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Control strawberry fruit decay caused by some mold fungi

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Abstract : Strawberry fruit (*Fragaria x ananassa* Duch.) have a very short postharvest life. This study was focused on strawberry fruit decay caused by some mold fungi during 2015 / 2016 season. The results of this study presented that, isolation of the causal agent of fungi that attacks strawberry fruits yielded three fungal genera i. e. *Alternaria solani*, *Botrytis cinerea* and *Rhizopus stolonifer*. *Botrytis cinerea* was higher fungal frequency occurred which record 85% followed by *Rhizopus stolonifer* 7%, while *Alternaria solani* was less fungal frequency occurred 5% in addition unknown 3%. It can be changes of all physical, bio-chemical properties and some mineral contents of infected fruits compared with healthy fruits (non-infected). Also the results indicated that, all alternative (Bio-) fungicides and chemical fungicides used as Bio-Arc, Bio-zeid, Plant-Guard**,** Switch and Ubarrin were found to be reduced the infection percent of all tested fungi that attacks strawberry fruits as well as decreased strawberry fruit decay compared with control (Un-treated). Increasing the reduction of infection with increasing the number of time spryer until the third time. Both Bio-Arc and Switch treatments were better than others. It can be reduced the infection percent of strawberry fruit decay from 26.4 to 9.6% equal 63.63% reduction at third time used. Bio-zeid was moderate affected which decreased strawberry fruit decay from 26.4 to 11.2 equal 57.57% reduction. Plant-Guard and Ubarrin were less affected which reduced the infection percent of strawberry fruit decay caused by these fungi from 26.4 to 14.6% infection equal 44.70% reduction respectively.
Key words: Strawberry, Fruit rot, Fungi, Quality characteristics, Control.

Introduction

Strawberry (*Fragaria* X *ananassa* Duchesne.) is an important world-wide grown and high-value crop, increasing in recent years. Also, strawberry is one of the most important vegetable crops in Egypt for local consumption and exportation. It is cultivated at least in five governorates. Strawberries are an excellent source of vitamin C, which contain an average 40–90 mg of vitamin C. This means that with a supply of 100g of strawberries, the daily needs of vitamin C will be covered. The major sugars in strawberry fruit are sucrose, glucose, and fructose, accounting for more than 99% of the total sugars in ripe fruit **(Agulheiro and Barreto, [1]).** The soluble solids and total sugars contents of strawberries tend to increase as the fruit matures **(Perez, et al., [2]).** Plant pathogens may infect fruits either prior to harvest under field conditions or after harvest during transit and storage. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries **(Droby, [3] and Zhu, [4]).** Fungi are the major microorganisms attacking strawberry plants and causing severe diseases at different developmental stages. Fruit rot disease caused by fungi is one of the major problems to strawberry cultivation and production reducing their quantity and quality and causing economic losses in the field, at harvest time, during marketing and exportation.

Alternaria spp., *Aspergillus* spp., *Botrytis cinerea, Fusarium* spp., *Penicillium* spp., *Phytophthora cactorum, Rhizoctonia solani, Rhizopus stolonifer,* and *Sclerotinia sclerotiorum* are the most fungal isolates causing strawberry fruit rots in Egypt **(Khafagi, [5]; Tadrous, [6] and Tarek, [7])**, in addition to *Pestalotia longisetula* **(Embaby, [8]).** Moreover, other fungi i. e. *Aspergillus, Botrytis, Colletotrichum, Geotrichum, Mucro, Penicillium, Pestaliopsis, Phytophthora and Rhizopus stolonifer* were isolated from different locations in all over the world and which infect this crop **(Fraire-Cordero, et al., [9] and Paivi, et al., [10]).** Biological control of plant pests and diseases relies on the use of one living organism to control another through antagonistic interactions. In 2010, only 14 microbial bio-control agents were included in the EU list of active substances against plant pathogens **(Nicot et al., [11] and Nicot and Bardin, [12]).** Biological control of plant pathogens by antagonistic microorganisms is a potential non-chemical means1 and is known to be a cheap and effective eco-friendly method for the management of crop diseases2. The use of biological control agents as an alternative to fungicides is increasing rapidly in the present day agriculture due to the deleterious effects of chemical pesticides (**Maurya, et. al., [13] and Sabry, et. al., [14]).**

This work was focused to identify the causal agent of fungi that attacks strawberry fruits, study the effect of these fungi on fruit quantity and quality (physical, chemical characteristics and mineral contents) and to investigate alternative control measures to reduce the application of fungicide against strawberry rotted fruits. The alternative control measures tested were biological control compared with some fungicides.

2-Material and Methods

2.1- Collected samples:

Healthy and diseased samples of strawberry fruits cv. Festival were collected from different farms at El-Dair village, Toukh, Governorate. Naturally rotted of strawberry fruit samples showing various types of rot symptoms were collected and photographed as showing in **Fig. (1).** Healthy and infected strawberry fruit samples were divided into five equal groups. Each 10 strawberry fruits were randomly selected. The fruit were transported to the laboratory according to **Hamouda, et. al., [15].**

Fig. (1): Healthy and naturally strawberry fruit decay symptoms caused by some mold fungi under field condition

2.2-Laboratory studies:

Percentage of strawberry rotted fruits (infection) were calculated as follow:-

Infection % was calculated as = Rotted fruits / Total of fruits x 100

2.3-Isolation and identification of the causal agent:

Diseased of strawberry fruit samples were surface disinfested using 70% Ethyl alcohol for 2 min, washed with sterile water and dried at room temp., then cut into small pieces. Sterilized pieces were sown on sterilized Potato dextrose agar medium (PDA) were transferred and purified on PDA medium. All fungal colonies were transferred and purified on PDA solid medium by using hyphal tip and/or single spore techniques. Purified fungi were kept on PDA slants at 5°C for further studies.

2.4- Identification:

All isolated fungi were identified in Plant Pathology Dept., National Research Center (NRC), El-Dokki, Egypt, based on cultural characteristics by using specific media **(Ronald, [16]).** Identification was carried out by light microscope and the literature according to **Gilman [17]; Barnett and Hunter [18] and Nelson et al., [19].** Percentages of fungal frequencies were calculated as follow:-

Total number of fungus/ Total number of isolated fungi x 100

2.5-Determination of fruit-quality parameters:

Effect of fungal infection on some physical, chemical characteristics as well as mineral contents of fruit were determined according to **Hamouda, et. al., [15].** Fruit weight (g), fruit firmness (g/cm^2) of fully ripe fruits measured with a Shatillon Penetrometer (NY, USA) Gauge- R. The fruit firmness was measured using a penetrometer (Fruit Pressure Tester 327, Effegì, Ravenna, Italy), with the data expressed in g **(Erica, [20]).** Total soluble solids (Brix°) samples were randomly chosen to measure the percentage of soluble solids content using the hand refract-meter. Total Sugar contents were determined color-metrically by the method described by **Ackerson [21]** and **Hamouda, et. al., [15].** As for mineral determination, fruit samples were taken and oven-dried at 70 C° until constant weight and ground to pass a 1 mm sieve then 0.1 g of the dry samples was taken and digested using a mixture of sulphuric acid (H_2SO_4 98 %) and hydrogen peroxide (H_2O_2 30 %) as described by **Thomas et al., [22].** Total nitrogen was determined using Kjeldahl method as described by **Page et al., [23].** Phosphorus content was determined applying colorimetric method (ammonium molybdate) using spectrophotometer **(AOAC, [24]).** Potassium was measured by flame photometer as described by **Page et al., [23].** Loss and reduction percent were calculated as follow:-

Loss = Healthy fruits – Infected fruits

Reduction % = Healthy fruits – Infected fruits / Healthy fruits x 100

2.6-Control the causal agent of strawberry fruit decay:

Five fungicides were used for controlling the causal agent of strawberry fruit decay under field condition; three of them were used as alternative fungicides (bio-fungicides) compared with two chemical fungicides **Table (1)** which prepared according to **Rathod, [25].** Biocides used were listed as Plant Guard (*T. harzianum*), Bio-Arc (*B. megaterium*), and Bio-Zied (*T. album*) which were obtained from Plant Pathology Dept., Fac. Of Agric., Ain Shams Univ. Three times for them were applied. Effects of these treatments on the percentage of strawberry rotted fruits (infection percent) were calculated as mentioned above.

Infection percent = Total of infected fruits / Total No. of collected fruits x 100

Table (1): General properties of some alternatives fungicides (Bio-control agents BCAs) and chemical fungicides used cited from Sesan [26] and Nicot, and Bardin [12].

Common or Trade name	Bio-Arc	Biozeid	Plant-Guard	Switch	Ubarrin
(a. I) Active ingredient	*Bacillus megaterium*	*Trichoderma album*	*Trichoderma harzianum*	Cyprodynil + Fludioxynil	Tolylfuanide
Conc.	$25x10^6$cf./g	$10 x10^6$cf./g	$30 x10^6$c/ml	62.5%	50%
Manufacturer	Organic for Biotechnology Co.	Organic for Biotechnology Co.	Bio Tech for Fertilizers and Biocides	Syngenta Crop Protection	Syngenta Crop Protection
Systemic action	No	No	No	No/ Slight	systemic (local)
Activity	Contact	Contact	Contact	Contact /slightly systemic	Contact /slightly systemic
Mode of Application	Foliar spray	Foliar spray	Foliar spray	Foliar spray	Foliar spray
Dose/100L	250g	250g	250ml	75g	250g
Crops	Several fruit & vegetable crops	Several fruit & vegetable crops	Strawberry	Several fruit & vegetable crops	Several fruit & vegetable crops

2.7-Efficacy of fungicide used for controlling strawberry fruit decay was calculated as follow:-

Efficacy percent (% E) = Percentage of infected fruits with Control (Un-treated) - Percentage of infected fruits with fungicide used / Percentage of infected fruits with Un-treated x 100

Results

1-Isolation and identification the causal agent of strawberry fruit decay:

Isolation of the causal agent of fungi that attacks strawberry fruits yielded three fungal genera **as shown in Table (2)** and **Fig. (2).** These are *Alternaria solani*, *Botrytis cinerea* and *Rhizopus stolonifer*. Percentage of fungal frequency resulted that, *Botrytis cinerea* was higher fungal frequency occurred which record 85% followed by *Rhizopus stolonifer* 7%, while *Alternaria solani* was less fungal frequency occurred and gave only 5% and unknown 3%.

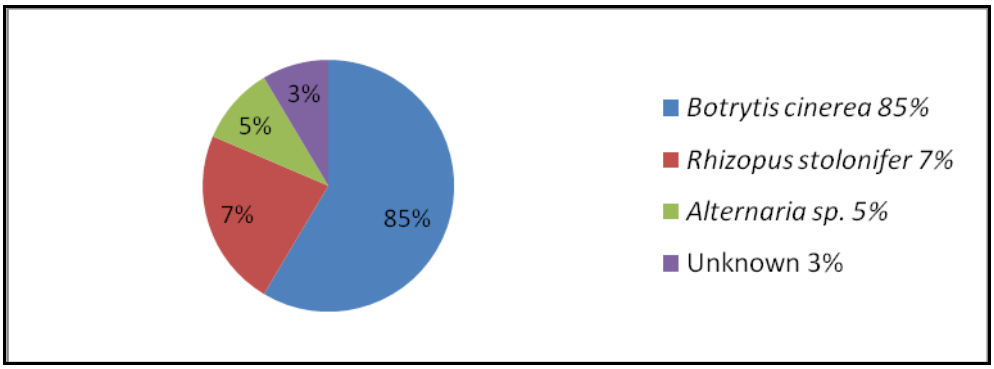

Fig. (2) Percentage of fungal frequency causing strawberry fruit rots

2-Effect of fungal infection on yield losses (fruit quantity and quality) of strawberry:

2.1-Physical characteristics:

Data in **Table (3)** indicate that, infected strawberry fruits (fungal infection) had significant lower diameter compared with healthy once. The average weight (g) of healthy fruit was 25.80 g, while the average

weight of infected fruit with *Botrytis cinerea* in ripe mature stage (mummified fruit) was 14.41g which was the highest marketable loosed 21.66g equal 83.95 of reduction followed by the average weight of infected fruit with *Rhizopus stolonifer* which record 8.85g and loosed 16.95g equal 65.70% reduction. The average weight of infected strawberry fruits with lowest marketable fruit weight were recorded with *Alternaria* sp. and *Botrytis cinerea* in maturing stage which record 14.41 and 14.37g and loosed 11.39 and 11.43g equal 44.15 and 44.30% reduction respectively. On the other hand, In the firmness analysis of these strawberry fruit, some of these treatments showed significant effects **(Table 3).** Data show that fruit firmness was the highest decreased from 338.70 to 327.70 (g/cm^2) in healthy and infected fruits by both *Alternaria* sp., and *Botrytis cinerea* in ripe mature stage (mummy symptoms) which loosed 11.0 g/cm^2 equal 3.25% of reduction respectively, *Botrytis cinerea* in maturing stage reduced from 338.70 to 330.70 g/cm^2 with 8.00 g/cm^2 losses and 2.42 % reduction. The lowest marketable fruit firmness was recorded with *Rhizopus stolonifer* which decreased from 25.80 to 8.85 g/cm^2 with 16.95 g/cm^2 losses in infected and non-infected (control) respectively with 65.70% % reduction.

Table (3): Effect of fungal infection on some physical characteristics

Parameters	Type of pathogen	H	I	L=H-I	% R
Average fruit weight (g)	*Alternaria* sp.	25.80	14.41	11.39	44.15
	Rhizopus stolonifer		8.85	16.95	65.70
	Botrytis cinerea		14.37	11.43	44.30
	Botrytis cinerea (mummified fruit)		4.14	21.66	83.95
Average fruit firmness (g/cm^2)	*Alternaria* sp.	338.70	327.70	11.00	3.25
	Rhizopus stolonifer		334.90	3.80	1.12
	Botrytis cinerea		330.70	8.00	2.42
	Botrytis cinerea (mummified fruit)		327.70	11.00	3.25

H = Healthy I = Infected L = Loss R = Reduction

3-Effect of fungal infection on some chemical characteristics in strawberry fruits:

Data in **Table (4)** presented that, infected strawberry fruits by some fungal infection (i. e. *Alternaria* sp., *Botrytis cinerea* and *Rhizopus stolonifer*) had lower TSS (9.79, 8.99, 9.79 and 10.03%) compared with healthy fruits (10.24%) it can be loosed 0.45, 1.25, 0.45 and 0.21g equal 4.40, 12.21, 4.40 and 2.05% reduction respectively. On the other hand data indicated that, all these fungi were found to be decreased the percentage of total sugar contents in comparing with healthy fruits. *Botrytis cinerea* (mummified fruit symptoms) was higher affecting which reduced the percentage of total sugar contents from 19.68% to 18.27% and loosed 1.41% equal 7.17 reduction percent followed by *Botrytis cinerea* which decreased the percentage of total sugar contents from 19.68% to 18.83% and loosed 0.85% equal 4.32% reduction. Also, decline in sugar contents from 19.68% in healthy fruits to 18.85 mg/100g in infected fruits with *Alternaria* sp. which loosed 0.83% with 4.22% reduction. *Rhizopus stolonifer* was higher affecting which reduced the percentage of total sugar contents from 19.68% to 18.96% and loosed 0.72% equal 4.32 reduction percent.

Table (4): Effect of fungal infection on some chemical characteristics in strawberry fruits

Parameters	Type of pathogen	H	I	L=H-I	% R
%T.S.S	*Alternaria* sp.	10.24	9.79	0.45	4.40
	Rhizopus stolonifer		10.03	0.21	2.05
	Botrytis cinerea		8.99	1.25	12.21
	Botrytis cinerea (mummified fruit)		9.79	0.45	4.40
% Total sugars	*Alternaria* sp.	19.68	18.85	0.83	4.22
	Rhizopus stolonifer		18.96	0.72	3.66
	Botrytis cinerea		18.83	0.85	4.32
	Botrytis cinerea (mummified fruit)		18.27	1.41	7.17

H = Healthy I = Infected L = Loss R = Reduction

Effect of fungal infection on some mineral content in strawberry rotted fruits

Mineral contents:

Data in **Table (5)** show that, all infected strawberry fruits by some fungal infection (i. e. *Alternaria* sp., *Botrytis cinerea* and *Rhizopus stolonifer*) gave lower values in total Nitrogen (N.), Phosphorus (P.) and Potassium (K. mg) comparing with healthy fruits. On the other hand, no significant differences were detected between healthy and infected fruits in their contents of Phosphorus (P.) and Potassium (K. mg) compared with healthy fruits. Nitrogen (N.) content was found to be decreased from 2.02 with healthy fruits to 1.73, 1.87, 1.79 and 1.69 (mg) and loosed 0.29, 0.15, 0.23 and 0.33 (mg) which record 14.36, 7.43, 11.39 and 16.34% reduction when strawberry fruits were infected by Alternaria sp., Rhizopus stolonifer and Botrytis cinerea (mature and ripping mummified fruit) respectively. Also, Phosphorus (P.) content was found to be decreased from 0.419 (mg) with healthy fruits to 0.397, 0.400, 0.386 and 0.390 (mg) and loosed 0.022, 0.019, 0.033 and 0.029 (mg) which gives 5.25, 4.54, 7.88 and 6.92% reduction when infected with the same tested fungi respectively. Finally Phosphorus (P.) content was found to be decreased from 2.21 (mg) with healthy fruits to 2.10, 2.01, 2.00 and 2.17 (mg) and loosed 0.11, 0.20, 0.21 and 0.04 (mg) which record 4.98, 9.05, 9.50 and 1.81% reduction when strawberry fruits were infected with the same tested fungi respectively.

Table (5): Effect of fungal infection on some mineral content in strawberry fruits

Parameters	Type of pathogen	H	I	L=H-I	% R
Nitrogen (N. mg)	*Alternaria* sp.	2.02	1.73	0.29	14.36
	Rhizopus stolonifer		1.87	0.15	7.43
	Botrytis cinerea		1.79	0.23	11.39
	Botrytis cinerea (mummified fruit)		1.69	0.33	16.34
Phosphorus (P. mg)	*Alternaria* sp.	0.419	0.397	0.022	5.25
	Rhizopus stolonifer		0.400	0.019	4.54
	Botrytis cinerea		0.386	0.033	7.88
	Botrytis cinerea (mummy fruit)		0.390	0.029	6.92
Potassium (K. mg)	*Alternaria* sp.	2.21	2.10	0.11	4.98
	Rhizopus stolonifer		2.01	0.20	9.05
	Botrytis cinerea		2.00	0.21	9.50
	Botrytis cinerea (mummified fruit)		2.17	0.04	1.81

H = Healthy I = Infected L = Loss R = Reduction

4-Control strawberry fruit decay

Data in **Table (6)** and **Fig. (4)** show that, all bio-fungicides (Bio-Arc, Bio-zeid & Plant-Guard) and chemical fungicides (Switch & Ubarrin) used were found to be reduced the infection percent of fungi that attacks strawberry fruits as well as decreased strawberry fruit decay and yield losses compared with control (Un-treated plants). Both Bio-Arc and Switch fungicides were better than others. It can be reduced the infection percent of strawberry fruit decay caused by the same fungi from 26.4 to 9.6% at third time sprayer used. Bio-zeid was moderate affected which decreased strawberry fruit decay from 26.4 to 11.2%. Both Plant-Guard and Ubarrin were found less affected which reduced the infection percent of strawberry fruit decay caused by these fungi from 26.4 to 14.6% infection respectively.

On the other hand data show that, continue decreased the percentage of strawberry rotted fruits with increased the time of sprayer. Bio-zeid was continue decreased the percentage of strawberry rotted fruits from 21.6% in the first time to 19% at the second time and 11.2% at the third time used respectively. Continue decreased the percentage of strawberry rotted fruits from 20.6% in the first time to 14.6% at the second time and 9.6% at the third time when treated strawberry rotted fruits by Switch fungicide. Plant-Guard was found to be decreased the percentage of strawberry rotted fruits from 20.1% in the first time to 20% and 14.6% at the second and third time respectively, followed by Ubarrin which decreased the percentage of strawberry rotted fruits from 19% in the first time to 16% at the second time and 14.6% at the third time while, Bio-Arc was continue decreased the percentage of strawberry rotted fruits from 15% in the first time to 13% and 9.6% at the second and third time respectively.

Table (6): Control strawberry fruits decay after three time sprayers

Commercial name	Percentage of rotted fruits with the time of sprayer		
	First sprayer	Second sprayer	Third sprayer
Bio-Arc	15	13	9.6
Bio-zeid	21.6	19	11.2
Plant-Guard	20.1	20	14.6
Switch	20.6	14.6	9.6
Ubarrin	19	16	14.6

Fig. (4): Effect of alternative and fungicides used in controlling strawberry fruit decay

5-Efficacy of fungicide used for controlling strawberry fruit decay

Table (7) indicated that, in the third sprayer both Bio-Arc and Switch were the most affected than others and the efficacy of them record 63.63%. Bio-zeid was moderate efficacy which gave 57.57%. Plant-Guard and Ubarrin were less efficacy which were recorded 44.70% efficacy.

Table (7): Efficacy percent of fungicide used

Commercial name	Efficacy percent with the time of sprayer		
	First time	Second time	Third time
Bio-Arc	34.21	43.23	63.63
Bio-zeid	5.26	17.03	57.57
Plant-Guard	11.84	12.66	44.70
Switch	9.65	36.25	63.63
Ubarrin	16.67	30.13	44.70

Discussion

Strawberry (*Fragaria* × *ananassa*), an important world-wide grown and high-value crop, is exposed to numerous infectious diseases. This study was focused on strawberry fruit decay caused by some mold fungi during 2015 / 2016 season. Isolation of the causal agent of the fungi that attacks strawberry fruits yielded three fungal genera i. e. *Alternaria solani, Botrytis cinerea* Pers. and *Rhizopus stolonifer*. Similar results were obtained by **Khafagi, [5]; Tadrous, [6] and Tarek, [7]** they reported that, *Alternaria* spp., *Aspergillus* spp., *Botrytis cinerea, Rhizopus stolonifer, Rhizoctonia solani, Phytophthora cactorum, Fusarium* spp., *Penicillium* spp. and *Sclerotinia sclerotiorum* are the most fungal isolates causing strawberry fruit rots in Egypt. Also, **Sesan [26]** reported that, the pathogens causing strawberry diseases are 35 fungal species, belonging to Ascomycetes (69%), Basidiomycetes (11%), Oomycetes (11%) and Zygomycetes (9%)**.** These isolates can be changes of all physical and bio-chemical properties in addition to some mineral contents of infected fruits compared with healthy (non-infected) fruits. **Khafagi, [5]; Tadrous, [6]; Tarek, [7]; Embaby, [8], Agulheiro and Barreto, [1]; Paivi, et al., [10] and Perez, et al., [2]** reported that, fruit rot disease caused by fungi is one of the major problems to strawberry cultivation and production reducing their quantity and quality and causing economic losses in the field, at harvest time, during marketing and exportation.

All alternative (Bio-) fungicides used as Bio-Arc, Bio-zeid, Plant-Guard and chemical fungicides i. e. Switch and Ubarrin were found to reduce the infection percent of all fungi that attacks strawberry fruits as well as decreased strawberry fruit decay compared with un-treated (control). Increasing the reduction of infection with increasing the time of fungicides used until the third time. Both Bio-Arc and Switch were better than other fungicides used. It can be reduced the infection percent of strawberry fruit decay caused by the same fungi from 26.4 to 9.6% equal 63.63% reduction at third time used. Biozeid was moderate affected while; Plant-Guard and Ubarrin were less affected. **Tronsmo and Dennis, [27]**; **Gullino et al., [28]** and **Shovan, [29]** stated that, the application of *Trichoderma* for the control of grey mould of strawberries has been shown to result in better control than the conventional fungicide or to be relatively less effective than a mixed fungicide spray**. Also, Iain and Peter, [30]** and **Shovan, [29]** reported that, the relatively few successes, however, have been reported in the field for mould control on strawberry using bacterial antagonists. The integration of bacterial bio-control agents with reduced levels of currently used fungicides has considerable potential, not least because the prokaryotic nature of bacteria may mean that they are not affected by the fungicides**.** Switch and Signum could successfully control *B. cinerea* in synthetic media. All of the fungicides were effective against other test pathogens as well. **Maas, J. L., [31], Agrios, [32] and Leroux, [33]** reported that, Bio-control using antagonistic microorganisms is one option, and previous success has been reported in reducing grey mould disease levels in strawberries using this approach. A number of microbial products available commercially for control of Botrytis diseases indicate that bio-control can succeed. Especially Trichoderma and Ulocladium have given the greatest success in Botrytis control. **Nicot, and Bardin, [12]** stated that, six of which are based on species of Trichoderma, are commercially available for the control of soil-borne pathogens of greenhouse crops in one country or more of the Mediterranean region. Another striking improvement is that many products are now available for bio-control of key airborne diseases (such as Botrytis and the powdery mildews) of a variety of greenhouse crops. Particularly interesting among them, are several micro-organisms (such as *Bacillus* sp.) with a wide range of action, covering a variety of pathogens on many different crops**.**

Conclusion and future study

Biological control agents and bio-products, represent environment friendly means for strawberry integrated protection against diseases. Technological tools lead to minimizing chemical input in integrated control of strawberry diseases. Based on the present study it may be concluded that *Bacillus megaterium* and *Trichoderma* species i. e. *Trichoderma album* and *Trichoderma harzianum* could be an effective bio-control agent against strawberry fruit decay caused by some mold fungi. Integrated control using *Bacillus megaterium* and *Trichoderma* spp. may provide alternatives to control plant diseases in the future.

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