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Research Article

Management of Chocolate Spot Disease in Faba Bean Plants by using Biological Control Means

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Abstract

Background: Faba bean (*Vicia faba* L.) is one of the most important grain legume crops in Egypt and many other countries of the world because the seeds offer a low-cost source of protein, lysine, carbohydrates, minerals, and vitamins. Chocolate spot disease is a stress-related fungal disease produced by *Botrytis fabae* that causes plant damage, limits photosynthetic activity, and reduces yield.

Results: *Trichoderma atroviride* greatly reduced mycelial growth by 90.00% *in vitro*, followed by *T. harzianum* (86.67%) and *T. album* (83.89%) on average. *In vivo*, all studied antagonists dramatically reduced *Botrytis fabae* disease incidence and severity in both seasons 2021/22 and 2022/23. *T. atroviride* showed the highest efficacy bioagent (73.55 and 85.15%), followed by *T. harzianum* (72.55 and 81.22%), in controlling *B. fabae* of faba bean plants in both seasons. In addition, the results also showed that all tested biological treatments had an impact on yield components and increased levels of chlorophyll, protein%, phenols, flavonoids, Peroxidase (PO), polyphenol Oxidase (PPO), chitinase, and -1, 3-glucanase activities compared to control treatment in both seasons. In this regard, spraying *T. atroviride* showed the highest efficacy as a bioagent, followed by *T. harzianum.* Contrary, *T. hamatum* showed the lowest efficacy compared to other treatments in both seasons.

Conclusion: This investigation was carried out to determine the effectiveness of several different antagonists, *i.e.*, *T. album, T. atrovirde, T. hamatum*, and *T. harzianum* (30 x 10^6 spore/ml), Blight Stop, and Bio Zeid, for controlling *Botrytis fabae* on bean plants and evaluating their effect on yield parameters, components, and quality.

Introduction

Faba bean (*Vicia faba* L.) is one of the most significant grain legume crops in Egypt and many other nations worldwide. In the Mediterranean region, it is frequently used in human diets as a source of protein [1,2] and it serves as a good supplier of nitrogen for the soil, particularly following systems of extensive grain farming [3]. Its seeds are recognized as an inexpensive source of protein as well as a food with high caloric and nutritional value, particularly in the diet, due to their high concentrations of lysine, carbs, minerals, vitamins, and a variety of bioactive components [4]. In Egypt, the crop's cultivable area was expected to be 86757 feddan and the total output was estimated to be 128137 tons, with an average yield of 1.48 tonnes of dry seeds per feddan [5].

Chocolate spot disease is a stress-related fungal disease that is caused by *Botrytis fabae* [6]. It is one of the most economically significant diseases, causing plant damage, limiting photosynthetic activity, and reducing faba bean yield [7-9]. Additionally, it is widely used in the northern Nile Delta region of Egypt, where low temperatures and high relative humidity reduce productivity by 22% - 25% [10]. One of the most important elements influencing the output of faba bean plants is pathogen infection [11], which directly affects protein production, causing yield production to drop by more than 50% [12].

Trichoderma species varied in their ability to inhibit *B. fabae* growth *in vitro*, ranging from 51.11 to 77.78%. *T. album* (Isolate 2) and *T. harzianum* (Isolate 6) also offered the highest degrees of inhibition. Additionally, 24 hours prior to inoculating with *B. fabae*, spraying faba bean plants with either the *Trichoderma* spp. or Bio Zeid as a bio fungicide significantly reduced the severity of the disease after 14 days in the range of 3.0 - 4% compared to the control (8.7%) in greenhouse conditions [13,14].

Trichoderma harzianum, T. viride, T. album, and *Bacillus subtilis* all dramatically decreased the disease severity% of *Botrytis fabae*, the causative of faba bean chocolate spot disease, under field conditions during the 2012/13 and 2013/14 seasons. The results also showed that all tested bio-inducer treatments had an impact on growth parameters, yield components, and increased levels of chlorophyll, phenols, flavonoids, Peroxidase (PO), polyphenol oxidase (PPO), chitinase and -1, 3-glucanase activities compared to control treatment [13,15].

The biocontrol agents had a significant effect on reducing chocolate spot diseases of faba bean plants and improving their potential on the number of pods per plant and grain weight compared to the untreated plants [16]. The goal of this study is to use alternatives to chemical pesticides, such as biological control, to preserve biological diversity as one of the safe control directions in accordance with European law for organic agriculture in controlling *Botrytis fabae*, and to reduce toxicity in the production chain in order to produce healthy and safe faba bean seeds of high quality and quantity in the long run for sustainable development.

Materials and methods

Plant resources were utilized in the experiment

Faba bean seeds source: Giza 716 seeds were obtained from the Legume Crop Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt.

Biological antagonists: *Trichoderma album, T. atroviride, T. hamatum,* and *T. harzianum* (30 x 10^6 spore/ml) were four different biocontrol agents that were added at the rate of 1 Lit./50 Lit. water and were graciously donated by Biological Control Production Unit Central Lab. of Organic Agriculture, CLOA; ARC.

Biocide preparations: There are some biocide formulations were used as a comparison with other treatments as follows:

a. Blight Stop was recommended as biocide preparation which contains wild fungal isolate (*Trichoderma harzianum* 30 x 10^6 spore/ml) that is added at the rate of 1 Lit./50 Lit. water and was kindly provided by Biological Control Production Unit Central Lab. of Organic Agriculture, CLOA; ARC.

b. Bio Zeid 25% WP, which was provided by kz, was suggested as a biocide preparation. It contains the wild fungus isolate "*T. album* 2.5% (w/w)" and is added at a rate of 250 g/100 L water.

Chocolate spot fungal pathogen isolation and identification

Leaf samples of faba beans infested naturally were collected from a field in Nubaria City, EL-Behira Governorate, Egypt. The infected leaves were collected, cut into small pieces, and surface disinfected with 3% sodium hypochlorite for 2 minutes before being repeatedly washed in disinfected distilled water. The pieces were dried between a pair of sheets of sterilized filter paper to get rid of excessive distilled water before plating on faba bean leaf extract agar medium "FBLA" as suggested by Hanouike and Hasanain [17]. Three slices were placed in each Petri dish, and they were incubated at 20 °C for 12 days. according to Sinclair and Dhingra [18], the hyphal tip approach was used to purify the isolated fungus.

Antagonistic effect of different antagonists against *Botrytis fabae*

The antagonist's suspensions were added to warm sterilized PDA medium at a 10% concentration and placed into Petri dishes (10 ml/plate) before solidification. A disc (5 mmØ) of B. fabae, taken from the periphery of a mycelium that was 7 days old on the same medium, was put in the center of each plate after it had solidified. Plates containing media without antagonists and inoculated only with *B. fabae* served as control treatment. Three plates were used for each treatment. Inoculated plates were incubated at 22 ± 2 °C. The research study was stopped when mycelial mats covered the surface in the control treatment. All of the petri dishes were checked, and the percentage decrease in the fungus's mycelial growth was determined using the formula provided by Ahmed [19] and Ahmed [20] as follows.

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

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

Assessments of *Botrytis fabae* isolates under greenhouse conditions

In order to produce a high quantity of spores, *Botrytis fabae* isolates from Nubaria City were propagated on "FBLA" medium for 12 days at 22 °C with a photoperiod of 12 h light/12 h dark as described by [21]. Five seeds of faba bean cv. Giza 716 were planted in pots (30 cm in diameter, three replicates of each cultivar). In order to preserve high relative humidity, the established plants were sprayed with the appropriate isolate's spore suspension 45 days after sowing and covered with polyethylene bags for 24 hours [22]. In this regard, biological control treatments were sprayed at the suggested concentrations five days after the pathogen was artificially inoculated. The following parameters were taken into account after the experiment:

a. **Disease incidence:** Ahmed, 2005 [19] and El-Shennawy, 2011 [23] used the following formula to calculate the percentages of disease incidence and survived plants in each treatment.

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

b. **Disease severity (DS %):** The disease severity of chocolate spot was determined after two weeks from inoculation when the initial symptoms appeared using the 0 – 9 scale described by Ding, et al. [24](1993), where 0= no visible leaf infection, 1= less than 10% infection, 2= less than 20% infection, 3= less than 30% infection 4= less than 40% infection, 5= less than 50% infection, 6= less than 60% infection, 7= less than 70% infection, 8= less than 80% infection and 9= infection more than 80% of the foliage. Disease severity scores were converted to percentage severity index (DSI) for analysis using the following formula developed by [25].

$$\text{D.S.I \%} = \frac{\sum(n \times v)}{9 \times N} \times 100$$

In this equation, n is the number of plants in each category, v is the numerical value of the symptom category, N is the total number of plants, and 9 is the maximum numerical value of the symptom category.

Field trials

Research studies were conducted during two winter growing seasons (2021/22 and 2022/23) to assess the efficacy of different antagonists for controlling chocolate spot of faba bean plants in a naturally infested farm with a history of high infestation with *Botrytis fabae* in Nubaria city, EL-Behira Governorate, Egypt.

The soil texture is sandy loamy, which is irrigated by a drip system from a Nile water source. The research study plot had a surface area of 24.75 m^2 and consisted of up to 3 rows that were 5 m and 1.5 m wide, spaced around 50 cm apart. Regarding each biological treatment, there were 3 replicated plots applied in addition to a control in a fully randomized block design study. Cultivated about 25 faba bean cv. Giza 716 seeds in each row on the 15th of October of both seasons [26]. The same organic fertilizers and irrigation schedule were used for each cultivar of faba beans. The faba bean plants were sprayed with suspensions of the four bioagent isolates—*Trichoderma album*, *T. atroviride*, *T. hamatum*, and *T. harzianum* (30 x 10^6 spore/ml)—and additionally, two commercial biocide products—Blight Stop and Bio Zeid—were applied at the previously mentioned recommended dosages to the faba bean plants at the beginning of the flowering stage 45 days after sowing and again 60 days after sowing at the end of the flowering stage. Super film as a surfactant and sticker material, was mixed with each treatment prior to spraying at a rate of 50 ml/100 L water. Plots that weren't actually dealt with (just sprayed with water) acted as controls.

The following characteristics were evaluated:

a. Disease parameters:

The percentages of Disease Incidence (DI) and Severity (DS) were calculated as mentioned above, in addition, the efficacy of the tested treatments was estimated using the following equations:

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

b. Yield components traits:

The plants were collected by hand approximately 150 days after seeding and left to dry for 5 days under natural conditions before the following parameters were examined; The number of pods per plant, Weight of pods/plant (g), the weight of 100 grains (g) in each plot and yield/plot (Kg) are all recorded. In addition, the percentage increase in all parameters was determined using El-Kholy's [9] formula:

$$\text{Increasing in the yield \%} = \frac{\text{Treament - Control}}{\text{Control}} \times 100$$

C. Chemical components determination:

All the following chemical assays for the faba bean plants were carried out in both the Central Laboratory of Organic Agriculture and the Central Laboratory for Agricultural Climate, ARC.

1. **Protein content determination:**

   The micro-Kjeldahl method was used to determine the total nitrogen in the seed and multiplied by 6.25 to obtain the percentage of crude according to AOAC [27].

2. **Determination of Chlorophyll (SPAD value):**

   At the flowering stage, after 75 days from sowing, the Chlorophyll content was measured by SPAD - -meter Model L13000L [28].

3. **Determination of total phenols:**

   The amount of total phenols in extracts was determined by the Folin – Ciocateu method as modified by Singleton, et al. [29].

4. **Determination of total flavonoids content:**

   The flavonoid content is estimated in milligrams of rutin equivalents per gram of the digested sample (mg RE/g) as described by [30] method.

5. **Enzymes determination:**

Determination of peroxidase (PO): Faba bean leaf samples were weighed from 0.1 g - 0.5 g and then stored at 20 °C until processed as described by Ni, et al. [31]. The activity of the peroxidase enzyme was estimated in accordance with the instructions provided by Devi [32].

Determination of polyphenol oxidase (PPO): The activity of polyphenol oxidase was estimated according to Devi [32].

Determination of chitinase: Chitinase enzyme activity was assessed by procedure [33]. Chitinase activity was measured as mM N-acetylglucose amine equivalent released/g fresh weight tissue/60 minutes.

Determination of β-1,3-glucanase: β-1, 3-glucanase enzyme activity was determined as described by Sun, et al. [34] and estimated to be expressed as glucose equivalent mM/g fresh weight tissue/60 minutes.

Statistical analysis

All the collected data were statistically analyzed and contrasted using the Least Significant Difference (L.S.D.) as mentioned by Snedecor and Cochran [35].

Results

Effect of antagonists on the linear growth of *Botrytis fabae*

The resulting data in Table 1 indicate that the different antagonists were significantly varied in their inhibitory effects against the linear growth of *B. fabae in vitro* test. In this respect, *T. atroviride* significantly caused the highest reduction of mycelial growth 90.00 % followed by *T. harzianum* (86.67 %) and *T. T. album* (83.89%) on the average. On the other hand, the biocide Bio-Zeid had the least effect and the average recorded decrease in the pathogen growth was 73.56% on the average).

Assessments of *Botrytis fabae* isolates under greenhouse conditions

The ability of *B. fabae* to induce disease incidence and severity was evaluated under greenhouse conditions. Data in Table 2 reveal that the most dangerous effects of *Botrytis fabae* have occurred in faba bean plants all tested biological control treatments (*T. Album*, *T. atroviride, T. hamatum,* and

Table 1: Effect of the different antagonists on the percentage of reduction in linear growth of *B. cinerea* after incubation at 22 ± 2 °C for 7 days.

Different antagonists	Linear growth (cm)	% Reduction
T. album	1.45	83.89
T. atroviride	0.90	90.00
T. hamatum	2.10	76.67
T. harzianum	1.20	86.67
Blight Stop (*T. harzianum*)	1.90	78.89
Bio-Zeid (*T. album*)	2.38	73.56
Control "Untreated"	9.00	00.00
L.S.D at 1%	0.22	0.25

Table 2: Effect of different antagonists on disease incidence and severity of chocolate spot disease in faba bean plants under greenhouse conditions.

Different antagonists	Disease Incidence (DI) (%)	Efficacy (%)	Disease Severity (DS) (%)	Efficacy (%)
T. album	11.10	87.51	8.80	87.81
T. atroviride	9.20	89.65	5.50	92.38
T. hamatum	16.30	81.66	10.30	85.73
T. harzianum	10.50	88.19	6.60	90.86
Blight Stop (*T. harzianum*)	10.8	87.85	7.10	90.17
Bio-Zeid (*T. album*)	12.40	86.05	9.40	86.98
Control "Untreated"	88.90	0.00	72.20	0.00
L.S.D at 5%	1.42		1.31	

T. harzianum, in addition, the two commercial preparations, Blight stop (*T. harzianum*), Bio Zeid (*T. album*) led to a significant reduction in the incidence and severity of chocolate spot disease on faba bean treatments under greenhouse conditions compared to the control treatment. *T. atroviride* recorded the highest efficacy (89.65 and 92.38%), followed by *T. harzianum* isolate (88.19 and 90.86%) in controlling disease incidence and severity in greenhouse conditions, respectively. On the other hand, *T. hamarum* showed the least efficacy (81.66 and 85.73%) in controlling the disease.

Effect of different treatments on faba bean chocolate spot disease under field conditions

Disease incidence and severity: Data in Table 3 indicate that all tested biological control treatments significantly reduced disease incidence and severity of *Botrytis fabae* in both seasons 2021/22 and 2022/23. In this regard, the different antagonistic isolates varied in their effect against disease parameters. *T. atroviride* showed the highest efficacy bioagent (73.55 and 85.15%) followed by *T. harzianum* (72.55 and 81.22%) in controlling *B. fabae* of faba bean plants during two seasons 2021/22 and 2022/23, respectively. On the other hand, *T. hamatum* showed the lowest efficacy in controlling the chocolate spot disease in both seasons, being 54.87 and 44.54%, respectively in comparison with the control treatment.

Yield components: The resulting data in Table 4 show the efficacy of different biological control treatments on controlling faba bean chocolate spot disease, in addition, shows an increase in yield components of the faba bean plant during the two growing seasons 2021/22 and 2022/23. In this regard, *T. atroviride* showed the highest efficacy in increasing the faba bean yield components, *i.e.* number of pods/plant (28 and 29), weight of pods/plant (182.00 and 188.50 g), dry weight of 100 seeds (117.60 and 121.80 g) and yield/plot (1446.48 and 1984.14 Kg), in addition, recorded the highest increase in yield (115,38 and 93.33%), respectively in both seasons in comparison with the control treatment. *T. harzianum* comes in second rank after *T. atroviride* in increasing the yield components compared to other biological treatments. On the other hand, *T. hamatum* showed the least effectiveness compared with untreated plants.

Table 3: Effect of biological control treatments on *Botrytis fabae* disease incidence and severity of faba bean plants under field conditions during 2021/22 and 2022/23 growing seasons.

Different antagonists	Disease incidence %				Disease severity %			
	2021/22	2022/23	Mean	Efficacy	2021/22	2022/23	Mean	Efficacy
T. album	14.50	14.20	14.35	69.88	6.30	6.10	6.20	72.93
T. atroviride	12.70	12.50	12.60	73.55	3.50	3.30	3.40	85.15
T. hamatum	21.60	21.40	21.50	54.87	12.80	12.60	12.70	44.54
T. harzianum	13.30	13.10	13.20	72.29	4.40	4.20	4.30	81.22
Blight Stop (*T. harzianum*)	16.80	16.60	16.70	64.95	8.50	8.30	8.40	63.32
Bio-Zeid (*T. album*)	18.90	18.70	18.80	60.54	10.90	10.70	10.80	52.84
Control "Untreated"	48.15	47.13	47.64	0.00	23.50	22.30	22.90	0.00
LSD at 5%	1.42	1.40			0.13	0.12		

Table 4: Effect of biological control treatments on yield components of faba bean plants under field conditions during 2021/22 and 2022/23 growing seasons.

Different antagonists	Number of pods/plant		Weight of pods/plant (g)		Dry weight of 100 seeds (g)		Yield/plot (Kg)			
	2021/22	2022/23	2021/22	2022/23	2021/22	2022/23	2021/22	Increase%	2022/23	Increase%
T. album	25	26	162.50	169.00	105.00	109.20	1291.50	92.31	1343.16	73.33
T. atroviride	28	29	182.00	188.50	117.60	121.80	1446.48	115.38	1498.14	93.33
T. hamatum	19	20	123.50	130.00	79.80	84.00	981.54	46.15	1033.20	33.33
T. harzianum	26	27	169.00	175.50	109.20	113.40	1343.16	100.00	1394.82	80.00
Blight Stop (*T. harzianum*)	23	24	149.50	156.00	96.60	100.80	1188.18	76.92	1239.84	60.00
Bio-Zeid (*T. album*)	21	22	136.50	143.00	88.20	92.40	1084.86	61.54	1136.52	46.67
Control "Untreated"	13	15	84.50	97.50	54.60	63.00	671.58	0.00	774.90	0.00
LSD at 5%	0.22	0.23	1.74	1.77	1.12	1.14	2.10		2.12	

The influence of different antagonists on total chlorophyll, protein, phenols, and flavonoids

The results presented in Table 5 demonstrate that, over the two growth seasons of 2021/22 and 2022/23, using any of the different antagonists greatly raised the total chlorophyll, protein%, phenols, and flavonoids of the treated faba bean plants in comparison to the untreated control treatment. In this regard, *T. atroviride* outperformed alternative biological treatments in both seasons, showing a greater increase in total chlorophyll (60.83 and 61.13), protein (32.39 and 33.02%), phenols (9.1 and 9.2), and flavonoids (7.5 and 7.7). The second treatment in this regard is *T. harzianum*. On the other hand, *T. hamatum* showed the least amount of influence on the chemical components as compared to untreated faba bean plants.

The reaction of enzymes to the impact of biological treatments on the development of *B. fabae* disease and the resistance of faba bean plants

The information presented in Table 6 indicates that applying any tested biological control treatments in the two growing seasons of 2021/22–2022/23, under field conditions, significantly increased the enzyme activities of faba bean plants in terms of Peroxidase (PO), polyphenol Oxidase (PPO), chitinase, and Β-1,3-gluconate when compared to untreated plants. *T. atroviride* was the most effective treatment and recorded the highest levels of enzyme activity when compared to untreated plants in both seasons, with increases in enzyme activity of Peroxidase (PO) of 2.10 and 2.30, Polyphenol Oxidase (PPO) of 0.27 and 0.29, chitinase (5.8 and 6.0), and Β-1,3-gluconate (6.9 and 7.1). On the other hand, *T. hamatum* was the least effective treatment when compared with untreated plants in both seasons.

Discussion

Recently, farmers' thoughts have changed by using non-chemical control methods, such as biological control, as one of the safe means to produce safe, healthy food of high quality and quantity, free of toxins in the production chain, which is in line with international standards for food safety, to increase export opportunities and bring in hard currency. The aim of the study is also to use biological control to prevent bean plants of chocolate brown spot disease to preserve biological diversity for sustainable development.

The resulting data in Table 1 indicate that *T. atroviride* significantly caused the highest reduction of mycelial growth followed by *T. harzianum* and *T. T. album*. This phenomenon could perhaps be clarified by considering that distinct infections with varying striations had distinct defense mechanisms against enzymes and toxic substances generated by distinct antagonists [19,20,36-39]. *Trichoderma* spp. destroyed the pathogen's cell wall by producing lytic enzymes like chitinases, peroxidase, polyphenol oxidase, and glucan 1-3 Β-glucosidases [20,38,40].

Data in Table 2 reveal that the application of *T. atroviride* recorded the highest efficacy, followed by *T. harzianum* isolate in controlling disease incidence and severity in greenhouse conditions, respectively. On the other hand, *T. hamarum* showed the least efficacy in controlling the disease. This may be due to an effect on germ-tube elongation and to a lesser extension of the germination rate [13]. *Trichoderma* spp. possess the ability to indirectly inhibit infections by competition for space and nutrient availability, changing the atmosphere, or encouraging plant development and increasing plant defensive mechanisms and antibiosis., or directly by inhibition of growth and sporulation of the pathogen mechanisms such

Table 5: Effect of different antagonists on total chlorophyll, protein, phenols, and flavonoids of faba bean plants under field conditions during 2021/22 and 2022/23 growing seasons.

Different antagonists	2021/22 growing seasons				2022/23 growing seasons			
	Total Chlorophyll	Total Protein	Total phenols	Total Flavonoids	Total Chlorophyll	Total Protein	Total Phenols	Total Flavonoids
T. album	54.95	28.66	7.9	6.9	56.01	29.33	8.3	7.1
T. atroviride	60.83	32.39	9.1	7.5	61.13	33.02	9.2	7.7
T. hamatum	39.74	24.37	6.5	5.6	41.18	25.77	6.6	5.8
T. harzianum	58.18	30.50	8.6	7.2	58.72	31.25	8.7	7.4
Blight Stop (*T. harzianum*)	50.27	27.31	7.3	6.3	52.16	27.98	7.4	6.5
Bio-Zeid (*T. album*)	43.49	25.84	6.8	6.1	44.84	26.44	7.0	6.0
Control "Untreated"	24.43	17.38	2.4	1.7	25.45	18.86	3.1	1.8
LSD at 5%	1.33	1.08	0.20	0.09	1.34	1.10	0.22	0.10

Table 6: Effect of different antagonists on Peroxidase (PO), Polyphenol Oxidase (PPO), chitinase, and B-1,3-glucanase enzyme activities of faba bean plants under field conditions during 2021/22 and 2022/23 growing seasons.

Different antagonists	2021/22 growing seasons				2022/23 growing seasons			
	PO	PPO	Chitinase	B-1,3-gluconase	PO	PPO	Chitinase	B-1,3-gluconase
T. album	1.93	0.20	5.3	5.8	1.95	0.22	5.5	6.0
T. atroviride	2.10	0.27	5.8	6.9	2.30	0.29	6.0	7.1
T. hamatum	1.75	0.11	3.8	4.3	1.77	0.13	4.0	4.7
T. harzianum	1.98	0.23	5.7	6.3	2.00	0.25	5.9	6.5
Blight Stop (*T. harzianum*)	1.85	0.17	4.6	5.3	1.87	0.19	4.8	5.6
Bio-Zeid (*T. album*)	1.81	0.14	4.1	4.9	1.83	0.16	4.3	5.2
Control "Untreated"	1.20	0.03	1.9	1.1	1.22	0.05	2.1	1.3
LSD at 5%	0.25	0.10	0.08	0.12	0.26	0.11	0.09	0.13

as mycoparasitism and enzyme production [13,19,41]. The earlier studies also revealed that antimicrobial metabolites produced by *Trichoderma* spp. have been efficient against the extensive range of phytopathogenic fungal organisms, such as *B. fabae*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Curvularia lunata*, *Bipolaris sorokiniana* and *Colletotrichum lagenarium* [14,22,42].

Data in Table 3 indicate that all tested biological control treatments significantly reduced disease incidence and severity of *Botrytis fabae* in both seasons 2021/22 and 2022/23. The findings can be explained by the bioagents' dual action, which leads to the production of growth regulators and the chemical action of antioxidants [15]. This action is evident in the enhancement of plant metabolism and physiology, as well as the induction of systemic resistance (ISR). It is known that *Trichoderma* spp. have the ability to directly affect target fungi through mycoparasitism, competition for key nutrients and colonization sites, and antibiosis as their main mechanisms for suppressing plant pathogenic fungus or stimulation of plant defense mechanisms [20,38,43]. Additionally, it has been observed that *Trichoderma* spp. produce antifungal compounds such as trichodermin, beta-glucosidase, endo chitinase, and alpha-1,3-glucanase [9,39]; in the meantime, the isolates struggle with one another for nutrients and space.

The resulting data in Table 4 show the efficacy of different biological control treatments on controlling faba bean chocolate spot disease, in addition, shows an increase in yield components of the faba bean plant during the two growing seasons 2021/22 and 2022/23. In this regard, *T. atroviride* showed the highest efficacy in increasing the faba bean yield components, *i.e.* number of pods/plant, the weight of pods/plant (g), dry weight of 100 seeds (g), and yield/plot (Kg), in addition, recorded the highest increase in yield (%), respectively in both seasons in comparison with the control treatment. *T. harzianum* comes in second rank after *T. atroviride* in increasing the yield components compared to other biological treatments. On the other hand, *T. hamatum* showed the least effectiveness compared with untreated plants. These results are in agreement with those obtained by [1,9,19] who found that biocontrol agents gave good control of chocolate spot disease and significantly increased grain yield. The efficacy of *Trichoderma* spp. is due to their potential to be antagonistic against *B. fabae*, which causes aba bean chocolate spot disease, in addition to improving growth as potential bio-fertilizers and plant growth promoter to enhance faba bean productivity [12,16,44].

The results presented in Table 5 demonstrate that, over the two growth seasons of 2021/22 and 2022/23, using any of the different antagonists greatly raised the total chlorophyll, protein%, phenols, and flavonoids of the treated faba bean plants in comparison to the untreated control treatment. In this regard, *T. atroviride* outperformed alternative biological treatments in both seasons, showing a greater increase in total chlorophyll, protein (%), phenols, and flavonoids. The second treatment in this regard is *T. harzianum*. On the other hand, *T. hamatum* showed the least amount of influence on the chemical components as compared to untreated faba bean plants. This finding is in agreement with El-Rahman and Mohamed [45] who discovered, however, that applying *Trichoderma harzianum* as a foliar treatment greatly decreased the severity of chocolate spot disease when compared to untreated infected plants. Additionally, inducers substantially raised the total chlorophyll and protein content in treated infected plants when compared to untreated.

This might be examined in light of the discoveries made by Hahlbrock and Scheel [46], who established that phenols and flavonoids are necessary for the formation of lignin, which is thought to be a crucial structural element of plant cell walls. These results are in harmony with [38,40] who explained that plant defense mechanisms begin with a fast build-up of phenols at the site of infection. These compounds operate as antioxidants, antimicrobials, and photoreceptors, limiting or slowing the pathogen's growth. However, Mohamed, et al. [47] noted that plants have two steps to their defense mechanism. It is thought that the first stage entails the quick build-up of phenols and flavonoids at the infection site, which serves to reduce the pathogen's growth rate and enable the activation of "secondary" methods that will more completely restrict the pathogen. Specific defense mechanisms, such as the creation of chemicals linked to pathogen stress, are activated as part of the secondary reactions.

The information presented in Table 6 indicates that applying any tested biological control treatments in the two growing seasons of 2021/22–2022/23, under field conditions, significantly increased the enzyme activities of faba bean plants in terms of Peroxidase (PO), Polyphenol Oxidase (PPO), chitinase, and B-1, 3-gluconate when compared to untreated plants. *T. atroviride* was the most effective treatment and recorded the highest levels of enzyme activity when compared to untreated plants in both seasons. On the other hand, *T. hamatum* was the least effective treatment when compared with untreated plants in both seasons. These findings are consistent with numerous studies that describe how *Bacillus subtilis, T. viride, T. albu*, and *Trichoderma harzianum* all significantly reduced the disease severity% of *Botrytis fabae*, the cause of faba bean chocolate spot disease, in field settings during the 2012/13 and 2013/14 seasons. Additionally, the results demonstrated that, in comparison to the control treatment, all tested bio-inducer treatments had an effect on yield components, growth parameters, and increased levels of phenols, flavonoids, Peroxidase (PO), Polyphenol Oxidase (PPO), chitinase, and -1, 3-glucanase activities [13,15,38,46].

These outcomes can be attributed to the main components of many fungi's cell walls, chitin and β-1,3 glucan, which are polymers of N-Acetylglucosamine (NAG). Since β-1,3 glucanase and chitinases have been demonstrated to be able to penetrate the fungal pathogens' cell walls, these enzymes have been suggested as plants' direct defense mechanisms. These findings also corroborate those of Ermias, et al. [48], who discovered that treating faba bean plants with *Bacillus subtilis* and *Trichoderma harzianum* caused a noteworthy increase in Peroxidase (PO), Polyphenol Oxidase (PPO), chitinase, and -1, 3-glucanase activities as compared to the plants that were not treated. Furthermore, an increase in peroxidase activity promotes lignification in response to infection with chocolate spots, which may limit the penetration of fungi. Plants can be made more resistant to future attacks by pre-treating them with avirulent pathogens (bio-inducers) or chemical compounds (abio-inducers). This can be done not just at the treatment site but also in tissues that are far from the original infection.

Conclusion

Several antagonists, such as *Trichoderma album, T. atrovirde, T. hamatum,* and *T. harzianum* (30 x 10^6 spore/ml), in addition to the two commercial biocide products, Blight Stop and Bio Zeid, were applied at a rate of 1 Lit./50 Lit. to control the chocolate spot of bean plants. Throughout the seasons 2022 – 2023 and 2021 – 2022, every antagonist that was tested considerably decreased the incidence and severity of *Botrytis fabae* disease. *In vivo*, all studied antagonists dramatically reduced *Botrytis fabae* disease incidence and severity in both seasons. *T. atroviride* showed the highest efficacy bioagent followed by *T. harzianum* in controlling *B. fabae* of faba bean plants during two seasons 2021/22 and 2022/23, respectively. Furthermore, the findings demonstrated that, in comparison to the control treatment in both seasons, all tested bio-control treatments affected the yield components and raised the levels of phenols, flavonoids, protein percentage, Peroxidase (PO), Polyphenol Oxidase (PPO), chitinase, and -1, 3-glucanase activities. The most effective bioagent in this regard was *T. atroviride* then *T. harzianum*. On the other hand, *T. hamatum* showed the least efficiency in comparison with the control treatment during the two growing seasons.

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