuging the ground materials at 10,000 rpm for 15 min, the supernatant was collected and used as an enzyme source.

Assay of peroxidase (PO)

According to the procedure outlined by **Hammerschmidt *et al*. (1982)** peroxidase activity was evaluated.

Assay of polyphenoloxidase (PPO)

The **Srivastava (1987)** method was used to measure polyphenoloxidase activity.

Total phenol

The standard graph created with various catechol concentrations was used to calculate the amount of phenol. The catechol equivalents of the phenol concentration were estimated as mg/g of fresh tissue **(Bray and Thorpe, 1954)**.

Statistical Analysis

According to **Snedecor and Cochran (1989)**, data were statistically analyzed by using the least significant difference (LSD) values at the 5%.

RESULTS AND DISCUSSIONS

Identification of the fungus

Microscopically examining the epidermal strips of infected leaves revealed that long septate conidiophores were seen emerging single, in cluster formations, or in bundles through the stomata of the lower leaf surface. These conidiophores were derived from branched endophytic hyphae. Macroscopically analysis was used to examine the morphological traits of the powdery mildew pathogen from sweet pepper. Endophytic mycelium and dimorphic conidia (pyriform to obclavate) were the traits seen in Fig. (1). this pathogen's teleomorphic stage, or sexual stage, has been observed in several hosts. These findings concur with those of **Correll *et al*., 1987; Tsror *et al*., 2004 and Little, 2006**.

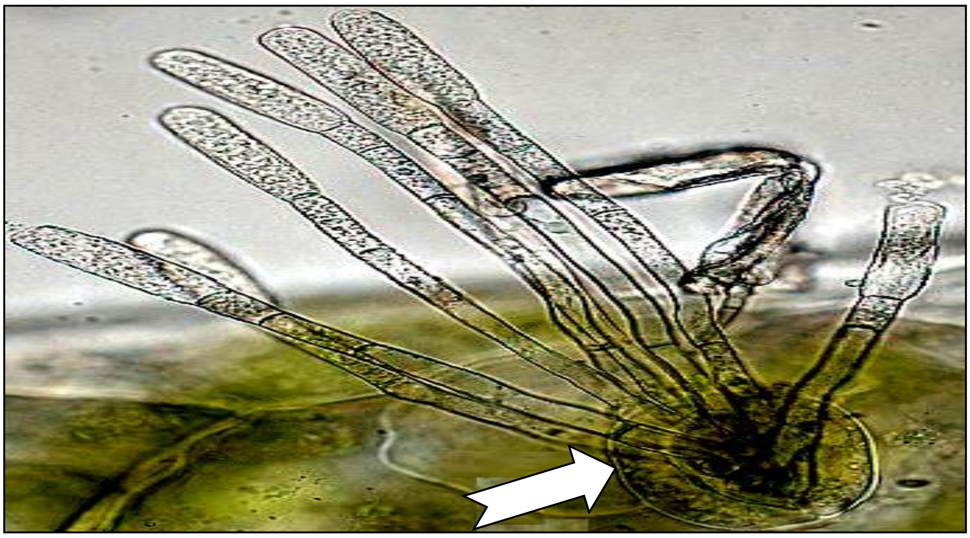


Fig. (1). Condispores of *Leveillula taurica* emerging through a stroma (arrow) of sweet pepper.

Efficacy of different treatments on conidial germination of *Leveillula taurica*

Presented data in Table (1) show that all treatments significantly reduced spore germination of *L. taurica* the main causal of sweet pepper cv. Godeon Hybrid F1 powdery mildew. The highest reduction was observed with treatment of biofungicide Blight stop followed by *Trichoderma harzianum* at reduction in spore germination of *L. taurica*, being 84.62 and 76.92%, respectively. On the contrary, micronic sulfur was showed the lowest inhibition in spore germination, being 53.85% in comparison with other treatments. These results are in harmony with those previously recorded by **Elad (2000), Kiss (2003); Sudha and Lakshmanan (2009)**.

The pronounced antifungal activity of *Trichoderma* spp. degraded the cell wall pathogen due to the production of lytic enzymes such as chitinases, peroxidase, polyphenoloxidase, glucan 1-3 B- glucosidases and protease **(Mausam *et. al.*, 2007; Brand *et al*., 2009 and Ahmed, 2018)**. Over all, the antagonist fungal filtrates were found significant in inhibiting germination of *L. taurica* conidial spores **(Kumar *et al*., 2006)**.

Bacillus subtilis occupied the second rank after *Trichoderma* spp., this might be due to that it produces a group of enzymes, which dissolve the cell wall of the pathogen **(Ahmed, 2013)**, antibiotics such as bacterocin and subtilisin, volatile compounds and phytotoxic substances **(Hoagland and Cutler, 2000)**.

Pseudomonas fluorescens occupied the third rank after the previous antagonists, this might be due to enable production of bacterial allelochemicals, including iron-chelating siderophores, antibiotics, biocidal volatiles, lytic enzymes and detoxification enzymes **(Compant, *et. al.*, 2005 and Ownley *et al.*, 2010)**.

Table (1). The effect of different treatments on spore germination of *Leveillula taurica*

Different treatments	Spore germination %	Reduction in spore germination %
T. harzianum	6.0	76.92
T. viride	7.0	73.08
B. subtilis	9.0	65.38
P. fluorescens	10.0	61.54
Micronic sulfur	12.0	53.85
Blight stop (*T. harzianum)*	4.0	84.62
Control (Untreated)	26.0	0.00
L S D at 0.01	1.10	2.14

The impact of several treatments on the powdery mildew of the sweet pepper cv. Godeon Hybrid F1 in greenhouse condition during the two seasons of 2019–2020 and 2020–2021

Disease parameters (incidence and severity)

The results in Table (2) demonstrate that, all tested biological control treatments (*Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens*, as well as commercial preparation, Blight stop (*T. harzianum*) and natural compounds (Micronic sulfur) led to a significant reduction in the incidence and severity of powdery mildew disease on sweet pepper cv. Godeon Hybrid F1 treatments in the 2019/20 and 2020/21 seasons compared to the control treatment.

Blight stop biocide had the best efficacy (82.89 and 83.70%), followed by *T. harzianum* isolate (80.25 and 79.26%) in reducing disease incidence and severity during the two seasons 2019/20 and 2020/21, respectively. On the contrary, micronic sulfur showed the least efficacy (68.72 and 58.15%) in controlling the disease. (68.72 and 58.15%) compared with other treatments rather than control treatment.

The field results can be interpreted according to both the effect of biotic factors which produce growth regulators **(Harman *et al.*, 2004; Mukherjee *et al.*, 2012 and Vinale *et al.*, 2014)** and the chemical effect of antioxidants **(Lopez *et. al*., 2019)**, which play a clear role in improving plant physiology, metabolism and induce systemic resistance (ISR) **(Kumar *et al*., 2006; Brand *et al*., 2009 and Kim *et al*., 2013)**.

Table (2). The effect of different treatments on disease incidence and severity of sweet pepper powdery mildew under greenhouse condition during 2019/2020 and 2020/2021 seasons

Different treatments	Disease incidence %				Disease severity %			
	2019/20	2020/21	Mean	Efficacy*	2019/20	2020/21	Mean	Efficacy*
T. harzianum	9.90	10.30	10.10	80.25	5.5	5.7	5.60	79.26
T. viride	11.40	12.20	11.80	76.93	6.1	6.3	6.20	77.04
B. subtilis	13.80	14.10	13.95	72.73	8.4	8.6	8.50	68.52
P. fluorescens	14.30	15.40	14.85	70.97	9.8	10.1	9.95	63.15
Micronic sulfur	15.50	16.50	16.00	68.72	11.2	11.4	11.30	58.15
Blight stop (*T. harzianum)*	8.70	8.80	8.75	82.89	4.3	4.5	4.40	83.70
Control (Untreated)	50.60	51.70	51.15	0.00	26.4	27.6	27.00	0.00
L S D at 0.05	1.41	1.42			0.10	0.12		

Vegetative growth characteristics

Regarding role of bio-control agents and natural compounds, data in Table (3) illustrate that there were significant effects on some growth characteristics in terms of plant height, fresh and dry weights per plant compared with untreated plants. The most effective treatments regarding the previous vegetative growth characteristics were recorded when plants were treated with Blight stop (75.78 cm, 474.5g and 146.68g), followed by that treated with *Trichoderma harzianum* which recorded (74.27 cm, 465.2g and 143.25g) in plant height, fresh and dry weight during the two growing seasons 2019/20 and 2020/21, respectively.

Moreover, the other treatments of sweet pepper plants on one raised bed with application of natural compound and bio-control agents gave good effect on the previous growth characteristics when compared with the control treatment. These results are in agreement with those reported by **Lorito (2005); Khandelwal *et al.* (2012); Babu and Pallavi (2013)** who mentioned that *Trichoderma* spp. causes conspicuous improvement in the aforementioned crop parameters during the two growing seasons.

In addition, these variations may be due to the variation in genetic pool of the sweet pepper cultivars and/or the effects of climatic change on vegetative growth. Similar results were reported by **Harman *et al.* (2006) and Farag *et al.*, (2018)**.

Table (3). The effect of different treatments on plant height, fresh weight and dry weight of sweet pepper plants under greenhouse condition during 2019/20 and 2020/21 growing seasons

Different treatments	Plant height (cm)			Fresh weight (g)			Dry weight (g)		
	19/20	20/21	Mean	19/20	20/21	Mean	19/20	20/21	Mean
T. harzianum	73.67	74.86	74.27	462.6	467.7	465.2	142.67	143.83	143.25
T. viride	71.12	72.13	71.63	453.3	455.5	454.4	140.53	141.55	141.04
B. subtilis	69.58	70.83	70.21	446.2	448.4	447.3	138.46	139.48	138.97
P. fluorescens	66.75	67.85	67.30	437.1	443.3	440.2	136.17	137.33	136.75
Micronic sulfur	65.33	66.13	65.73	385.5	396.8	391.2	135.22	136.53	135.88
Blight stop (*T. harzianum)*	75.22	76.33	75.78	473.4	475.6	474.5	145.63	147.73	146.68
Control (Untreated)	57.13	58.20	57.67	219.1	221.2	220.2	123.62	124.75	124.19
L S D at 0.05	1.60	1.62		3.12	3.22		2.31	2.33	

Yield and its components

Presented data in Table (4) indicate that, spraying any of the tested biological control and natural compounds according to the organic agriculture law as a recommended biocide treatment significant increased yield components *i.e.* No. of fruits/plant, fruit weight (g), yield/plant (kg) and total yield/greenhouse (ton) compared to untreated sweet pepper cv. Godeon Hybrid F1 plants during the two growing seasons 2019/20 and 2020/21.

Blight stop as biocide was recorded the highest significant increase in yield components and being, 47.65, 144.6, 6.89 and 3.31 in (No. of fruits/plant, fruit weight (g), yield/plant (kg) and yield/greenhouse (ton)), respectively and followed by *T. harzianum* isolate and being, 44.15, 140.6, 6.21 and 2.98 in (No. of fruits/plant, fruit weight (g), yield/plant (kg) and yield/greenhouse (ton)), respectively in comparison with control treatment. In the contrary, micronic sulfur treatment showed the lowest effect compared to the other treatments rather than control treatment during the two seasons. These results are in harmony with those of **Reuveni *et al*., 1998 and Ahmed, 2018** who observed the mode of action of biocontrol agents due to produce growth regulators and resistant inducer which help to increase the yield productivity and quality **(Halil *et al*., 2011)**.

Table (4). The efficacy of different treatments on yield and its parameters of sweet pepper under greenhouse condition during 2019/20 and 2020/21 seasons

Different treatments	No. of fruits /plant			Fruit weight (g)			Yield/plant (kg)			Yield/greenhouse (ton)		
	19/20	20/21	Mean	19/20	20/21	Mean	19/20	20/21	Mean	19/20	20/21	Mean
T. harzianum	43.1	45.2	44.15	139.5	141.7	140.6	6.01	6.40	6.21	2.88	3.07	2.98
T. viride	41.8	42.0	41.90	137.8	140.9	139.4	5.76	5.92	5.84	2.76	2.84	2.80
B. subtilis	38.4	39.6	39.00	135.7	132.3	134.0	5.21	5.24	5.23	2.50	2.52	2.51
P. fluorescens	35.1	37.3	36.20	134.3	131.1	132.7	4.71	4.89	4.80	2.26	2.35	2.30
Micronic sulfur	34.7	36.9	35.80	129.1	128.8	129.0	4.48	4.75	4.62	2.15	2.28	2.22
Blight stop (*T. harzianum)*	46.6	48.7	47.65	143.2	145.9	144.6	6.67	7.11	6.89	3.20	3.41	3.31
Control (Untreated)	22.2	23.3	22.75	82.4	83.3	82.9	1.83	1.94	1.89	0.88	0.93	0.90
L S D at 0.05	1.66	1.88		2.28	2.26		0.43	0.45		0.10	0.12	

Fruit quality parameters

The effect of different treatments on sweet pepper quality parameters of total chlorophyll, total soluble solids and ascorbic acid under greenhouse conditions during 2019/20 and 2020/21 seasons

Presented data in Table (5) including total chlorophyll (mg/100 g FW), total soluble solid (TSS) and ascorbic acid (mg/100 g FW) show that, a great increase in sweet pepper cv. Hybrid F1 plants contents due to the role of different biological control and natural compounds on controlling sweet pepper powdery mildew. Treated plants with Blight stop biocide was the best one on controlling powdery mildew disease and increasing in total chlorophyll (26.55 and 26.99 mg/100 g FW), total soluble solid (TSS, 8.1 and 8.2%) and ascorbic acid (140.5 and 141.4 mg/100 g FW), respectively, followed by *T. harzianum* biocontrol agent during the two successive growing seasons 2019/20 and 2020/21 compared with untreated plants.

On the opposite trend, micronic sulfur as natural compound showed the least effective treatment in total chlorophyll (24.15 and 24.45 mg/100 g FW), total soluble solid (TSS), (6.1 and 6.2%) and ascorbic acid (131.6 and 132.8

mg/100 g FW), respectively during two seasons in comparison with untreated plants.

These results indicated that all treatment kept sweet pepper plants healthy and supported its optimal growth, which could be emphasized by too low chemical contents in the control treatment. This finding could be clarified by **(kumar *et al*., 2008; Ali and Ayoub, 2017 and Ahmed, 2018)** who confirmed that the use of alternatives to chemical pesticides such as biological control and natural compounds have high efficacy in controlling powdery mildew disease by changing the biochemical metabolism of the plant to improve the systemic resistance acquired by the plant which help to increase the content of chlorophyll, total soluble solid and ascorbic acid in plants, in addition to that it produces healthy, safe food that is free of toxicity and it is environmentally friendly **(Nielsen, 2017)**.

Influence of biological preparations and micronic sulphur on total phenol and enzyme activity in response to the emergence of the *Leveillula taurica* disease and sweet pepper cv. Godeon Hybrid F1 plant resistance

According to the role of biological control and natural compound in controlling the *Leveillula taurica* of sweet pepper, which led to a significant increase in the enzymatic activity of both peroxidase (PO) and polyphenoloxidase (PPO), as well as, an increase in the phenolic content within the treated plants in comparison with control treatment during the two growing seasons 2019/20 and 2020/21 under greenhouse conditions. Data in Table (6) show that Blight stop was the best treatment in increasing activity of PO (1.22 and 1.32), PPO (0.71 and 0.73) and total phenol (0.49 and 0.50) more than other treatments, while micronic sulfur was the lowest effective one compared with untreated plants.

The obtained results are in agreement with those obtained by **Lopez *et al.* (2019)** who insured that the application of biological control effects on powdery mildew of sweet pepper plants in addition to increasing its chemical content **(Ahmed, 2018)**. Treating sweet pepper plants with sulfur as environmentally friendly compounds led to control of powdery mildew disease and increased the metabolic activity of sweet pepper plants and recorded the highest content of total phenols and oxidative enzyme activity compared to the other treatments **(Sudha and Lakshmanan, 2009 and Farag *et al*., 2018)**.

Table (5). The effect of different treatments on total chlorophyll, total soluble solids and ascorbic acid of sweet pepper under greenhouse condition during 2019/20 and 2020/21 seasons

Different treatments	Total chlorophyll (mg/100 g FW)		Total soluble solids (TSS %)		Ascorbic acid (mg/100g FW)	
	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
T. harzianum	26.00	26.55	7.6	7.8	138.8	140.2
T. viride	25.87	25.99	7.2	7.3	137.4	138.3
B. subtilis	25.33	25.55	6.7	6.9	135.7	136.5
P. fluorescens	24.88	25.00	6.3	6.5	133.8	134.9
Micronic sulfur	24.15	24.45	6.1	6.2	131.6	132.8
Blight stop (*T. harzianum)*	26.55	26.99	8.1	8.2	140.5	141.4
Control (Untreated)	15.00	16.35	4.1	4.4	85.0	87.1
L S D at 0.05	1.06	1.07	0.01	0.02	2.19	2.28

Table (6). Effect of different treatments on peroxidase (PO), polyphenoloxidase (PPO) activity and total phenol of sweet pepper under greenhouse condition during 2019/20 and 2020/21 seasons

Different treatments	Peroxidase (PO) activity mg/ml		Polyphenoloxidase (PPO) activity (mg/ml)		Total phenols (mg/100 g FW)	
	2019/20	2020/21	2019/20	2020/21	2019/20	2020/21
T. harzianum	1.11	1.12	0.68	0.69	0.47	0.48
T. viride	1.02	1.03	0.63	0.65	0.45	0.46
B. subtilis	0.97	0.99	0.55	0.57	0.42	0.43
P. fluorescens	0.93	0.95	0.51	0.53	0.39	0.41
Micronic sulfur	0.83	0.85	0.44	0.47	0.36	0.37
Blight stop (*T. harzianum)*	1.22	1.32	0.71	0.73	0.49	0.50
Control (Untreated)	0.30	0.32	0.27	0.29	0.12	0.13
L S D at 0.05	0.24	0.25	0.11	0.12	0.10	0.11

CONCLUSION

The objective of this study was to assess a number of biocontrol agents, including *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, as well as, the biofungicide Blight stop and micronic sulfur treatments were used to control the powdery mildew disease of the sweet pepper cv. Godeon Hybrid F1 during the two successful growing seasons in 2019/2020 and 2020/2021 as an alternative to fungicides to reduce the toxicity of chemicals in the food chain and produce enough food of high quality and quantity. All the treatments showed efficacy in reducing the incidence and severity of *Leveillula taurica*-caused powdery mildew disease and improving the yield and its components, quality, chemical components, and enzyme activity of sweet pepper cv. Godeon Hybrid F1 plants as compared to untreated plants. Concerning chemical composition, total soluble solids (TSS), total chlorophyll and ascorbic acid, as well as the biochemical analysis, *i.e.* total phenol, enzyme activities of peroxidase (PO), and polyphenoloxidase (PPO), were all highest in the treated sweet pepper with Blight stop at the rate of 1 L: 50 liter water during both growing seasons compared to the control treatment.

Funding: Not applicable

Ethics approval and consent to participate
Not applicable.

Consent for publication: Not applicable.

Competing interests: The author declares that he has no competing interests.

REFERENCES

Ahmed, M.F.A. (2013). Studies on non-chemical methods to control some soil borne fungal diseases of bean plants *Phaseolus vulgaris* L. Ph.D. Thesis. Faculty of Agriculture, Cairo Univ., 137 pp.

Ahmed, M.F.A (2018). Evaluation of some biocontrol agents to control Thompson seedless grapevine powdery mildew disease. Ahmed Egyptian Journal of Biological Pest Control, 28(93):694-700.

Ali, A.M. and Ayoub, F.H. (2017). Effect of Some Bio-Control Agents, Natural Salts and Planting Densities on Controlling Sweet Pepper Powdery Mildew and Some Horticultural Characteristics Under Greenhouse Conditions. Egypt. J. Phytopathol., 45 (1): 117-134

A.O.A.C. (2005). Official method of Analysis. 18th Edition, Association of Officiating Analytical Chemists, Washington DC, Method 935.14 and 992.24.

Babu, K.N. and Pallavi, P.N. (2013). Isolation, identification and mass multiplication of Trichoderma an important bio-control agent. Int. J. Pharm. Life Sci., 4(1):2320-2323.

Brand, M.; Messika, Y.; Elad, Y.; David, D.R. and Sztejnberg, A. (2009). Spray treatments combined with climate modification for the management of *Leveillula taurica* in sweet pepper. Eur. J. Plant Pathol., 124: 309–329.

Bray, H.G. and Thorpe, W.V. (1954). Analysis of Phenolic compounds of interest in Metabolism. Meth Biochem Anal 1:27 –52.

Compant, S.; Duffy, B.; Nowak, J.; Cl´ement, C. and Barka, E. A. (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases: Principles, mechanisms of action and future prospects. Applied and Environmental Microbiology, 71(9):4951–4959.

Correll, J.C.; Gordon, T.R. and Elliott, V.J. (1987). Host range, specificity and biometrical measurements of *Leveillula taurica* in California. Plant Dis 71:248 – 251.

Elad, Y. (2000). Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection, 19:709-714.

Farag, M.F.; Taha, M.B.; Kamel, S.M. and Mohamed, A.G. (2018). Application of Compost for Controlling Powdery Mildew of Pepper and its Effect on Productivity. Egypt. J. Phytopathol., 46 (2): 131-155.

FAO (2021). World Food and Agriculture. Statistical Year Book. Food and Agriculture Organization of the United Nations, Rome, 368p.

Halil, K.; Recep, K.; Fatih, D.; Rafet, A. and Fikrettin, S. (2011). Effects of plant growth promoting rhizobacteria on fruit set, pomological and chemical characteristics, color values, and vegetative growth of sour cherry (*Prunus cerasus* cv. Kütahya). Turk. J. Biol., 35: 283-291.

Hammerschmidt, R.; Nuckles, E.M. and Kuc, J. (1982). Association of enhanced peroxidase activity with induced systemic resistance of Cucumber to *Colletotrichum lagenarium*. Physiol Plant Pathol., 20:73 – 82.

Harman, G.E. (2006). Overview of mechanisms and uses of *Trichoderma* spp. Phytopathology, 96: 190–194.

Harman, G.E.; Howell, C.R.; Viterbo, A.; Chet, I. and Lorito, M. (2004). Trichoderma species opportunistic, avirulent plant symbionts. Nat Rev. Microbiol.; 2:43–56.

Hoagland, R.E. and Cutler, S.J. (2000). Plant microbial compounds as herbicides. In: Allelopathy in Ecological Agriculture and Forestry. Narwal, S.S., Hoagland, R.E., Dilday, R.H., Reigosa, M.J. (Eds.), *Proceedings of the III International Congress on Allelopathy in Ecological Agriculture and Forestry, Dharwad, India, 18–21 August 1998. Kluwer Academic Publications, London, UK*, pp. 73–99.

Khandelwal, M.; Datta, S.; Mehta, J.; Naruka, R.; Makhijani, K.; Sharma, G.; Kumar, R. and Chandra, S. (2012). Isolation, characterization & biomass production of *Trichoderma viride* using various agro products- A bio control agent. Advan. Appl. Sci. Res., 3(6):3950-3955.

Kim, D.H.; Park, J.H.; Lee, J.S.; Han, K.S.; Han, Y.K. and Hwang J.H. (2009). Effect of Temperature, Relative Humidity on Germination and Development of Powdery Mildew (*Leveillula taurica*) on Pepper and Its Inoculation Method. Res. Plant Dis., 15(3): 187-192.

Kim, Y.S.; Song, J.G.; Lee, I.K.; Yeo, W.H. and Yun, B.S. (2013). *Bacillus* sp. BS061 suppresses powdery mildew and gray mold. Mycobiology, 41(2): 108-111.

Kiss, L. (2003). A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manag. Sci., 59: 475–483.

Kumar, A.M.; Ganeshan, G.; Reddy, K.N. and Ramachandra, Y.L. (2008). Integrated Disease Management for the Control Powdery Mildew (*Leveillula taurica* (Lev.) Arn.) in Bell Pepper. The Asian and Australasian Journal of Plant Science and Biotechnology, 2(2):107-112.

Kumar, A.M.; Reddy, K.N.; Sundaresha, S. and Ramachandra, Y.L. (2006). Compatibility of Fungal Antagonists Filtrates against Germination of Powdery Mildew Spores, *Leveillula taurica* (Lev.) Arn. of Bell Pepper. Plant Pathology Journal, 5: 248-252.

Litchenthaler, H.K. (1987). Chlorophyll and carotenoids: Pigments of photosynthetic membrane. Methods in Enzymology, 148: 350-382.

Little, C.R. (2006). Occurrence of *Leveillula taurica* cleistothecia on Honey Mesquite in Southern Texas. P1. Dis., 90: 1358.

Lopez, C.G.; Castellanos, L.N.M.; Ortiz, N.A.F. and Gonzalez, J.A.G. (2019). Control of powdery mildew (*Leveillula taurica*) using *Trichoderma asperellum* and *Metarhizium anisopliae* in different pepper types. BioControl, 64:77–89.

Lorito, M. (2005). Molecular biology of the interactions between Trichoderma, Phytopathogenic, Fungi and Plants: Opportunities for Developing Novel Disease Control Methods. Second Global Conference. Plant Health-Global Wealth. Abstracts, 162-163.

Mausam, V.; Brar, S.; Tyagi, R.; Surampalli, R. and Valero, J. (2007). Antagonistic fungi, *Trichoderma* spp.: Panoply of biological control. Biochemical Engineering J., 37(1): 1-20.

Mukherjee, P.K.; Horwitz, B.A. and Kenerley, C.M. (2012). Secondary metabolism in Trichoderma a genomic perspective. Microbiology, 158:35–45.

Nielsen, S.S. (2017). Vitamin C determination by indophenol method. in food analysis laboratory manual. Springer, Cha., 143–146.

Offor, C.E.; Okechukwu, P.C.U. and Esther, U.A. (2015). Determination of ascorbic acid contents of fruits and vegetables. International Journal of Pharmaceutical and Bio-Medical Science, 5: 1-3.

Ownley, B.H.; Gwinn, K.D. and Vega, F.E. (2010). Endophytic fungal entomopathogens with activity against plant pathogens: ecology and evolution. BioControl, 55:113–128.

Rajput, J.C. and Poruleker, Y. R. (1998). Capsicum in Handbook of vegetable science and technology (D.K. Salunkhe and S.S. Kadam, eds.) Marcel Dekker, Inc. New York, p.721- 729.

Reuveni, R.; Doi, G. and Reuveni, M. (1998). Local and systemic control of powdery mildew (*Leveillula taurica*) on pepper plants by foliar spray of mono-potassium phosphate. Crop Prot., 17:703 – 709.

Snedecor, G.W. and Cochran, W.G. (1989). Statistical Methods, 8th ed. Iowa State Univ. Press, Ames, Iowa USA, 503 p.

Souza, D.V.L. and Cafe-Filho, A.C. (2003). Resistance to *Leveillula taurica* in the genus capsicum. Plant Pathol., 52:613.

Sudha, A. and Lakshmanan, P. (2009). Integrated disease management of powdery mildew (*Leveillula taurica* (Lev.) Arn.) of chilli (*Capsicum annuum* L.). Arch. Phytopathol. Plant Prot., 42:299–317.

Srivastava SK. (1987). Peroxidase and polyphenol oxidase in *Brassica juncea* injected with *Macrophomina phaeseolina* (Tassio) and their implications in disease resistance. J. Phytopathol., 120:249 – 254.

Tsror, L.; Lebiush, M.S. and Shapira, N. (2004). Control of powdery mildew on organic pepper. Management of plant diseases and arthropod pests by BCAs. IOBC/WPRS Bull, 27:333–336.

Vinale, F.; Marra, R. and Ruocco, M. (2014). Trichoderma secondary metabolites active on plants and fungal pathogens. Open Mycol. J., 8:127–139.