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Biological Control of Gray Mould Disease Caused by *Botrytis cinerea* on Strawberry Fruits

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Abstract

Different bio-agents and biocides were applied under greenhouse and field conditions in two successive seasons (2010/11 &2011/12) to study their efficiency in controlling the gray mould disease on strawberry fruits. *Trichoderma asperellum* was the most effective one among the tested bio-agents, where it reduced the disease incidence DI% and disease severity DS% caused by *Botrytis cinerea* during the two seasons followed by *T. hamatum* and *Bacillus subtilis*. Blight Stop biocide proved to be the most efficient biocide in decreasing the gray mould disease and increasing strawberry fruit yield, while, Rhizo-N was the least effective biocide in controlling the gray mould disease on strawberry fruits. Increasing the interval time between sprayings increased disease incidence, where 6-days interval time were more effective than 12 days in decreasing the gray mould infection with all tested biocides. Spraying the plants with Blight Stop combined with Plant Guard was the most effective combination among the tested biocides in decreasing the gray mould incidence and gave highest yield. Fruit rot incidence on grown plants, under field conditions were decreased by spraying plants with each one of the tested biocides or combinations between them.

Keywords: Strawberry, Bio-agents, Bio-biocides, Gray mould, *Botrytis cinerea*, Disease incidence

Introduction

Gray mould caused by *Botrytis cinerea* Pers., Fr. is one of the most important diseases attacking strawberry fruits and causing severe field losses (**Elad *et al*., 2004; Willamson *et al*., 2007; Zhang *et al*., 2007**). Management of gray mould is based principally on chemical control; however, using fungicides in controlling the gray mould infection might be undesirable as a result of their toxic residues in addition to continuous initiation of resistant isolates of the pathogen (**Paul *et al*., 1998; Ma and Michailides, 2005; Myresiotis *et al*., 2007; Pande *et al*., 2010)**. Fungicide application at flowering stage may reduce pollen viability and consequently hinder fruit formation (**Kovach *et al*., 2000**). Control of fungal pathogens is based on the use of agronomic practices and pesticides, but widespread application of chemicals inundates the agro-eco systems with toxic compounds that affect the balance of the natural food chain. Treating strawberry plants with fungicides prior to harvesting led to presence of toxic residues in harvested fruits, due to the short time elapsing between treatment and consumption. Thus, using the natural micro flora which present on fruits will play an important role in controlling the different fungal pathogens as new safe ways of control. It is important therefore to isolate and characterize microorganisms having antagonistic activities to use them as biocontrol agents (**Paul *et al.*, 1998**). Most sustainable and environmentally acceptable control may be achieved using biocontrol agents (BCAs) as described by **Haggag (2002) and Haggag (2003).**

The objective of the current work is to evaluate the potentialities, *Trichoderma* spp., *Bacillus subtilis* and some commercial biocides in controlling the gray mould infection on strawberry fruits under greenhouse and field conditions.

Materials and Methods

1. Source of tested bio-agent cultures and biocides:

Bio-agents *i.e., Trichoderma harzianum, T. viride, T. hamatum,* and *Bacillus subtilis* were obtained kindly from the Central Laboratory of Organic Agriculture, Agriculture Research Centre while *Trichoderma asperellum* T 34 was isolated from the new formulated biocide which named Biocontrol T34 (under evaluation in Agriculture Research Center).

Biocides listed in **Table 1**, *i.e.* Plant Guard (*T. harzianum*), Rhizo-N (*B. subtillis*), Bio-Arc (*B. megaterium*), Bio-Zied (*T. album*) and Blight Stop (Mixture of *Trichoderma*) were obtained from Plant Pathology Research Institute at Giza**.**

2. Effect of spraying strawberry plants with bio-agents on gray mould infection under greenhouse conditions:

All experiments were conducted under artificial inoculation in the greenhouse and yield experiments of Horticulture Research Station at El Kasasin (Ismailia Governorate), Agric. Research Center, to evaluate the suitable treatment in controlling the gray mould infection on strawberry fruits under greenhouse conditions.

A greenhouse experiment was carried out in a complete randomized block design with three replicates for each treatment. Pots (Ø35 cm) were filled with 5 kg sterilized sandy-clay soil (1:1) and were sown with five cv. Sweet Charlie transplants. A suspension of each one of the tested bio-agents *i.e*., *T. viride, T. harzianum, T. hamatum,* and *T. asperellum* T 34 were prepared directly from 7 days old culture grown on PDA medium and adjusted at concentration of 6 x 10^8 spores/mL according to **Abd El-Kader (1997).** The plants were sprayed with spore suspension of *B. cinerea* at the rate 1 x 10^3 spores/mL at the beginning of flowering stage. Then, the prepared suspension was sprayed. *Bacillus subtillis* was used at concentration 1×10^8 cfu/mL.

Disease severity was recorded according to an empirical scale with six degrees: 0, healthy strawberry; 1, 1 to 20% fruit surface infected; 2, 21 to 40% fruit surface infected; 3, 41 to 60% fruit surface infected; 4, 61 to 80% fruit surface infected; 5, more than 81% of the strawberry surface infected and showing sporulation **(Romanazzi *et al*., 2000)**. The empirical scale allowed the calculation of the McKinney's index, expressed as the weighted average of the disease as a percentage of the maximum possible level **(McKinney, 1923)**. This parameter also includes information on both disease incidence and disease severity.

-Preparation of pathogen inoculum:

The inoculum of *B. cinerea* was separately prepared by plated on potato dextrose agar amended with 100 mg/L streptomycin (SPDA). All inoculated plats were incubated at 24°C for two weeks to obtain sufficient fungal growth. A two week old, sporulating plate (one week in black light / one week in 12 h in fluorescent light and 12 h in dark cycle) was flooded with sterilized distilled water containing one drop of Tween 20 (Fisher scientific, Pittsburg, PA), **(Janisiewiez, 1998)**.

Disease incidence % and disease severity % were estimated after 20-25 days from treatment of strawberry plants and every 2-3 days.

-Preparation of spore suspension of bio-agent fungi:

Spore suspensions of bio-agent fungi were prepared by growing on potato dextrose medium (250 mL) for 10 days at 25ºC. The conidia were released in sterilized water through filtration using muslin cloth to exclude mycelial growth. The conidial suspension was adjusted to be 2 × 10^6 spores/mL using Haemocytometer slide technique according to **(Ibrahim, 1993)**.

Table 1. Commercial name, active ingredient, biocides dose and manufacturers of tested biocides:

Commercial name	Active ingredient	Dose	Manufacturer
Bio-Zied (powder)	*Trichoderma album*	250g/100 liter water 10 million cfu/g	Organic for Biotechnology Co.
Bio-Arc (powder)	*Bacillus megaterium*	250g/100 liter water 25 million cfu/g	Organic for Biotechnology Co.
Rhizo-N (powder)	*Bacillus subtillis*	4 g/ liter water 30 X 10^6 cell/kg	El-Nasr Co. for fertilizers and Biocides
Plant Guard (liquid)	*Trichoderma harzianum*	3.0 mL/Liter water 30x10^6 cfu/mL	Bio Tech for Fertilizers and Biocides
Blight Stop (liquid)	Mixture of *Trichoderma* spp.	1L/100L water	Central lab. of Organic Agri. (ARC)

3. Effect of spraying strawberry fruits with biocides on gray mould infection under greenhouse conditions:

Biocides *i.e.,* Plant Guard (*T. harzianum*), Rhizo-N (*Bacillus subtillis*), Bio-Arc (*Bacillus megaterium*), Bio-Zied (*T. album*) and Blight Stop (Mixture of *Trichoderma*) were prepared with recommended dose and used as spraying on strawberry fruits before and after inoculation with 1x10^3 spore/mL of *B. cinerea* to test their effects on gray mould incidence. Results were recorded as mentioned before.

4. Effect of the interval time between sprayings with biocides on gray mould infection under greenhouse conditions:

In this trial, spraying strawberry plants with the tested biocides was applied every 6 and 12 days to see which is more effective.

5. Effect of the tested biocides with different concentrations on gray mould infection under greenhouse conditions.

Forty eight pots (Ø35 cm) filled with 5 kg of sterilized soil were sown with cv. Sweet Charlie transplants. The plants were sprayed with spore suspension of *B. cinerea* at the rate 10,000 spores/mL at the beginning of flowering stage and after 48 h. Plants were sprayed with suspension of each one of the tested biocides every 7 days (4 times). Three pots were left as control which sprayed with spore suspension of *B. cinerea* only.

6. Effect of spraying strawberry plants with some combined biocides on gray mould infection:

Spraying the strawberry plants with the combined biocides in form of "Bio-Zied + Blight Stop", "Plant Guard + Bio-Zied", "Blight Stop + Plant Guard" was done after artificial inoculation

with spore suspension of *B. cinerea* as mentioned before. Two days after inoculation, treatments were sprayed at 7 days intervals (4 times). The control treatment was sprayed with water only. The first spray started at the beginning of flowering stage.

7. Effect of the interval time between sprayings with the tested biocides on gray mould infection under field conditions:

Frigo strawberry transplants of Sweet Charlie cv. were planted than left to grow. The biocides were Bio-Zied 2.5 g/L, Bio-Arc 2.5 g/L, Rhizo-N 4 g/L, Plant Guard 5 mL/L and Blight Stop 1L/100L were sprayed at the beginning of flowering stage every 6 and 12 days. Three plots were left as a check treatment and sprayed with the same amount of water

8. Effect of the tested biocides with different concentrations on gray mould infection under field conditions:

Frigo strawberry transplants of Sweet Charlie cv. were planted. Bio-Zied 1.5, 2.5 and 3.5 g/L, Bio-Arc 1.5, 2.5 and 3.5 g/L, Rhizo-N 2, 4 and 6 g/L, Plant Guard 1.5, 2.5 and 5 mL/L and Blight Stop 5, 10 and 15 mL/L were sprayed at the beginning of flowering stage. Four sprays were carried out during the growing season. Three replicates (21 m=1/200 fed.) were used for each particular treatment. Three plots were sprayed with water only to serve as a check treatment.

9. Effect of spraying strawberry plants with some combined biocides on gray mould infection under field conditions:

Three combinations of the tested biocides *i.e*., Bio-Zied + Blight Stop, Plant Guard + Bio-Zied, Blight Stop + Plant Guard with the pre-mentioned rates were sprayed on strawberry plants under field conditions as mentioned before. Results were recorded as mentioned before. Disease incidence % and disease severity % were estimated after 20-25 days from strawberry plants treatment and every 2-3 days as mentioned before.

Results

1. Effect of spraying strawberry plants with different bio-agents on gray mould infection under greenhouse conditions:

The obtained results in **Table 2** show that, all tested bio-agents were able to control effectively the gray mould infection on strawberry cv. Sweet Charlie under greenhouse conditions compared with control treatment (without bio-agents). *T. asperellum* was the most effective where it reduced DI% and DS% of the gray mould infection caused by *B. cinerea* followed by *T. hamatum* and *B. subtilis*. Generally, data in season 2011/12 were more effective than season 2010/11 and this was also true for fruit yield. *T. harzianum* and *T. viride* had the lowest effect in this respect.

Table 2. Effect of spraying strawberry plants with bio-agents on gray mould infection and fruit yield of c.v. Sweet Charlie during two successive seasons (2010/11 and 2011/12) under greenhouse conditions.

Bio-agent	2010/11				2011/12			
	Disease incidence%	Disease severity%	Yield Mg/ha.	%Yield increase	Disease incidence%	Disease severity%	Yield Mg/ha.	%Yield increase
T. harzianum	10.93	7.25	49.93	113.86	11.18	8.10	48.58	102.08
T. viridi	11.86	8.15	49.46	111.82	14.33	8.25	48.46	101.58
T. hamatum	10.26	6.85	50.88	117.94	10.42	6.80	50.58	110.39
T. asperellum T34	9.52	6.50	52.07	123.04	9.76	6.40	51.55	114.46
Bacillus subtilis	10.37	7.15	50.29	115.39	10.85	7.50	48.74	102.77
Control	58.42	43.30	23.35	0.00	56.02	41.80	24.04	0.00
L.S.D. at 5%	5.17	3.06	2.03	-	3.46	3.56	2.52	-

2. Effect of the tested biocides with different concentrations on gray mould infection:

Data shown in **Table 3** reveal that all tested biocides affected strawberry gray mould incidence, under greenhouse conditions. However, Blight Stop was the most effective biocide in this respect, since it significantly reduced the percentages of DI and DS. Also, DI% and DS% were decreased by increasing the concentration of biocide. The most effective concentration was 15 mL/L of Blight Stop. This particular treatment decreased the percentages of DI and DS by 10.0% and 7.3%, respectively in first season and 10.33% and 7.28%, respectively in the second season. Blight Stop gave the highest fruit yield during the two seasons. However, Rhizo-N was the least effective biocide were it showed the highest percentages of DI in the two seasons.

3. Effect of the interval time between sprayings with biocides on gray mould infection:

Data shown in **Table 4** indicate that the 6-days interval time between spraying decreased DI% and DS%. However, DI% and DS% were increased by increasing the spraying interval time. The 6-days interval was the most effective. Also, Blight Stop was the most effective in the two treatments of

spraying. At 12-days interval time, this particular treatment decreased DI% and DS% to be 13.42 and 14.88%, respectively in the first season and 8.65, 9.50%, respectively in the second season.

All tested biocides gave effective results with 6-days interval time as compared with 12- days' interval time.

4. Effect of spraying strawberry plants with some biocides on strawberry gray mould infection:

Data in **Table 5** show that all tested biocide combinations significantly affected strawberry fruit rot incidence. The combined form of Blight Stop + Plant Guard was the most effective combination, where it significantly decreased DI and DS by 12.10 and 9.80%, respectively in the first season and 11.90 and 10.5%, respectively in the second season. "Blight Stop + Plant Guard" gave the highest yield 49.3 and 48.3 Mg/ha during season 1 and season 2, respectively. On the other hand, Bio-Zied + Plant Guard was the least effective one where it resulted in the highest DI and DS 13.30 and 10.30% respectively in the first season and 12.50 and 11.30% respectively in the second season.

Table 3. Effect of the tested biocides with different concentrations on gray mould infection caused by *B. cinerea* of C.V. Sweet Charlie during two successive seasons (2010/11 and 2011/12) under greenhouse conditions**.**

Biocide	Conc.	2010/11			2011/112		
		Disease incidence (DI%)	Disease severity (DS%)	Yield Mg/ha.	Disease incidence (DI%)	Disease severity (DS%)	Yield Mg/ha.
Bio-Arc	1.5g	24.07	19.51	42.58	26.52	20.18	40.15
	2.5g	18.32	12.51	45.79	23.46	17.80	41.82
	5.0g	18.60	12.64	45.51	22.68	16.40	42.25
Bio-Zied	1.5g	23.53	19.11	42.86	27.39	21.24	39.67
	2.5g	20.34	16.34	44.67	18.15	10.33	45.82
	5.0g	16.35	16.31	47.03	16.43	15.70	42.94
Rhizo-N	2.0g	29.11	13.92	39.75	29.86	16.11	42.70
	4.0g	28.02	13.60	40.41	28.52	11.34	44.51
	6.0g	26.53	13.78	41.20	26.73	8.50	46.03
Plant-Guard	1.5mL	19.82	13.94	44.96	20.24	11.20	45.20
	2.5mL	18.69	9.81	45.58	18.79	8.11	47.10
	5.0mL	12.09	8.31	49.29	11.04	7.78	48.60
Blight Stop	5.0mL	19.60	13.90	45.08	17.94	12.44	44.84
	10.0mL	15.25	10.50	47.53	12.50	7.84	47.81
	15.0mL	10.00	7.30	50.48	10.33	7.28	48.89
Control	-	58.40	43.30	23.32	56.00	41.80	24.04
L.S.D. at 5%		4.84	1.40	1.90	2.28	1.11	1.27

Table 4. Effect of the interval time between sprayings with biocides on strawberry gray mould infection caused by *B. cinerea* of c.v. Sweet Charlie during two successive seasons (2010/11 and 2011/12) under greenhouse conditions.

Interval time	Biocide	Disease incidence%		Disease severity%		Yield Mg/ha.	
		2010/11	2011/12	2010/11	2011/12	2010/11	2011/12
6 days	Bio-Arc	12.04	13.54	8.40	8.95	49.31	48.48
	Bio-Zied	9.45	12.33	6.50	8.45	50.77	49.15
	Rhizo-N	18.91	18.45	13.38	12.50	45.46	45.72
	Plant Guard	7.38	10.78	3.86	6.86	51.93	50.03
	Blight Stop	6.98	10.11	3.50	6.50	52.17	50.41
	Control	58.40	56.00	43.80	43.30	23.32	24.04
12 days	Bio-Arc	25.85	25.44	10.40	19.50	40.51	40.75
	Bio-Zied	23.89	23.83	10.45	18.75	41.58	41.60
	Rhizo-N	28.91	28.95	16.55	22.80	38.84	38.82
	Plant Guard	18.77	16.13	8.75	10.85	44.39	45.82
	Blight Stop	13.42	14.88	8.65	9.50	47.29	46.51
	Control	58.4	56.00	43.80	43.30	23.32	24.04
LSD at 5%		2.93	1.33	2.79	2.86	2.05	3.07

Table 5. Effect of spraying strawberry plants with some combined on gray mould infection of c.v. Sweat Charlie fruits during two successive seasons (2010/11 and 2011/12) under greenhouse conditions.

Biocide	Recommended dose	2010/11			2011/12		
		Disease incidence%	Disease severity%	Yield Mg/ha.	Disease incidence%	Disease severity%	Yield Mg/ha.
Bio-Zied+Plant Guard	2.5g+5.0mL	13.30	10.30	48.55	12.50	11.30	47.81
Blight Stop +Plant Guard	10.0mL+5.0mL	12.10	9.80	49.29	11.90	10.50	48.12
Blight Stop +Bio- Zied	10.0ml+2.5g	12.30	10.10	49.17	12.10	10.90	48.03
Control	-	58.40	43.30	23.32	56.00	41.80	24.04
L.S.D. at 5%	-	4.02	1.75	0.69	3.35	2.32	2.43

5. Effect of spraying strawberry plants with some different bio-agents on gray mould infection:

Data presented in **Table 6** show that, all tested bio-agents controlled effectively the incidence of gray mould infection under field conditions. In the first season *T. asperellum* was the most effective bio-agent in decreasing DI% and DS% of gray mould where it recorded 14.29 and 7.45%, respectively. *T. hamatum* came in second rank where it decreased the DI and DS to be 14.63 and 7.66%, respectively while, *Bacillus subtilis* came in the third rank where it decreased the DI and DS to be 15.87 and 7.75%, respectively. *T. viride* and *T. harzianum* were the least effective one. In the second season, results were similar to that of season 1 although the yields were generally lower than those of season 1.

Table 6. Effect of spraying strawberry plants with some different bio-agents on gray mould infection and yield of c.v. Sweet Charlie during two successive seasons (2010/11and 2011/12) .

Bio-agent	2010/11				2011/12			
	Disease incidence%	Disease severity%	Yield Mg/ha.	% Yield increase	Disease incidence %	Disease severity %	Yield Mg/ha.	% Yield increase
T. harzianum	17.23	9.80	46.89	62.81	18.44	11.35	45.48	49.22
T. viridi	16.54	8.50	47.36	64.46	17.58	10.15	45.17	47.66
T. hamatum	14.63	7.66	51.36	78.35	15.15	8.10	48.91	60.55
T. asperellum T 34	14.29	7.45	51.84	80.00	13.79	7.30	52.07	70.80
B. subtilis	15.87	7.75	49.50	71.90	16.48	8.40	45.79	50.0
Control	35.53	25.70	28.80	0.00	33.32	22.40	30.49	0.00

6. Effect of spraying strawberry plants with different concentration of bio-agents on gray mould infection:

The obtained data of greenhouse experiment were confirmed under filed conditions. Data in **Table 7** show that Blight Stop was the most effective biocide at the two growing seasons, under field conditions, since it achieved the lowest percentages of incidence in case of concentrations 10 mL/L and 15 mL/L giving DI% and DS% of 15.05 and 15.25%, respectively in the first season and 17.50 and 15.53%, respectively in the second season. Blight Stop resulted in the highest fruit yield giving 37.94 and 38.58Mg/ha, respectively. Rhizo-N was the least effective one where it caused the highest DI (32.53%) in the first season followed by Bio-Arc which gave 29.49% of DI in the second season.

7. Effect of the interval time between spraying of the tested biocides on gray mould infection:

Data in **Table 8** show that 6-days interval time decreased DI% and DS%. The DI% and DS% were increased by increasing the interval time of spraying. The 6-days interval time was the most effective one, decreasing DI% and DS%. The Blight Stop was the most effective in the two intervals of spraying. It decreased DI by 18.98, 18.33% for season 1 and 2, respectively 12.6, 12.5% for season 1 and 2, respectively.

All biocides exhibited the most effective results with the 6-day interval time.

8. Effect of the tested biocide combinations on strawberry gray mould infection under field conditions:

Data in **Table 9** show that the combined formula of Blight Stop + Plant Guard was the most effective treatment under field conditions, where it achieved the lowest DI (19.30 and 19.80% for season 1 and 2, respectively). Also, Blight Stop + Plant Guard resulted in the highest fruit yield during the two growing seasons (40.0 and 36.6 Mg/ha, respectively). The Bio-Zied + Plant Guard treatment was the least effective treatment resulting in the highest DI (20.0 and 21.50%, for season 1 and 2, respectively) and the least yield in both studied seasons.

Table 7. Effect of spraying strawberry plants with different concentrations of some biocides on gray mould infection of C.V. Sweet Charlie during two successive seasons (2010/11and 2011/12) under field conditions.

Biocide	Concentration	2010/11			2011/12		
		Disease incidence%	Disease severity%	Yield Mg/ha.	Disease incidence%	Disease severity%	Yield Mg/ha.
Bio-Arc	1.5g	30.07	25.50	30.99	29.46	23.44	32.20
	2.5g	24.32	19.53	33.80	26.52	20.12	33.56
	5.0g	24.60	19.67	33.65	25.68	20.01	33.94
Bio-Zied	1.5g	29.53	24.85	31.46	27.39	23.41	33.15
	2.5g	20.34	16.34	35.56	20.15	16.62	36.46
	5.0g	20.35	16.33	35.53	26.43	20.51	33.61
Rhizo-N	2.0g	32.53	27.85	30.13	27.73	22.41	33.01
	4.0g	31.11	26.75	30.75	23.52	17.81	34.91
	6.0g	29.20	23.41	31.70	26.86	22.11	33.42
Plant-Guard	1.5mL	21.82	15.71	34.91	22.24	17.87	35.51
	2.5mL	19.69	14.56	35.87	18.79	12.50	37.08
	5.0mL	16.09	12.11	37.56	18.04	12.42	37.44
Blight Stop	5.0mL	19.60	13.94	35.89	22.94	18.72	35.20
	10.0mL	15.25	10.33	37.82	17.50	12.33	37.68
	15.0mL	15.05	10.30	37.94	15.53	10.31	38.58
Control	-	35.50	45.70	28.80	33.30	42.40	30.46
L.S.D. at 5%		3.12	1.89	0.58	4.37	1.64	NS

Table 8. Effect interval time between spraying of the tested biocides on strawberry gray mould infection during two successive seasons (2010/11and 2011/12) under field conditions.

Interval	Biocide	Disease incidence%		Disease severity%		Yield Mg/ha.	
		2010/11	2011/12	2010/11	2011/12	2010/11	2011/12
6 days	Bio-Arc	25.04	23.54	17.33	14.13	33.92	35.39
	Bio-Zied	24.45	22.95	17.55	14.77	33.61	35.20
	Rhizo-N	26.91	25.61	18.71	18.64	32.63	33.99
	Plant Guard	22.38	20.08	14.88	14.50	34.65	36.51
	Blight Stop	18.98	18.33	12.60	12.50	36.18	37.29
	Control	35.50	33.30	25.70	22.40	28.80	30.46
12 days	Bio-Arc	29.89	30.89	23.75	18.40	31.32	31.92
	Bio-Zied	28.85	29.35	22.60	17.30	31.54	32.18
	Rhizo-N	33.91	32.67	24.50	19.50	29.51	30.73
	Plant Guard	24.77	22.87	17.85	14.95	33.58	35.22
	Blight Stop	23.42	22.50	15.30	14.90	34.20	35.30
	Control	35.50	33.30	25.70	22.40	28.80	30.46
LSD at 5%		1.47	2.49	0.92	1.30	1.64	0.97

Table 9. Effect of tested biocide combinations on strawberry gray mould infection and fruit yield during two successive seasons (2010/11and 2011/12) under field conditions.

Biocide	Recommended dose	2010/11			2011/12		
		Disease incidence%	Disease severity%	Yield Mg/ha.	Disease incidence%	Disease severity%	Yield Mg/ha.
Bio-Zied + Plant Guard	2.5g +5.0mL	20.00	9.20	35.94	21.50	12.50	35.94
Blight Stop + Plant Guard	10.0mL+5.0mL	19.30	8.50	39.98	19.80	10.30	36.63
Blight Stop + Bio-Zied	10.0mL+2.5g	19.50	10.50	36.41	19.90	11.10	36.89
Control	-	35.50	25.70	28.80	33.30	22.40	30.46
L.S.D. at 5%	-	3.22	3.61	1.11	2.63	0.64	0.51

Discussion

Gray mould, caused by *Botrytis cinerea* is one of the most important strawberry diseases inciting great losses before or after harvesting of strawberry fruits (**Elad *et al.*, 2007 and Zhang *et al.*, 2007**). Although, *B. cinerea* can infect almost all aerial plant parts and the most damaging infections occur at

flowering stage (**Myresiotis *et al.*, 2007**). Under greenhouse conditions, pre-spraying of strawberry plants with different biocides before inoculation *B. cinerea* was more effective in controlling the gray mould disease than that inoculated at the same time with the pathogen. Blight Stop was the most effective biocide as it showed the least fruit rot after with the 6-days interval followed by Plant Guard. In both cases, disease incidence and severity were less than the control. This result is in agreement with **Nelson and Powelson (1988) and Blacharski *et al.*, (2001)** who noticed that applied two fungicides (Captan and Thiram) at the peak of bloom period, decreased the incidence of preharvest Botrytis fruit rot and increased marketable yield. All bio-agents effectively decreased infection caused by *B. cinerea* on cultivar Sweet Charlie under greenhouse conditions. *B. subtilis* was the most effective one compared with *T. harzianum* and *T. viride*. It decreased DI and DS. These results are in agreement with **Levy *et al.* (2004); Ju *et al.*, (2007); Choi *et al.*, (2009); Saloma and Eugenio (2010) and Ragab *et al.*, (2011)**, reported that biological control using *T. harzianum* isolate T39 controlled several foliar pathogens like *B. cinerea*, powdery mildew and *Sclerotinia sclerotiorum* by inducing the resistance locally or systemically through decreasing the reactive oxygen species (ROS) in uninfected leaf tissue or enhancing the formation of phytohormones.

The mechanisms of biological control with bacteria may involve (1) competition for nutrients and space, (2) antibiotics production, **(Wilson and El-Ghaouth, 1993).**

Results concerned the effect of the spray interval time indicated that Blight Stop was the most effective one when spraying after 6 days where decreased the infection and increased the yield. On the other hand, Rhizo-N was the least effective in this respect, and Plant Guard was moderately effective.

In the current investigation, it was found that the biological control in field experiments of strawberry gray mould of caused by *B. cinerea* was achieved by spraying the flowers with a conidial suspension of the biocide Blight Stop followed by Plant Guard. Therefore, biological control could be applied to reduce fungal diseases on fruits. Infection and severity of gray mould decreased using Blight Stop. The low effect of Rhizo-N was reported by **Niemi and Lahdenpera (2000) and Younis (2002)**. Antagonists that compete with saprophytic growth of *Botrytis* spp. may reduce pathogen growth and/or sporulation in crop debris **(Kohl *et al.*, 1995; Morandi *et al.*, 2003),** resulting a decrease of disease progress rate. Using these antagonists is advantageous because of the continuity of the interaction between pathogen and antagonist in the crop debris (**Fokkema, 1993).** Suppressing either colonization or sporulation of *B. cinerea* is a valid strategy for biological control of the pathogen in strawberry and other hosts **(Sutton and Peng, 1993; Kohl and Fokkema, 1998; Morandi *et al.*, 2003).** In strawberry production, young leaves can be infected by *B. cinerea.* Therefore, the high levels of suppression of pathogen sporulation in leaves would effectively reduce inoculum produced in crop debris and consequently contribute to decrease DI on flowers as well as fruits (**Mertely *et al.*, 2002; Legard *et al.*, 2005).**

As for the effect of biocide combinations Blight Stop + Plant Guard was the most effective in reducing the incidence of strawberry gray mould on cv. Sweat Charlie caused by *B. cinerea* under greenhouse conditions. The combination of Blight Stop + Bio-Zied came second in effect. While, Bio-Zied + Plant Guard was the least effective one in this respect. The same trend was true concerning the effect of biocide combinations on yield. Results under field conditions followed the same trend obtained under greenhouse conditions. These results are in harmony with those of **Guetsky *et al.*, (2001) Latha *et al.*, (2011) and El-Sayed (2013)** who showed positive correlation between increase bio-agent concentrations. The highest decrease in disease incidence was obtained when either *T. harzianum* or *B. subtilis* used at high concentration (6 x 10^5 c.f.u).

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المكافحة البيولوجية لمرض العفن الرمادي المتسبب عن الفطر بوطرايتس سينيريا على ثمار الفراولة

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طُبِقت العوامل البيولوجية المختلفة والمبيدات الحيوية التجارية تحت ظروف الصوبة والحقل في موسمين متتالين (2011/2010 & 2011/ 2012) لدراسة فعاليتها في مكافحة مرض العفن الرمادي على ثمار الفراولة. وقد أظهرت النتائج أن عزلة الفطر ترايكودرما أسبيريولوم. تليها ترايكودرما هاماتوم قد أظهرتا أفضل كفاءة في مكافحة المرض الذي يسببه الفطر بوطرايتس سينريا ، وفي نفس الوقت جاء استخدام البكتريا باسلس سائتلس في المرتبة الثالثة. ومن ناحية أخرى، كان المبيد الحيوي بلايت ستوب هو الأكثر فعالية في هذا الصدد حيث أدى إلى تقليل نسبة الإصابة مع زيادة المحصول. وعلى العكس من ذلك فإن المبيد الحيوي رايزو– إن كان الأقل تأثيرا حيث لم يؤدي استخدامه إلى تقليل نسبة الإصابة بالعفن الرمادي. كما أظهرت النتائج أن الرش بالمبيدات الحيوية كل 6 أيام أفضل من الرش كل 12 يوم في تقليل إصابة الثمار بالمرض. وقد أظهرت النتائج أيضا أن خليط البلايت ستوب مع البلانت جارد أعطى كفاءة أعلى من استخدام أي منهما منفردا في مكافحة المرض وزيادة المحصول.