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Efficacy of some Biological Control Treatments on Grey Mold Disease (*Botrytis cinerea*) of Strawberry Fruits

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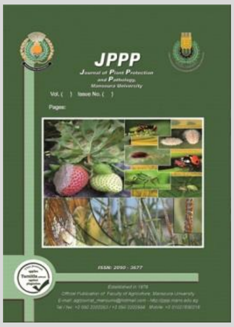

ABSTRACT

Strawberry (*Fragaria ananassa* Duch) is one of Egypt's and the world's greatest for business, nutritionally, and health-wise temperate fruit crops. *Botrytis cinerea* caused one of the most common types of grey mould, causes detrimental diseases impacting strawberry plants, resulting in economic losses in strawberry productivity. This study examined the effectiveness of some biological agents, such as *Trichoderma album*, *T. atroviride*, *T. hamatum*, *T. harzianum* and Blight stop (*T. harzianum*, 30x10^6 spores/ml), *in vitro* for suppression of *B. cinerea* linear growth. The most effective treatment was *T. atroviride*, which significantly decreased the mycelial development of *B. cinerea* to 90.83%, and then *T. harzianum* (89.50%), on average. On the contrary, *T. hamatum* had the least effect, with an average reduction in fungal growth of 81.33%. All tested biocontrol agents significantly decreased the disease incidence and severity of *B. cinerea* in strawberry cultivars compared to untreated plants under field conditions during the two seasons 2021/22 and 2022/23, however, *T. atroviride* showed the best efficacy, followed by *T. harzianum*. All of the biological control treatments that were investigated led to a significant rise in the values of total soluble solids (TSS), total acidity, ascorbic acid, total chlorophyll, sucrose (%), total phenol and increased strawberry yield during the two seasons. The research was done to identify the best bioagent for protecting strawberry plants from *B. cinerea* fungal diseases.

Keywords: Strawberry; *Botrytis cinerea*; biological control

INTRODUCTION

Strawberry (*Fragaria ananassa* Duch.) is one of the most commercially beneficial fruits, nutritionally and healthily significant temperate fruit crops in the world. The demand for strawberries increased in Egypt due to its economic importance, whether for domestic consumption or export (Petrasch *et al*., 2019). The overall strawberry-growing area in Egypt is 12579 ha. with a production of 470913.10 tons (FAO STAT 2021).

One of the greatest serious diseases is grey mould, which is brought on by *Botrytis cinerea* affecting strawberry plants, results in economically losses in strawberry production (Abada, 2002, Barakat and Al-masri, 2017, Chen *et al*., 2018, Petrasch *et al*., 2019 and Rhouma *et al*., 2022).

Trichoderma harzianum and *T. koningii* (T21) are used to control grey mold *in vitro* in addition to reducing the severity of *Botrytis cinerea* disease on strawberry plants *in vivo* in comparison to a control treatment (Alizadeh *et al*., 2007). *T. asperellum* foliar spraying significantly reduced *Botrytis cinerea* on harvested and stored strawberry fruits while also boosting yield relative to the control treatment. *T. asperellum* has the capacity to be exploited as a biological control agent for post-harvest diseases as well as to extend storage time to 7 days after harvest (Kowalska, 2011).

Treatment of strawberry plants with *Bacillus halotolerans* KLBC XJ-5 impeded *B. cinerea's in vitro* mycelial development and conidial germination. Additionally, it greatly promoted enzyme activity (polyphenol oxidase, phenylalanine ammonia lyase, -1, 3-glucanase, and chitinase), as well as components associated with disease resistance (total phenols, flavonoids) and improved the nutritional quality of ascorbic acid, titratable acidity, and total soluble solids in comparison with untreated plants (Wang, *et al*., 2021). This action may be a result of their capacity to create a variety of antibacterial and antifungal substances, including fengycin, surfactin, and iturin (Calvo *et al*., 2017 and Gotor-vila *et al*., 2017). According to Ahmed and El Fiki (2017), when *Trichoderma harzianum* antagonists were applied to strawberries, there was a much higher rise in total phenols, total nitrogen percentage, and total chlorophyll than in the control group. The application of several biological control agents to strawberry plants, *Bacillus subtilis*, *B. megatherium*, *Trichoderma album*, *T. asperellum* (T34), and *T. viride* demonstrated the best levels of *in vitro* linear growth inhibition of *Botrytis cinerea* fruit rot pathogens. The tested treatments outperformed the control treatments of Switch (synthetic fungicide) and tap water in terms of disease incidence (D.I.) of fruit rots in the field. According to the findings, spraying strawberry fruits before harvest with *T. asperellum* (T34) is the most efficient treatment for preventing fruit degradation while lowering colour change, decay, and preserving decent shape, firmness, acidity, TSS%, and weight loss (Rashid *et al*., 2022).

The current research aims to reduce the toxicity at the food chain caused by the use of fungicides in agricultural operations in order to produce enough food of higher quality and improve the balance of biological variety. In addition, investigation was done to identify the best bio agent that

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might protect strawberry plants from *B. cinerea* fungal diseases.

MATERIALS AND METHODS

Plant materials

Three cultivars of strawberry plants were used in this study, and they were obtained from PICO Company, including Red merlin, Elyana and Fortuna.

Biological antagonists

Four different biocontrol agents, *T. album, T. atroviride, T. hamatum and T. harzianum* ($30x10^6$ spores/ml), as well as the biocide Blight stop, which contains wild fungal isolate (*T. harzianum* $30x10^6$ spores/ml) that was added at the rate of 1 Lit./50 Lit. water, were kindly provided by Biological Control Production Unit Central Lab. of Organic Agriculture, CLOA; ARC.

Isolation and identification of *Botrytis cinerea*, the organism that causes strawberry grey mould disease

The *B. cinerea* isolate used in this investigation was gathered from various sites within Beheira Governorate, Egypt. Rotten strawberry fruits, showing grey mold disease symptoms, were cut off from the plants and put in a fresh plastic bag. Taking advantage of a sterilized scalpel, infested fruits were cut up into small bits and moved to sterile petri dishes, where they were surface sterilized with 0.5% sodium chlorine exposure for 3 minutes, washed three times using sterile water distillation after that and put in sterile petri dishes containing 10 ml of potato dextrose agar (PDA). After that, Petri dishes were incubated for 7 days at 22 ± 2°C. Purified mycelia that was developing at the colonies' profit margins was subcultured on three sterilized petri dishes containing 10 ml PDA and incubated for the second time at 22 ± 2°C for 7 days under fluorescent lighting. The fungus was purified using the single spore method, and it was kept for further study on the PDA slope in test tubes at 5°C (Dhingra and Sinclair, 1995). Pure isolates have been distinguished based on the outside appearance of mycelium and spores (Barnett and Hunter, 1987).

The influence of several bioagents on *B. cinerea* antagonistic behavior

The antagonists and commercial prepared solutions have been added to warm sterilized PDA medium at a rate of 10% and added into petri dish plates (10 ml/plate) before solidification. After solidification, a disc (5 mm) of *B. cinerea* collected from the perimeter of a 7-day-old cultured on identical medium had been positioned in the middle of each dish. Plates contained media without antagonists and inoculated only with *B. cinerea* were served as control treatment. On average, there were three plates utilized for each specific treatment. Incubation was carried out at 27±2 °C. The investigation was stopped when mycelial mats covered the medium surface in control treatment, each plate was inspected and percentage of reduction in mycelial growth of pathogenic fungal averages was calculated using the method defined by Ahmed (2005) and Ahmed (2013) as follows:

$$\% \text{ Reduction in linear growth of pathogenic fungi} = \frac{G1 - G2}{G1} \times 100$$

Where: G1: growth of the pathogenic fungus in control only, G2: growth of the pathogen against the tested antagonists.

Field trials

Unless otherwise specified, all field experiments were conducted on 1st October 2021/22 and 2022/23 two growing seasons, respectively, at a private farm (Om Sabr village), Badr Center, Beheira Governorate, Egypt, to evaluate some biocontrol agents on grey mould disease of strawberry plants, where the soil is light loamy soil with natural infestation. This area has Nile water available by a drip irrigation system. The field experimental designed was totally randomized using three replicates for each treatment. Plots each 1.2 x 5 m were used as replicate (Abada, 2002). Each replicate contains 50 strawberry seedlings. There were 1.8 meters between each plot. All strawberry seedlings receive the same organic fertilizers and irrigation regime as recommended. Strawberry plants received two foliar treatments during the season, the first during the bloom stage and the second two weeks after the plant finished flowering. Strawberry cultivars were sprayed with suspensions of four bioagent isolates—*Trichoderma album, T. atroviride, T. hamatum, T. harzianum* ($30x10^6$ spores/ml) and Blight stop—at the recommended doses. Super film was added with each treatment prior to spraying at a rate of 50 ml/100 L water as a surfactant agents and adhesive substance. Plots that weren't previously treated (just sprayed with water) acted as control. Strawberry fruits were harvested at the market's stage of maturity (about 3/4 of full fruit color). The total strawberry yield (ton/feddan) for each treatment was recorded for each growing season.

Disease severity (DS %) and disease incidence (DI)

The disease index rating, which was created to assess the average diameter of the infected areas on fruit surface after 14 days of prior treatments, was used to determine disease parameters on rotten fruits, according to Townsend and Heuberger (1943) and Promyou *et al.*, 2023, who rated disease severity on a scale of 0-3, where 0 = no symptoms of rot, 1 = sporadic tiny rots (less than 25%), 2 = Rots are aggregating and covering 25–50% of the fruit area and 3 = The fruit area had more than 50% of the infection. The severity percentage of each foliar disease was calculated using the following formula:

$$\mathbf{D.S.I}\ \% = \frac{\Sigma(\mathbf{n} \times \mathbf{r})}{\mathbf{3} \times \mathbf{N}} \times \mathbf{100}$$

Where, D.S.I = disease severity index, n = number of fruits in each category, r = numerical value of each category, 3= numerical value of highest category and N = total number of infected fruits.

The percentages of disease incidence and efficacy were calculated using the following equations:

$$\text{Disease Incidence (DI)}\% = \frac{\text{No. of rotted fruits}}{\text{Total No. of examined fruits}} \times 100$$

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

Biochemical component analysis

All bio-chemical tests of strawberry plants were performed at the Central Laboratory of Organic Agriculture (CLOA), Agricultural Research Center (ARC), Egypt. In an experiment to compare the biochemical components with disease incidence, leaves samples from treated plants were taken from each treatment. A hand refractometer made by Carlzeiss was used to calculate the total soluble solids (TSS%). (A.O.A.C. 2005). Strawberry titration acidity percentage was calculated as a percentage of citric acid using the method of (A.O.A.C. 2005). Ascorbic acid (mg/100 g FW) was assessed using the Offor *et al.* (2015) method, while chlorophyll content was evaluated using the Wellburn (1994) method. The amount of crude protein was calculated by multiplying the total nitrogen via 6.25. (A.O.A.C. 2005). The total sugars in the collected samples were calculated using the standard Dubois *et al.* (1956) method and expressed for each treatment as milligrammes of glucose per gramme of dry weight (mg/g DW). The method created by Singleton *et al.* (1999) was also used for estimating total phenols, which were measured for each treatment as milligrammes of gallic acid per gramme of dry weight (mg GA/g DW) in accordance with the gallic acid reference curve.

Statistical analysis

The least significant difference (L.S.D.) method was used to statistically analyse and compare the data in accordance with Snedecor and Cochran (1989) recommendations.

RESULTS AND DISCUSSION

Effect of different antagonists on the linear growth of the *B. cinerea*:

The data in Table 1 show that, there were considerable differences between the different antagonists' inhibitory effects on the linear growth of *B. cinerea in vitro*. In this regard, *T. atroviride* significantly reduced mycelial growth by 90.83%, followed by *T. harzianum* (89.50%), Blight Stop "*T. harzianum*" (87.60%), and *T. album* (85.70%), on average. In contrast, *T. hamatum* was least effective, with an average reduction in pathogen growth of 81.33%.

This phenomena may be clarified through the fact that several pathogens with distinct outlines have varied protective mechanisms against the enzymes and poisonous chemicals provided by various antagonists. **(**Ahmed, 2013 and Awad, 2017**).** *Trichoderma* spp. produced lytic enzymes such chitinases, peroxidases, polyphenoloxidases, and glucan 1-3 B-glucosidases that destroyed the pathogen's cell wall **(**Elad, 2000 and Verma *et al*., 2007**)**. *Trichoderma* spp. are recognized to be capable of causing systemic acquired resistance (SAR) **(**Ahmed and El Fiki, 2017**)**, and this is thought to be one of the most important modes of action for this biocontrol agent (Matei and Matei, 2008). This has been reported previously for a broad range of plant-pathogen systems (Harman *et al.*, 2004, Balode, 2010 and Sylla *et al.*, 2015).

Table 1. The impact of the different antagonists on the proportion of *B. cinerea* linear growth that was reduced after 7 days of incubation at 22±2°C.

Different antagonists	%Reduction in growth of *B. cinerea*
T. album	85.70
T. atroviride	90.83
T. hamatum	81.33
T. harzianum	89.50
Blight Stop (*T. harzianum*)	87.60
Control "Untreated"	00.00
L.S.D at 1% for	0.44

The effectiveness of some antagonists on strawberry fruit grey mold under field conditions during 2021/22 and 2022/23 two growing seasons:

1. Diseases parameters

The presented data in Table 2 illustrate that all tested antagonist treatments (*Trichoderma album, T. atroviride, T. hamatum, T. harzianum* and Blight stop (*T. harzianum* 30x10^6 spores/ml)) greatly surpassed untreated plants in decreasing the disease parameters (incidence and severity) of fruit grey mold disease in different cultivars in the two growing seasons of 2021/22 and 2022/23. In this way, strawberry cv. Fortuna was the most tolerant to *B. cinerea* disease, followed by the Elyana cultivar. On the contrary, strawberry cv. Red merlin showed more sensitivity to the fruit grey mold disease. In reducing disease parameters throughout the two seasons 2021/22 and 2022/23, respectively on strawberry cv. Fortuna, *T. atroviride* showed the best efficacy (65.11 and 81.88%), followed by *T. harzianum* isolate (61.70 and 73.14%). On the contrary, *T. hamatum* had the lowest effectiveness (45.92 and 50.16%) in containing the diseases.

Table 2. The effectiveness of tested antagonists on the incidence and severity of *Botrytis cinerea* in different strawberry cultivars under field conditions during the 2021/22 and 2022/23 growing seasons.

Strawberry cultivars	Tested antagonists	Disease incidence %				Disease severity %			
		2021/22	2022/23	Mean	Efficacy	2021/22	2022/23	Mean	Efficacy
Fortuna	*T. album*	29.8	29.3	29.55	53.13	7.2	6.5	6.85	55.66
	T. atroviride	22.6	21.4	22.00	65.11	2.9	2.7	2.80	81.88
	T. hamatum	34.7	33.5	34.10	45.92	7.8	7.6	7.70	50.16
	T. harzianum	24.5	23.8	24.15	61.70	4.4	3.9	4.15	73.14
	Blight Stop (*T. harzianum*)	26.9	25.2	26.05	58.68	5.3	4.8	5.05	67.31
	Control (Untreated)	63.5	62.6	63.05	00.00	15.5	15.4	15.45	00.00
Elyana	*T. album*	32.5	32.0	32.25	49.61	7.5	7.3	7.40	54.04
	T. atroviride	24.8	23.7	24.25	62.11	3.1	2.9	3.00	81.37
	T. hamatum	37.3	36.4	36.85	42.42	8.0	7.9	7.95	50.62
	T. harzianum	26.7	26.5	26.60	58.44	4.5	4.4	4.45	72.36
	Blight Stop (*T. harzianum*)	27.2	27.0	27.10	57.66	5.5	5.2	5.35	66.77
	Control (Untreated)	64.5	63.5	64.00	00.00	16.2	16.0	16.10	00.00
Red merlin	*T. album*	33.7	32.8	33.25	53.66	7.8	7.6	7.70	55.75
	T. atroviride	25.5	24.5	25.00	65.16	3.5	3.1	3.30	81.03
	T. hamatum	38.2	37.7	37.95	47.11	8.3	8.0	8.15	53.16
	T. harzianum	27.3	26.9	27.10	62.23	4.7	4.5	4.60	73.56
	Blight Stop (*T. harzianum*)	28.5	27.6	28.05	60.91	6.0	5.8	5.90	66.09
	Control (Untreated)	72.5	71.0	71.75	00.00	17.5	17.3	17.40	00.00
LSD at 5%		2.12	2.11			0.76	0.74		

These findings potentially due to hereditary traits present in these cultivars, which were able to fend against *B. cinerea* infection (Seijo *et al*., 2008). This phenomena may be clarified through the fact that several pathogens with distinct outlines have varied protective mechanisms against the enzymes and poisonous chemicals provided by various antagonists. **(**Ahmed, 2013 and Awad, 2017**).** *Trichoderma* spp. produced lytic enzymes such chitinases, peroxidases, polyphenoloxidases, and glucan 1-3 B-glucosidases that destroyed the pathogen's cell wall **(**Elad, 2000; Verma *et al*., 2007 and Sylla *et al.*, 2015**)**.

Trichoderma spp. are recognized to be capable of causing systemic acquired resistance (SAR) **(**Ahmed and El Fiki, 2017**)**, and this is thought to be one of the most important modes of action for this biocontrol agent (Matei and Matei, 2008 and Balode, 2010).

This has been reported previously for a broad range of plant-pathogen systems (Harman *et al.*, 2004). Biocontrol agents function as mycoparasites, sporulation and germination inhibitors, nutrition competitors, agents that secrete anti-fungal mycotoxins, and inducers of defense systems in strawberry plants (Feliziani and Romanazzi, 2016 and De Angelis *et al.*, 2022). The findings of this study suggest that foliar spray can promote plant development. The timing of treatments is a crucial component of their effectiveness; the first application should be made at the start of the season (Freeman *et al*., 2004). In light regarding this, Khirallah *et al.* (2016) demonstrated that *T. harzianum* susceptibility to different fungicides varied based on the doses and operative elements of the fungicides tested. Additionally, it was shown that *T. harzianum* moderate susceptibility to fludioxonil and cyprodinil is caused by active components from two distinct families that have two separate mechanisms of action (Robinson-Boyer *et al*., 2009).

2. Total soluble solid (T.S.S), total acidity and ascorbic acid (mg/100 g FW):

The finding data in Table 3 state that all tested biological treatments significantly increased strawberry fruit quality parameters *i.e.* total soluble solid (TSS), total acidity and ascorbic acid (vitamin C) when compared to the untreated plants. The strawberry biography works in this way. Fortuna was the cultivar with the greatest level of ascorbic acid (vitamin C), total soluble solid (TSS), and total acidity (a measure of how resistant a strawberry cultivar is to *B. cinerea*) as well as the highest rise in these parameters in strawberry fruit quality. Elyana and Red Merlin were the next two cultivars in line. Spraying strawberry plants cv. Fortuna with *T. atroviride* resulted in the greatest increases in TSS, being 13.0 and 12.5%, total acidity, being 0.98 and 0.96% and ascorbic acid, 48.91 and 48.83% percentages, respectively during both seasons, followed by *T. harzianum*. In contrast, *T. hamatum* had the least efficient when applied twice compared to other treatments rather than untreated plants during the two seasons 2021/22 and 2022/23.

These findings are consistent with those provided by Chen *et al.* (2018), who found that BCAs efficiently controlled *B. cinerea* and simultaneously promoted the plants growth as evidenced by a significant increase in TSS, total acidity and ascorbic acid (vitamin C) of strawberry plants. According to Jiang *et al.* (2001), the more ascorbic acid is present in treated fruits, the more effectively fruit rot is controlled. Fruits treated with the control had the greatest decrease in ascorbic acid concentration throughout all storage durations, despite the significance of all treatments and the storage term. These results are consistent with those of Rashid *et al.* (2022), who enhanced that strawberry fruit pre-harvest spraying with *T. asperellum* (T34) was the most successful therapy for postponing degradation of fruit while lowering colour change and decay and preserving look, hardness, acidity, TSS%, and weight loss are all positives.

Table 3. Efficacy of tested antagonists on total soluble solid (T.S.S), total acidity and ascorbic acid (mg/100 g FW) of strawberry cultivars under field conditions during 2021/22 and 2022/23 growing seasons.

Strawberry cultivars	Tested antagonists	2021/22 growing season			2022/23 growing season		
		T.S.S (%)	Total acidity %	Ascorbic acid (mg/100 g FW)	T.S.S (%)	Total acidity %	Ascorbic acid (mg/100 g FW)
Fortuna	*T. album*	11.5	0.79	47.76	11.0	0.78	47.48
	T. atroviride	13.0	0.98	48.91	12.5	0.96	48.83
	T. hamatum	11.0	0.75	47.49	10.5	0.74	47.35
	T. harzianum	12.5	0.83	48.87	12.0	0.81	48.81
	Blight Stop (*T. harzianum*)	12.0	0.81	47.88	11.5	0.80	47.75
	Control (Untreated)	6.0	0.23	26.48	5.5	0.21	27.11
Elyana	*T. album*	11.0	0.78	47.42	10.5	0.76	47.28
	T. atroviride	12.5	0.95	48.76	12.0	0.93	48.53
	T. hamatum	10.5	0.74	47.25	10.0	0.72	47.15
	T. harzianum	12.0	0.82	48.57	11.5	0.79	48.49
	Blight Stop (*T. harzianum*)	11.5	0.80	47.59	11.0	0.77	47.47
	Control (Untreated)	5.5	0.18	26.33	5.0	0.16	26.56
Red merlin	*T. album*	10.5	0.76	47.33	10.0	0.75	47.17
	T. atroviride	12.0	0.93	48.11	11.5	0.91	48.05
	T. hamatum	10.0	0.72	47.14	9.5	0.70	47.00
	T. harzianum	11.5	0.80	48.00	11.0	0.76	47.83
	Blight Stop (*T. harzianum*)	11.0	0.78	47.53	10.5	0.74	47.25
	Control (Untreated)	5.0	0.15	26.25	4.5	0.12	26.41
LSD at 5%		1.0	0.07	1.22	0.9	0.05	1.20

3. Total chlorophyll, total nitrogen and total sugars:

The presented data in (Table 4) illustrate that all different biological control treatments raised levels of total chlorophyll (mg/g FW), total nitrogen % (mg/100g DW) and total sugars (mg/g DW) of different strawberry cultivars compared to control treatments during the two growing seasons 2021/22 and 2022/23. *T. atroviride* provided the highest levels of total chlorophyll (39.83 and 39.50), total nitrogen (3.39 and 3.36) and total sugars (4.98 and 4.95) in the majority of the instances in this trend of strawberry cv. Fortuna, followed by *T. harzianum*. In contrast to the other biological treatments, *T. hamatum* illustrated the least effectiveness recording (38.03 and 38.00 in total chlorophyll), (2.83 and 2.78 in total nitrogen) and (4.29 and 4.27 in total sugars), respectively, throughout the two subsequent seasons of cultivation 2021/22 and 2022/23 compared with the control treatment. These results match those obtained by Robinson-Boyer *et al*., 2009 and Ahmed and El Fiki (2017) who demonstrated that, Increases in total phenols, total proteins, total sugars, and total chlorophyll have been combined with biological treatments of strawberry plants that had a significant impact on plant protection and disease reduction (Matei and Matei, 2008). Bioagents may provide the nutrients and biological ingredients necessary to increase photosynthesis in the host plants, reducing disease incidence and severity (%) and the loss of photosynthesis leaf area, and enhancing the chemical components in order to lessen the pathogen's negative impacts (Scholes and Rolfe, 2009, Barakat and Al-Masri, 2017 and Rashid *et al.*, 2022).

Table 4. Efficacy of tested antagonists on total chlorophyll (mg/g FW), total nitrogen % (mg/100g DW) and total sugars (mg/g DW) of some strawberry cultivars *in vivo* during the 2021/22 and 2022/23 growing seasons.

Strawberry cultivars	Tested antagonists	2021/22 growing season			2022/23 growing season		
		Total Chlorophyll (mg/g FW)	Total nitrogen % (mg/100g DW)	Total sugars (mg/g DW)	Total Chlorophyll (mg/g FW)	Total nitrogen % (mg/100g DW)	Total Sugars (mg/g DW)
Fortuna	*T. album*	38.78	2.98	4.57	38.72	2.83	4.53
	T. atroviride	39.83	3.39	4.98	39.50	3.36	4.95
	T. hamatum	38.03	2.83	4.29	38.00	2.78	4.27
	T. harzianum	39.44	3.36	4.83	39.27	3.18	4.81
	Blight Stop (*T. harzianum*)	39.25	3.12	4.79	39.12	3.00	4.75
	Control (Untreated)	25.29	1.05	2.10	24.33	1.03	2.08
Elyana	*T. album*	37.75	2.83	4.51	37.56	2.81	4.49
	T. atroviride	38.87	3.28	4.93	38.83	3.17	4.90
	T. hamatum	37.23	2.79	4.25	37.25	2.75	4.23
	T. harzianum	38.29	3.15	4.79	38.18	3.12	4.77
	Blight Stop (*T. harzianum*)	38.18	3.09	4.73	38.11	3.00	4.71
	Control (Untreated)	24.54	1.02	2.13	24.08	1.00	2.11
Red merlin	*T. album*	36.77	2.81	4.45	36.73	2.93	4.41
	T. atroviride	37.93	3.07	4.88	37.81	2.97	4.83
	T. hamatum	36.54	2.71	4.21	36.35	2.68	4.19
	T. harzianum	37.81	2.95	4.75	37.77	2.89	4.73
	Blight Stop (*T. harzianum*)	37.45	2.83	4.68	37.56	2.79	4.65
	Control (Untreated)	23.62	0.98	2.15	22.12	0.95	2.13
LSD at 5%		0.33	0.11	0.13	0.32	0.10	0.12

4. Total phenol and fruit yields:

Regarding the impact of biological control on the recoverable yields/fed and fruit grey mould of strawberry cultivars (*Trichoderma album*, *T. atroviride*, *T. hamatum*, *T. harzianum*, and Blight stop (*T. harzianum* $30x10^6$ spores/ml)). The findings in (Table 5) for total phenol (mg/DW) also showed that all tested treatments significantly increased total phenol (mg/DW) and strawberry fruit yields in both seasons 2021/22 and 2022/23 compared to untreated plants. In the above way, the most beneficial treatments raised the total phenol and fruit yields when strawberry plants were sprayed with the bioagent treatment *T. atroviride* twice, followed by *T. harzianum* compared to the other treatments. On the contrary, *T. hamatum* treatment showed the least effectiveness in total phenol and fruit yields in both seasons 2021/22 and 2022/23 compared to control treatment. Strawberry cv. Fortuna was the most resistant one to *B. cinerea* disease and observed the greatest improvement in total phenol and fruit yield followed by the Elyana cultivar. On the contrary, strawberry cv. Red merlin showed more sensitivity to the fruit grey mold disease and showed the least effect one in this way during the both seasons.

Table 5. The impact of tested antagonists on total phenols (mg/g DW) and fruit yield of strawberry cultivars under field conditions during 2021/22 and 2022/23 growing seasons

Strawberry cultivars	Tested antagonists	2021/22 growing season			2022/23 growing season		
		Total phenols (mg/g DW)	Strawberry fruit		Total phenols (mg/g DW)	Strawberry fruit	
			Yield (ton/fed.)	Efficacy		Yield (ton/fed.)	Efficacy
Fortuna	*T. album*	59.98	7.09	104.91	59.93	7.00	103.49
	T. atroviride	64.85	7.48	116.18	64.32	7.43	115.99
	T. hamatum	58.95	6.83	97.40	58.55	6.81	97.97
	T. harzianum	63.83	7.25	109.54	63.78	7.18	108.72
	Blight Stop (*T. harzianum*)	62.48	7.17	107.23	62.36	7.11	106.69
	Control (Untreated)	36.12	3.46	00.00	36.10	3.44	00.00
Elyana	*T. album*	58.98	6.85	99.71	57.85	6.55	92.08
	T. atroviride	63.83	7.25	111.37	63.32	7.00	105.28
	T. hamatum	57.78	6.65	93.88	57.12	6.12	79.47
	T. harzianum	62.50	7.13	107.87	62.00	6.81	99.71
	Blight Stop (*T. harzianum*)	61.53	7.05	105.54	61.18	6.75	97.95
	Control (Untreated)	36.00	3.43	00.00	35.83	3.41	00.00
Red merlin	*T. album*	56.65	5.45	36.93	55.48	5.00	26.58
	T. atroviride	62.31	6.00	50.75	60.69	5.55	40.51
	T. hamatum	56.11	5.25	31.91	52.69	4.56	15.44
	T. harzianum	61.05	5.53	38.94	58.22	5.34	35.19
	Blight Stop (*T. harzianum*)	59.32	5.35	34.42	57.57	5.28	33.67
	Control (Untreated)	35.50	3.98	00.00	35.00	3.95	00.00
LSD at 5%		0.44	0.16		0.42	0.14	

The outcomes were in line with those predicted by Ahmed and El-Fiki, 2017 and El-Morsy *et al.*, 2022, in order to lessen the pathogen's negative impacts and increase total phenol and fruit harvests, they found that bioagents might raise nutrition and essential components needed to improve photosynthetic activity in the host plants. The increase in production may possibly be attributable to strong roots that can absorb and provide an acceptable quantity of raw nutrients or to the efficient synthesis of raw nutrients in the presence of high levels of protein and chlorophyll, which resulted in an increase in fruit yield (Kowalska, 2011 and Ahmed, 2018).

CONCLUSION

The biological control, *i.e. Trichoderma album*, *T. atroviride*, *T. hamatum*, *T. harzianum* and Blight stop (*T. harzianum* 30x10^6 spores/ml) when sprayed with recommended doses at the rate of 1 Lit./50 Lit. water at two different spray regimes with 15 days between sprays on some strawberry cultivars such as Fortuna, Elyana and Red Merlin, considerably reduced the disease parameters (incidence and severity) of *Botrytis cinerea* of strawberry cultivars in comparison with untreated plants in both seasons. All the biological factors that were tested resulted in a considerable increase in values of total soluble solids (TSS), total acidity, ascorbic acid, total chlorophyll, sucrose (%), total phenol and increased strawberry yield during the two seasons. Spraying with *T. atroviride* was the most effective treatment, followed by *T. harzianum*. On the contrary, *T. hamatum* was the least effective biocide treatment compared to the other treatments in both seasons 2021/22 and 2022/23.

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فاعلية بعض معاملات المكافحة البيولوجية لمرض العفن الرمادى (بوترايتس سيناريا) لثمار الفراولة

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الملخص

الفراولة هي واحدة من أعظم محاصيل الفاكهة المعتدلة في مصر والعالم من حيث الأهمية الأقتصادية ، الغذائية والصحية. يعتبر الفطر بوترايتس سيناريا من أكثر الأنواع المسببة لمرض العفن الرمادي شيوعًا والتى يسبب أمراضًا ضارة تؤثر على نباتات الفراولة، مما يؤدي إلى خسائر اقتصادية في إنتاجية الفراولة. تناولت هذه الدراسة فعالية بعض العوامل البيولوجية، مثل عزلات فطر الترايكوديرما "ألبم، أتروفيردى ، هماتم و هارزيانم " و المستحضر التجارى "بلايت أستوب" (30×10 6) في المختبر فى تثبيط النمو الميسليومى للفطر بوترايتس سيناريا. أظهر الفطر ترايكودرما أتروفيردى فعالية عالية فى تقليل نمو فطر البوترايتس سيناريا بشكل ملحوظ بنسبة 90.83%، يليه فى الفاعلية ترايكودرما هارزيانم (89.50%) في المتوسط. وعلى النقيض من ذلك، كان الفطر ترايكودرما هماتم أقلهم تأثيرا ، حيث ادى الى تقليل النمو الميسليومى للفطر بوترايتس سناريا الى 81.33%. أدت جميع معاملات المكافحة الحيوية إلى انخفاض معنوي في نسبة وشدة مرض العفن الرمادى للفطر بوترايتس سناريا في أصناف الفراولة مقارنة بالنباتات غير المعاملة تحت الظروف الحقلية خلال الموسمين 22/2021 و23/2022 ، ولكن كان الاكثر كفائة هو فطر ترايكودرما أتروفيردى متبوعا بفطر ترايكودرما هارزيانم. كما أدت جميع المعاملات البيولوجية التي تم تقييمها إلى ارتفاع معنوي في قيم المواد الصلبة الذائبة الكلية (TSS) ، الحموضة الكلية ، حامض الاسكوربيك ، الكلوروفيل الكلي ، السكروز (%) ، الفينول الكلي وزيادة محصول الفراولة خلال الموسمين.تم إجراء البحث لتحديد العامل الحيوي الأفضل لحماية نباتات الفراولة من العفن الرمادى لفطر بوترايتس سيناريا.

الكلمات المفتاحية: الفراولة ، بوترايتس سيناريا ، المكافحة البيولوجية