Egyptian Journal of Phytopathology, Vol. 50, No. 2, pp 79-87 (2022).
DOI 10.21608/ejp.2022.161990.1069

ORIGINAL PAPER

Potential of Bioagents Application Pre-Harvest Strawberry on Fruit Rots and Quality under Storage Conditions

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Received: 10 September 2022 / **Accepted**: 22 November 2022 / **Published online**: 01 December 2022.

ABSTRACT

In the present study, strawberry (*Fragaria× ananassa* Duch.) plants c.v. Festival, were grown in a local farm (Meet Kenana, Toukh county, Qaliobia governorate, Egypt) during the two-winter successive seasons of 2020 and 2021. The effect of pre-harvest spraying with different biological control agents on postharvest decay and quality of strawberry fruits under storage conditions was investigated. Postharvest decay caused by *Botrytis cinerea* (grey mold) and *Rhizopus stolonifer* (soft rot) is the most important factor affecting strawberry fruits in the field, during harvest transportation and storage. The plants were pre-harvest sprayed with some biological control agents; *Bacillus subtilis*, *Bacillus megatherium*, *Trichoderma album* and *Trichoderma asperellum* (T34), *Trichoderma viride*, compared to Switch (synthetic fungicide) and tap water as control treatment. All biological control agents showed the highest linear growth inhibition of fruit rots pathogens under laboratory conditions. The tested treatments gave the best effects as they decreased the disease incidence (D.I.) of fruit rots in the field. The fruits were harvested at a commercial maturity 3/4 color stage and stored at 0 °C and 95-98 % RH for 20 days. The obtained results indicated that pre-harvest spraying of strawberry fruits with *Trichoderma asperellum* (T34) was the most effective treatment for delaying fruit deterioration throughout reducing color changing, decay maintaining good appearance, firmness, acidity, TSS% and weight loss.

Keywords: Strawberry, *Fragaria* × *ananassa*, Fruit rots, biological control, *Trichoderma* spp., *Bacillus* spp.

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INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is one of the most important nontraditionally vegetable cash crops in Egypt and the demand has increased for local consumption and exportation. Fungal diseases of strawberry fruits in pre and postharvest caused by *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternata*, are responsible for severe economic losses. Strawberry fruit rot diseases develop abundantly causing fruit decay accompanied by profuse sporulation of the pathogens which cause significant losses. The post-harvest quality losses of strawberry are mostly due to high metabolic activity, mechanical injury, physiological disorders, and fungal decay (Hernández-Muñoz *et al*., 2008).

Grey mold caused by *Botrytis cinerea* is one of the most severe postharvest diseases in strawberry production causing losses for both plant and fruits (Romanazzi *et al*., 2013 and Rhouma *et al*., 2022) and Kowalska, 2011). The application of postharvest fungicides is not allowed, due to several normative restrictions (Felizial and Romanazzi, 2016). Controlling grey mold is usually carried out by the application of chemical fungicides. Continuous application of chemical fungicides has been increasing numbers of fungicides-tolerant grey mold strains and pollutes the air and causes severe effects on the environment and human health (Vinale *et al*., 2008; Kowalska, 2011; Woo *et al*., 2014 and Rhouma *et al*., 2022). Biological control agents are one of the most effective methods that effectively control postharvest diseases without any bad effect on the environment or the consumer, antagonistic to pathogens that cause postharvest fruit spoilage (Rahul *et al*., 2015 and Feliziani and Romanazzi, 2016). *Trichoderma* spp. are among the most used microbial biological control agents in agriculture. They are used as bio-pesticides, bio-fertilizers, growth enhancers and stimulants of natural resistance and considered safety for plants and animal (Sawant, 2014; Woo *et al*., 2014). *Bacillus* spp. are considered effective biocontrol agents against fungal pathogens due to their capability enzymes, and volatile and they also are safety to human health and environment friendly (Fravel, 1988; Kim *et al*., 2003 and Wang *et al*., 2010). *Trichoderma* spp. and *B. subtilis* improve shelf life of roots and vegetables by several researchers such as Jiang *et al*. (2001) on Litchi, Wang *et al*. (2010) on melon, Kawalska (2011) on strawberry.

The aim of this work is studying the effect of pre-harvest spraying strawberry with some biological control agents on post-harvest quality attributes of strawberry fruits during storage at 0 °C and 95-98 % RH for 15 days.

MATERIALS AND METHODS

Source of fungus pathogen:

Pathogenic isolates of *Botrytis cinerea* and *Rhizopus stolonifer* previously isolated from diseased strawberry fruits with gray mold and soft rot were used for all control experiments.

Effect of pre-harvest spraying with some biological control agents on postharvest decay of strawberry fruits:

Under field conditions for two successive seasons in 2020 and 2021, the effect of pre-spraying strawberry plants at pre-harvest stage with the tested biocontrol agents to controlling fruit rot diseases during harvesting and storage was studied. Five biocontrol agents, *Bacillus subtilis*, *Bacillus megaterium*, *Trichoderma album*, *Trichoderma asperellum* (T34) and *Trichoderma viride*, were used and compared by Switch (synthetic fungicide) and tap water as control treatment were tested for their abilities to control strawberry fruit rots during storage. This experiment was carried out at (Meet Kenana, Toukh county Qaloubiya governorate, Egypt). Each plot consisted of three rows (one row is 5×0.6 m) was used as an experimental unit. Strawberry plants were sprayed three times, the first at bloom stage, the second was done ten days fter the first spray while the third one was done ten days after the second spray. Plots, cultivated with untreated strawberry plants were used as control. After harvest, the fruits were inoculated separately with the two tested pathogens each alone. Three replicates were used for each treatment (250 g fruits for each) and packed in polyethylene bags. Natural and artificially inoculated strawberry fruits were incubated at 0°C and 90 - 95% RH for 20 days, Disease incidence was recorded according to Spalding and Reeder (1974) as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected fruits}}{\text{Total number of fruits of the treatment}} \times 100$$

Sources and preparation of bioagents:

Five bio-agents, namely, Bio-zeid (Bioside) *Trichoderma album* obtained from Organic Biotechnology Co., Egypt, Bio-Arc (*Bacillus megatherium*) obtained from Organic Biotechnology Co., Egypt, *Bacillus subtilis* obtained from Kafr-Elzayat Co., Egypt, Biocontrol T34 (*Trichoderma asperellum*) obtained from Shoura Chemicals Co., Egypt and *Trichoderma viride*, isolate supplied from Post-harvest Diseases Department, ARC, Giza, Egypt were used in the present investigation. *Trichoderma viride* isolate was maintained in refrigerator at 4°C on potato dextrose agar (PDA) medium until using. *T. viride* isolate was grown in liquid gliotoxin fermentation medium (GFM) (Brain and Hemming, 1945) under complete darkness conditions for nine days at 28°C to stimulate toxin production (Abd El-Moity and Shatla, 1981). *T. viride* culture was formulated as powder form to use as stable formulation of bio-fungicide by mixing blended culture media with talc powder at the rate of 1/1 (v/w) adjusted to contain 30×10^6 CFU/g using the method developed by Abd El-Moity (1985). The experiment was conducted in a local farm of loamy soil in Qaliobia governorate, in the two-winter successive seasons 2020/2021. Fresh transplants of strawberry (*Fragaria x ananassa*) cv. Festival, obtained from Pico Company, were transplanted on the 1st of October in both seasons, 2020 and 2021. The transplants were grown in plots. The area of the plot was 9 m^2 and consisted of three rows. Each raw was 5 m long and 60 cm width and plants were spaced 25 cm on both of drip irrigation hose. A complete randomized block design with three replicates was adopted, where treatments were distributed randomly among the plots. The beds were covered with clear plastic mulch 60 micron in thickness, 45 days after transplanting. Foliar applications with bio-fungicides were done as follows:

1- Bio-Zeid (*Trichoderma album*, 10×10^6 CFU/g) at rate 250 g/100L.

2- (*Bacillus subtilis*, 30×10^6 CFU/g) at rate 400 g/100 L.

3- Bio-Arc (*Bacillus megaterium*, 25×10^6 CFU/g) at rate 250 g/100L.

4- Biocontrol T34 (*Trichoderma asperellum*, 1×10^9 CFU/g) at rate 150 g/100L.

5- Isolate of *Trichoderma viride* 30×10^6 CFU/mL) at rate 100 g/100L.

6- Switch (50% Iprodione, synthetic fungicide) at rate 90 g/100 L.

7- Control (spraying with water)

Plants of each treatment were sprayed Two times during flowering time and 15 days before harvesting. Strawberry plants received the normal agricultural practices during the two growing seasons. Strawberry fruits were harvested at a commercial maturity stage (3/4 of

full fruit color). Uniform fruits in size without physical defects or fungal infection from each treatment were selected and directly packed in the field in plastic punnets approximately 250 g of strawberries/ punnets and placed in carton box. Each eight punnets were put in a carton box (2 kg) and then transferred to the laboratory of the vegetable handling department, within 2 h of harvest. The samples of each treatment were arranged in a complete randomized design with 3 replicates each contained 3 punnets. These punnets were stored at 0°C and 95-98 RH for 20 days and inspected every five days for the following characteristics:

1- Weight loss % =

$$\frac{\text{Initial fruit weight-fruit weight at sampling date}}{\text{Initial fruit weight}} \times 100$$

2- Skin color was measured using a Minolta Chroma Meter, Model CR-200, calibration was done by a white plate before use. Color changes were quantified by calculating lightness and hue angle in tested samples during storage. (L.) refers to the lightness, ranging from 0 (black) to 100 (white), and hue angle is defined as a color wheel, with red-purple at an angle 0°C, yellow at 90°C, plush-green at 180°C, and blue at 270°C (McGuire, 1992).

3- Firmness was determined by TA-100 firmness analyzer instrument using a penetrating cylinder of 1 mm diameter, to a constant distance (measuring the compression force (at 3 and 5 mm) inside the pulp of the fruits, and at a constant speed 1 mm/s-1 at the peals of resistance was recorded per (g/cm^2).

4- Total soluble solid (TSS)% was determined directly from each sample by using a hand refractometer according to the method mentioned by Anon (2000).

5- Titration acidity percentage in strawberry was measured as a percentage of citric acid according to Anon (2000).

6- Ascorbic acid content (Vitamin C.) was determined using 2,6-Dichlorophenol-indophenol as described by Anon (2000).

Effect of *T. viride* and commercial bio-fungicides on growth of *Botrytis cinerea* and *Rhizopus stolonifer in vitro*

Antagonistic behavior of *T. viride* in bio-fungicide formulation and four commercial biological formulations *bacillus subtills*, 2.0 g/l) *Bacillus megaterium* 2.5g/l, *Trichoderma album* 2.5 g/l and *Trichoderma asperellum* (T34) 1.0 g/l as bio-fungicides were obtained from Biotech Company, El-Sadat City, Egypt) were evaluated against *Botrytis cinerea* and *Rhizopus stolonifer*. in-vitro by dual culture techniques (Alwathnani and Kahkashan, 2012).

Different concentrations of evaluated formulations were used to studying the antagonistic effect against *Botrytis cinerea* and *Rhizopus stolonifer* obtained from the periphery of seven days old colonies which were isolated and purified during this work. The mentioned biological formulations in case of aqueous extracts were used to study their efficiency on reducing radial growth of *Botrytis cinerea* and *Rhizopus stolonifer* in-vitro in comparison with control treatment. Dilution of any biological formulations was separately incorporated into PDA medium before solidification, and then powered in sterilized Petri-dishes. Three dishes (replicates) were used for each concentration, then inoculated at the center with equal mycelia discs (5.0 mm in diameter) taken from the periphery of seven days old *Botrytis cinerea* and *Rhizopus stolonifer* cultures each alone then incubated at 20±2°C. Fungal radial growth was measured when the fungal growth covers all medium surfaces in the control plate, three independent replicates were used. Percentage of reduction in mycelial growth of the tested fungus was calculated as follows:

The mycelial radial growth (mm) of the tested pathogen in treated and control plates were recorded after seven days of incubation, reduction (%) of the tested pathogens was calculated according to Hmouni *et al*. (1996) by using the following formula:

$$\text{Inhibition (\%)} = \frac{\text{linear growth of control} - \text{linear growth of treatment}}{\text{linear growth of control}} \times 100$$

Statistical analysis:

The obtained data were subjected to analysis of variance. The mean values were compared using LSD method at 5% level. The data were tabulated and statistically factorial analysis according to Snedecor and Cochran (1989), quality control Data of the field experiment and cold storage experiment were statistically analyzed by using MSTAT statistical software and the treatments means were compared by using LSD at 0.05 level of probability according to Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

***In vitro* evaluation of antagonistic activity of certain bioagents:**

the antagonistic activity of formulated *Trichoderma viride* isolate comparing with other commercial bioagents, *Bacillus subtilis*, *Bacillus megaterium*, *Trichoderma album* and *Trichoderma asperellum* (T34) showed significant effect against the growth of *Botrytis cinerea* and *Rhizopus stolonifer* isolates as shown in Table (1). All tested bioagents had more or less similar antagonistic activity; except for *B. subtilis* isolate which showed higher antagonistic effect than the others against each of *B. cinerea* and *R. stolonifer* in the same concern, followed by *Bacillus megaterium* being 83.88 and 70.27% inhibition, respectively.

Table (1): Linear growth (mm) of *Botrytis cinerea* and *Rhizopus stolonifer* on PDA medium seeded with certain bioagents after incubation at 20±2°C for 7 days.

Treatment	Linear growth (mm)			
	Botrytis cinerea	Inhibition%	*Rhizopus stolonifer*	Inhibition%
Bacillus megaterium	14.50	83.88	26.75	70.27
Trichoderma album	19.25	78.61	32.5	63.88
Bacillus subtilis	13.00	85.55	21.0	76.66
Trichoderma asperellum	15.25	83.05	29.5	67.22
Trichoderma viride	18.00	80.00	27.75	69.16
Switch	0.00	100.00	0.00	100.00
control	90.00	-	90.00	-
LSD at 5%	1.51		1.82	

Development of grey mold and soft rot diseases in strawberry fruits seems to be affected by microbial content. Spraying certain bioagents on strawberry plants 4 weeks before harvesting decreased infection percentage with *B. cinerea* and *Rhizopus stolonifer* during cold storage at 0-1°C for 20 days as shown in Tables (2 and 3). During season 2020, infection of control treatment of naturally infected strawberry fruits in control treatment with *B. cinerea* reached 18.55%. Infection of strawberry fruits collected from sprayed strawberry plants with the tested bioagents was less than 7% during the cold storage at 0-1°C for 20 days. This means that the tested bioagents reduced infection percentage of strawberry fruits with *Rhizopus stolonifer* with 100% in some treatments, which is considered so high effectiveness. *Trichoderma album* was significantly the least suppressive bioagent to control *B. cinerea* and *Rhizopus stolonifer* on naturally infected strawberry fruits during seasons 2020 and 2021. On the other hand, *Bacillus subtilis* isolate showed the highest suppressive effect on infection percentage of strawberry fruits during cold storage with *Botrytis cinerea* and *Rhizopus stolonifer* where infection of sprayed strawberry fruits with *Bacillus subtilis* during cold storage was 0.0% and 0.0% comparing with grey mold 12.5% and 6.25% infection of artificially inoculated fruits in seasons 2020 and 2021, respectively. *Bacillus subtilis* isolates showed more effectiveness to inhibit infection with *Rhizopus stolonifer* than *Trichoderma* isolates. *Trichoderma viride* isolates showed more effectiveness to inhibit infection with *B. cinerea* than all isolates. Effectiveness of *Trichoderma viride* was close to that obtained by *Bacillus subtilis*. Similar trends were obtained on artificially inoculated strawberry fruits with *B. cinerea* and *Rhizopus stolonifer* before cold storage. Infection of sprayed strawberry fruits with gray mold and treated with *Trichoderma viride* during cold storage was 6.25% and 6.25% comparing with 100% and 90% infection of artificially inoculated strawberry fruits in seasons 2020 and 2021, respectively and 3.13% and 4.69% comparing with 42.25% and 44.25% infection of artificially inoculated strawberry fruits with *Rhizopus stolonifer* in seasons 2020 and 2021, respectively. All tested bioagents highly reduced infection percentage of strawberry fruits with *B. cinerea* and *Rhizopus stolonifer* during cold storage at 0-1ºC for 20 days. *Trichoderma viride* and *Bacillus subtilis*. were significantly the most suppressive bioagent against *B. cinerea* and *Rhizopus stolonifer* on naturally infected strawberry during seasons 2020 and 2021. *Trichoderma album* isolate was less efficient than both *Trichoderma viride* to control *B. cinerea* and *Rhizopus stolonifer* on naturally infected strawberry fruits in both seasons. Concerning artificially inoculated strawberry fruits with *B. cinerea* and *Rhizopus stolonifer*, all tested bioagents strongly inhibited disease development during cold storage with no significant differences among the tested bioagents in seasons 2020 and 2021. *Bacillus subtilis* was more efficient against *Rhizopus stolonifer* on artificially inoculated strawberry

than other tested bioagents. While *Trichoderma viride* was more efficient against *B. cinerea* on artificially inoculated strawberry than other tested bioagents. It could be concluded that all tested bioagents on strawberry plants markedly prevented strawberry fruits infection with *B. cinerea* and *Rhizopus stolonifer* during cold storage at 0-1°C for 20 days. Biological control is considered one of the promising treatments to control postharvest diseases of fruits. *Bacillus subtilis*, *Bacillus megaterium*, *Trichoderma album*, *Trichoderma asperellum* (T34) *Trichoderma viride*, were the most effective treatments *in vitro*. All tested isolates showed strong effect to control *B. cinerea* and *Rhizopus stolonifer* either *in vitro* or *in vivo* evaluation. So, using any of the tested isolates will help extremely in controlling postharvest decay of strawberry fruits. Such results are in compliance with Kowalska (2011) and Mahdy *et al*. (2014) for *Trichoderma asperellum* T34 on strawberry, and Wang *et al*. (2010) for *Bacillus subtilis* on melon

Table (2): Effect of spraying certain bioagents on strawberry plants 4 weeks before harvest on infection (%) with grey mold and soft rot diseases during cold storage at 0-1°C under 90-95% relative humidity for 20 days, season 2020.

Treatment	Infection (%)							
	B.C	Effc. %	N.I	Effc. %	R.S	Effc. %	N.I	Effc. %
Bacillus megaterium	12.5	87.50	6.25	66.21	6.25	85.20	0.00	100
Trichoderma album	18.75	81.25	6.25	66.21	14.06	66.72	2.67	76.43
Bacillus subtilis	12.5	87.50	12.5	32.43	1.56	96.31	0.00	100.00
Trichoderma asperellum	6.25	93.75	6.25	66.21	3.13	92.59	0.00	100.00
Trichoderma viride	6.25	93.75	0.00	100.00	3.13	92.13	2.08	81.64
Switch	6.25	93.75	0.00	100.00	0.00	100	0.00	100.00
control	100.00	-	18.50	-	42.25	-	11.33	-
LSD at 5%	9.80		7.39		5.03		1.46	

B.C = *Botrytis cinerea*; Effc. % = Efficiency %; R.S = *Rhizopus stolonifer*; N.I = natural infection

Table (3): Effect of spraying certain bioagents on strawberry plants 4 weeks before harvest on infection (%) with grey mold rot and soft rot disease during cold storage at 0-1°C under 90-95% relative humidity for 20 days season 2021.

Treatment	Infection (%)							
	B.C	Effc. %	N.I	Effc. %	R.S	Effc. %	N.I	Effc. %
Bacillus megaterium	12.5	86.11	6.25	53.11	6.25	85.87	2.08	82.82
Trichoderma album	12.5	86.11	6.25	53.11	9.38	78.80	4.17	65.56
Bacillus subtilis	12.5	86.11	6.25	53.11	3.13	92.92	0.00	100.00
Trichoderma asperellum	18.75	79.16	0.00	100.00	4.69	89.40	0.00	100.00
Trichoderma viride	6.25	93.05	0.00	100.00	4.69	89.40	1.33	89.02
Switch	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
control	90.00		13.33		44.25		12.11	
LSD at 5%	7.41		8.69		7.08		3.00	

B.C = *Botrytis cinerea*; Effc. % = Efficiency %; R.S = *Rhizopus stolonifer*; N.I = natural infection

Weight loss:

Results in Table (4) show that a gradual increase in weight loss % was noticed as the storage period was increased. This increase is mostly associated with respiration and moisture evaporation (Hernández-Muñoz *et al*., 2008). Concerning treatments, no significant differences were recorded among all tested treatments. All treatments and storage period were significant and indicated that strawberry fruits treated with *Bacillus subtilis* recorded the lowest weight loss % after 5 days and at the end of storage at 0°C and 95-98 % RH in both seasons. Such results are in line with the work of Wang *et al*. (2010) who demonstrated that *B. subtilis* effectively suppressed respiration rate in melon fruits during storage at room temperature for 10 days.

Firmness:

Data in Table (5) reveal that, softening of strawberry fruits was developed gradually during the storage period. Therefore, a progressive decline in fruit firmness was noticed because of ripening, which mainly occurs as a result of degradation of middle lamella of the cell wall of cortical parenchyma cells, that resulting in a dramatic increase in pectin solubilization, and small decrease in the content of hemicelluloses (Koh and Melton, 2002; Hernández-Muñoz *et al*., 2008). Samples treated

with biological control agents efficiently maintained fruit firmness, as compared with samples treated with chemical fungicide and control treatments. Also, pre-harvest treatments with *Trichoderma asperellum* T34 and *Bacillus subtilis* effectively reduced softening of fruits in both seasons. This result agrees with the findings of Wang *et al*. (2010) for *Bacillus subtilis* on melon. While all treatments and storage time were significant and showed that *Trichoderma asperellum* T34 was the most effective treatment in maintaining the firmness of strawberry fruits during storage.

Table (4): Efficiency of pre-harvest spraying with bio-agents on weight loss % of strawberry fruits during refrigerated storage.

Treatments (A)	Season 2020					Season 2021				
	Storage period in days (S)					Storage period in days (S)				
	0	5	10	15	Mean	0	5	10	15	Mean
Bacillus megaterium	-	1.24	2.01	2.45	1.90	-	1.20	1.94	2.42	1.85
Trichoderma album	-	1.19	2.14	1.31	1.88	-	1.13	2.09	2.29	1.81
Bacillus subtilis	-	0.55	2.06	2.36	1.66	-	0.78	2.03	2.27	1.69
Trichoderma asperellum	-	1.20	1.78	2.27	1.75	-	1.19	1.76	2.24	1.73
Trichoderma viride	-	1.25	1.95	2.34	1.85	-	1.20	1.89	2.31	1.80
Switch	-	1.29	2.60	2.66	2.18	-	1.25	2.48	2.65	2.13
Control (water)	-	1.51	2.45	2.90	2.29	-	1.46	2.43	2.54	2.14
Mean	-	1.18	2.14	2.47	-	-	1.17	2.09	2.39	-
L.S.D at 5%	A=n.s.; S=0.51; A*S=1.35					A=n.s.; S=0.43; A*S=1.13				

Table (5): Efficiency of pre-harvest spraying with bio-agents (biological control agents) on firmness (g/cm2) of strawberry fruits during refrigerated storage.

Treatments (A)	Season 2020					Season 2021				
	Storage period in days (S)					Storage period in days (S)				
	0	5	10	15	Mean	0	5	10	15	Mean
Bacillus megaterium	8.00	7.33	6.66	6.01	7.00	8.00	7.00	6.33	6.00	6.83
Trichoderma album	8.33	8.00	7.00	6.00	7.33	8.33	7.33	7.00	6.33	7.25
Bacillus subtilis	9.00	8.00	7.65	6.02	7.66	9.00	8.00	7.33	6.51	7.71
Trichoderma asperellum	9.66	0.63	8.00	7.00	8.42	9.33	8.00	7.66	6.65	7.93
Trichoderma viride	8.66	8.03	7.00	6.00	7.42	8.34	7.50	7.00	6.51	7.34
Switch	8.00	7.33	6.33	5.36	6.72	8.00	7.00	6.17	5.33	6.63
Control (water)	8.00	7.33	5.66	5.00	6.50	8.00	7.00	6.00	5.82	6.71
Mean	8.53	7.85	6.90	5.90		8.42	7.40	6.77	6.17	
L.S.D at 5%	A=0.46; S=0.35; A*S=0.93					A=0.4; S=0.32; A*S=0.81				

Total soluble solids (TSS):

The efficacy of biological control agents as fungicides alternatives is summarized in Table (6). TSS % was significantly affected by the storage period. Thus, a considerable decrement was occurred in TSS % in strawberry fruits as the storage period extended as a result of respiration. Concerning treatments, it is obvious that fruits obtained from plants sprayed with *Trichoderma asperellum* T34 significantly maintained the highest value of TSS % as compared with other treatments. These results were true in both seasons. all treatments and storage period were significantly in both seasons. Data in (Table,7) indicate that titratable acidity (TA %) was not affected by the storage period or the used treatments and the interaction also, was not significant in both seasons of study.

Titratable acidity (TA):

As shown in Table (7) TA declined as the storage period extended. This decline TA may be due to the consumption of organic acids during respiration process. In respect to treatments, on statistical differences were detected among all tested treatments and the control treatment. The interaction between treatments and storage period was significant and showed that pre-harvest foliar application of *Trichoderma* spp. at the three drenches kept higher TA% for 15 days in seasons.

Ascorbic acid contents:

Data in Table (8) reveal that ascorbic acid content was dramatically decreased as the prolongation of the storage period. This decrease is due to the oxidation of ascorbic acid by enzymes, which can be induced by pathogens in litchi (Zhi-fang *et al*., 1988). Regarding

treatments, biological control agents maintained ascorbic acid contents as compared to control. Moreover, strawberry fruits that pre-harvest sprayed with *Trichoderma asperellum* T34 were superior in maintaining the highest content of ascorbic acid. The higher content in ascorbic acid in treated fruits the effective control of fruit rot (Jiang *et al*., 2001). all treatments and the storage period were significant and showed that control treatment of fruits recorded the highest reduction in ascorbic acid content during all storage periods.

Table (6): Efficiency of pre-harvest spraying with biological control agents on TSS % of strawberry fruits during refrigerated storage.

Treatments (A)	Season 2020					Season 2021				
	Storage period in days (S)					Storage period in days (S)				
	0	5	10	15	Mean	0	5	10	15	Mean
Bacillus megaterium	7.00	6.33	6.03	5.82	6.30	7.33	6.33	5.66	5.32	6.17
Trichoderma album	7.00	6.50	6.17	5.83	6.33	7.00	6.50	6.33	6.00	6.46
Bacillus subtilis	6.82	6.66	6.65	6.16	6.59	7.33	6.66	6.51	6.00	6.63
Trichoderma asperellum	7.83	7.00	7.01	7.00	7.21	7.33	7.34	7.17	6.66	7.13
Trichoderma viride	7.33	6.33	6.00	6.00	6.42	7.00	6.67	6.66	6.33	6.65
Switch	7.00	6.33	6.17	5.33	6.21	7.00	6.16	6.00	5.33	6.13
Control (water)	6.83	6.34	6.16	5.00	6.90	6.66	6.00	6.03	5.32	6.00
Mean	7.11	6.51	6.31	5.87	-	7.08	6.53	6.32	5.86	-
L.S.D at 5%	A=0.63; S=0.49; A*S=1.3					A=0.71; S=0.52; A*S=1.4				

Table (7): Efficiency of pre-harvest spraying with biological control agents on acidity % of strawberry fruits during refrigerated storage.

Treatments (A)	Season 2020					Season 2021				
	Storage period in days (S)					Storage period in days (S)				
	0	5	10	15	Mean	0	5	10	15	Mean
Bacillus megaterium	0.7	0.61	0.59	0.51	0.6	0.61	0.55	0.52	0.47	0.54
Trichoderma album	0.71	0.63	0.55	0.52	0.61	0.73	0.61	0.53	0.49	0.59
Bacillus subtilis	0.72	0.65	0.6	0.51	0.62	0.71	0.65	0.61	0.55	0.63
Trichoderma asperellum	0.73	0.71	0.63	0.53	0.65	0.73	0.69	0.65	0.63	0.68
Trichoderma viride	0.75	0.64	0.60	0.45	0.61	0.71	0.65	0.61	0.47	0.61
Switch	0.73	0.63	0.53	0.41	0.57	0.63	0.61	0.53	0.41	0.54
Control (water)	0.64	0.61	0.53	0.51	0.57	0.7	0.62	0.45	0.33	0.52
Mean	0.71	0.64	0.57	0.49	-	0.69	0.62	0.55	0.48	-
L.S.D at 5%	A= n.s; S = n.s.; A*S = n.s.					A= n.s.; S = n.s.; A*S = n.s.				

Table (8): Efficiency of pre-harvest spraying with biological control agents on Ascorbic acid content (mg/100g f.w.) in strawberry fruits during refrigerated storage.

Treatments (A)	Season 2020					Season 2021				
	storage period days (S)					storage period days (S)				
	0	5	10	15	Mean	0	5	10	15	Mean
Bacillus megaterium	45.94	40.07	33.25	26.5	36.43	46.43	36.08	31.81	28.59	35.73
Trichoderma album	46.49	41.89	30.27	29.13	36.95	45.49	39.83	32.49	26.25	34.02
Bacillus subtilis	47.35	40.87	33.25	31.26	38.18	46.83	39.01	35.29	27.74	37.22
Trichoderma asperellum	47.76	40.88	35.63	31.35	38.91	47.49	41.68	34.27	29.49	38.23
Trichoderma viride	46.54	40.57	33.46	30.88	37.86	45.52	41.18	28.9	28.61	36.05
Switch	46.55	40.13	29.53	27.38	35.9	47.5	35.26	30.01	28.99	35.44
Control (water)	46.55	41.82	28.26	26.56	35.8	45.46	36.14	26.48	26.41	33.62
Mean	46.74	40.89	31.95	29.01		46.39	38.46	31.32	28.01	
L.S.D at 5%	A=2.95; S=2.23; A*S=5.91					A=3.02; S=2.36; A*S=6.21				

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest

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