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Efficacy of different biological control agents against major postharvest pathogens of grapes under room temperature storage conditions

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Summary. Grapes were treated post harvest with a variety of biological agents to determine their efficacy in reducing yield loss. The agents *Pseudomonas, Bacillus, Trichoderma* and yeast isolates were individually screened against a number of postharvest pathogens including *Aspergillus carbonarius*, *Penicillum expansum*, and *Fusarium moniliforme*. *B. subtilis* strains EPC-8 and EPCO-16 showed high mycelial growth suppression of *A. carbonarius* and *P. expansum in vitro*. The fungal antagonist *Trichoderma viride* strain (Tv Tvm) was the most effective, inhibiting mycelial growth by 88.8 per cent. The biological control agents were tested in pre, post and combined inoculation studies against postharvest pathogens of grapes. In the pre inoculation, *B. subtilis* (EPC-8) reduced the disease incidence of *A. carbonarius* causing rot, *T. harzianum* (Th Co) was effective against *P. expansum,* and *T. viride* (Tv Tvm) was effective against *F. moniliforme*. The same trend of effectiveness was also found in the post-inoculation and combined inoculation tests.

Key words: *Aspergillus carbonarius, Penicillium expansum*, *Fusarium moniliforme Bacillus subtilis, Trichoderma* spp.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crop in the world, but between 20 to 30% of fresh grapes are lost every year due to inadequate postharvest storage (El-Ghouth and Wilson, 1995). In cold stored grapes (-1°C), the main decay pathogens are *Botrytis cinerea* Pers., *Cladosporium herbarum* Link, *Alternaria alternata* Keissler, *Rhizopus stolonifer, Aspergillus carbonarius* (Bainier), *Aspergillus* sp. and *Penicillium expansum* Link. Further losses occur during grading, packing, transport and finally marketing as fresh produce or in processed form (Ghosh, 1999). In general terms, post-harvest diseases destroy 10–30% of the total yield of crops and in some perishable crops especially in developing countries, losses are more than 30% (Kader, 2002; Agrios, 2005). Postharvest losses caused by the above pathogens have been estimated at over 40% in mangoes (Singh, 1960), 30% in citrus (Ramanna *et al*., 1973), 50% in pineapple (Mallikarjunaradya *et al*., 1979), 10 to 18% in apple (Kaul and Munjal, 1982), and 7 to 12% in grapes (Anandaswamy *et al*., 1972). The worldwide post-harvest loss of perishables due to fungi is between 10 and 50% (Tripathi *et al.,* 2008). Thakur and Saharan (2008) estimated that postharvest losses in grapes are about 39% of yield, and 30% of value. Research has focused on developing alternative control methods against pre- and postharvest decay in grapes as well as in other crop (Leibinger *et al.,* 1997). Biological control has emerged as an effective strategy to combat major postharvest decays of fruits (Janisiewicz and Korsten, 2002). Postharvest biocontrol is especially attractive because harvested fruits are readily accessible to treatment with antagonists and many postharvest pathogens the infect fruits through wounds after harvest (Janisiewicz and Jeffers, 1997 and Nunes *et al.,* 2001). Several biological control agents reduce de-

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cay caused by gray mold on strawberry (Guinebretiere *et al.,* 2000). Several isolates of bacteria and yeasts control postharvest pathogens on a range of perennial and annual crops (El-Ghaouth *et al.,* 1997). Decay from by *B. cinerea* and *P. expansum* in pome fruits has been controlled by bacterial and yeast antagonists in laboratory and pilot storage tests (El-Ghaouth *et al.,* 2000). The *Trichoderma harzianum* (Rifai) bioagents are extremely versatile biocontrol agents suppressing diseases caused by a number of airborne plant pathogens, including anthracnose and grey mould in strawberry (Freeman *et al*., 2004). The variety of controlling primary postharvest diseases caused by *Rhizopus stolonifer*, *B. cinerea* and *P. expansum* on a variety of fresh fruit was achieved with an invert emulsion formulation of *T. harzianum* (Rifai) (Batta, 2007). Sivakumar *et al*. (2000) reported that isolate TrH 40 of *T. harzianum* is antagonistic against the postharvest pathogens *Gliocephalotrichum microchlamydosporum, Colletotrichum gloeosporioides* and *Botryodiplodia theobromae*. Arokia Raj (2000) found that the bacterial antagonist *B. subtilis* caused the highest reduction of mycelial growth of *Colletotrichum musae* and *B. theobromae,* cutting them by 87.7% and 81.4% respectively. Against this background, the present study has been carried out to evaluate the effect of bioagents as pre-, combined, and post-inoculation for the management of postharvest diseases. The techniques identified in this study need to be developed but they can substantially reduce post- harvest losses of grapes from disease.

Materials and methods

Pathogen isolation

Grape berries showing typical symptoms of disease were surface sterilized with 0.1% mercuric chloride for one min and washed three times in separate sterile distilled water containers. Sterile pieces of symptomatic grape berries were plated on sterile of potato dextrose agar (PDA) and incubated at room temperature (28±2°C) to recover the fungal pathogens. Pure cultures of the isolated fungi were obtained by single spore according to Riker and Riker (1936) and maintained in potato dextrose slants. Fungal pathogens involved in postharvest diseases of grapes were isolated from fruits collected from different markets and they were identified. *Aspergillus carbonarius* (Bainier) Thom. causing black mould, *Penicillium expansum* Link., and Thom. causing blue mould and *Fusarium moniliforme* Sheldon., causing dry rot were the most frequently fungi isolated from the grapes.

Pathogenicity tests

Grape berries were inoculated with the pathogens following the methods suggested by Franck *et al.,* 2005. Postharvest pathogens were inoculated by the pinprick method. Healthy grape berries were washed in running tap water, surface sterilized with 0.1 per cent mercuric chloride and washed with sterile distilled water, the thrice before inoculation of the pathogen. Injuries were made with a sterile needle up to a diameter of 5 mm and a disc of mycelium was immediately placed over it. The inoculated area was covered with moist cotton and the grapes were placed inside sterile perforated polythene bags (200 gauge) and sprayed with sterile distilled water so as to provide the required humidity. The stems were tied with threads and the berries were incubated at room temperature (28±2°C).

Isolation and collection of biocontrol agents

Trichoderma harzianum (Th Co) was isolated from soil samples collected from grapevine fields in Coimbatore, India. Five soil suspensions were prepared by adding 1.0 g of soil to 10 mL of sterile distilled water and shaking for 15 min and each suspension was serially diluted to 10^{-5}, and 0.1 mL from the final dilution was spread on a PDA Petri dish and incubated at 28°C for 5 days (Wijesundera *et al.,* 1991). The isolated fungi were purified by single spore isolation according to Riker and Riker (1936) and maintained in potato dextrose slants. *T. harzianum* (Th Co) was identified based on its morphological and reproductive characters (Elad *et al*., 1981). The epiphytic micro-organisms were isolated by shaking five grape berries in 10 mL of sterile distilled water for 1 h at 200 rpm on a rotary shaker (Peng and Sutton, 1991). The yeast solution was centrifuged at 5000 rpm for 10 min. The supernatant was serially diluted to 10^{-5} and 1 mL of each dilution was spread on yeast extract peptone dextrose agar (YPDA) containing 20 g glucose, 10 g yeast extract, 10 g protease peptone and 15 g agar amended with 250 mg Penicillin G (to suppress growth of bacteria) in 1 L of distilled wa-

ter. The Petri dishes were incubated at room temperature for 4 days and yeast-like colonies were selected randomly according to color and morphological characteristics, removed with a sterile needle, and transferred to fresh YPDA plates to obtain pure cultures. Colonies were held at 4°C until used. The *Trichoderma viride* (Tv Tvm) strain, *Pseudomonas fluorescens* isolate (Pf1) and *Bacillus subtilis* isolate (EPCO-16 and EPC-8) were obtained from the Department of Plant Pathology, TNAU, Coimbatore collection.

In vitro evaluation of antagonists

Trichoderma viride, T. harzianum, *P. fluorescens* (Pf1), *B. subtilis* (EPC8 and EPCO16), *Candida albicans* (CY1 and CY2), and a *Saccharomyces* sp. (SY1 and SY2) were tested for their efficacy by the dual-culture technique (Dennis and Webster, 1971) in PDA. Three replications were done with each antagonist. The Petri dishes were incubated at room temperature (28±2°C) and the zone of inhibition was measured (Kishore *et al.*, 2005). Radial mycelial growth of the pathogen and the percent reduction over the control was calculated using the following formula:

Inhibition over control (%) = C-T/C × 100

where C = mycelial growth of pathogen in control, and T = mycelial growth of pathogen in dual culture.

Effect of biological agents on disease incidence

Effect of pre inoculation

Fully ripened Muscat grape berries were selected and individually treated with bacterial cells (8×10^8 cfu mL^{-1}) and fungal spores (3×10^5 cfu mL^{-1}) of one of each antagonist by dipping 100 berries in liquid culture containing the antagonist. Two days later, the berries were inoculated with a spore suspension (3×10^5 cfu mL^{-1}) of each pathogen. Six replications of each treatment using a completely randomized design were kept in a sterile perforated polythene bag and incubated for 2, 4, 6, 8 and 10 days at room temperature. The percent disease index (PDI) was calculated for each treatment as the percentage of the number of berries showing disease symptoms in relation to the total number of fruits in that treatment using the following empirical, disease grade: 0, bunch without rot; 1, 0–10% rotted berries; 2, 10–25%; 3, 25–50%; 4, 50–75% and 5, more than 75% rotted berries. The disease index was calculated using Mc Kinney's (1923) formula: PDI = sum of numerical ratings ×100/total number of bunches observed × maximum disease grade. In the combined inoculation tests and the post-inoculation tests the PDI was calculated in the same way.

Effect of combined inoculation

Fully ripened Muscat grape berries were selected and treated with bacterial cells (8×10^8 cfu mL^{-1}) and fungal spores (3×10^5 cfu mL^{-1}) of one of each of the antagonists, by dipping the 100 berries in the liquid culture of the antagonist. The berries were then immediately treated with spore suspension (3×10^5 cfu mL^{-1}) of each pathogen. Six replications were carried out for each treatment, using completely randomized design. The treated and inoculated berries were kept in sterile perforated polythene bags and incubated for 2, 4, 6, 8 and 10 days at room temperature. The PDI was calculated.

Effect of post-inoculation

Fully ripened Muscat grape berries were selected and inoculated with spore suspension of each pathogen (3×10^5 cfu mL^{-1}). Two days later, the berries were treated with bacterial cells (8×10^8 cfu mL^{-1}) and fungal spores (3×10^5 cfu mL^{-1}) of each antagonist. Six replications were carried out for each treatment. The inoculated and treated grape berries were kept in sterile perforated polythene bag and incubated for 2, 4, 6, 8 and 10 days at room temperature. The PDI was calculated.

Statistical analysis

Data were statistically analyzed using IRRISTAT (Version 92, developed by the International Rice Research Institute Biometrics Unit, The Philippines) and treatment means were compared by Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984). Analysis was done using the values in the table transformed by the arcsin method.

Results

The fungal pathogens involved in postharvest diseases of grapes were isolated from fruits collected from different markets and they were identified. The pathogenicity was done and the Koch's postulates were fulfilled.

Effect of antagonists against mycelial growth of postharvest pathogens of grapes

Nine potential biocontrol isolates, *Pseudomonas fluorescens* (Pf1), *Bacillus subtilis* (isolates EPCO-16 and EPC-8*), Trichoderma viride* (Tv Tvm), *T. harzianum* (Th Co), *Candida albicans* (isolates CY1 and CY2) and *Saccharomyces* sp. (isolates SY1 and SY2) were screened against *A. carbonarius, P. expansum* and *F. moniliforme.* The effectiveness of the biocontrol agents against mycelial growth of *A. carbonarius* was studied *in vitro* (Table 1). *B. subtilis* strain EPC-8 was the most effective, suppressing 88.7% of *A. carbonarius* mycelia growth. *Trichoderma harzianum* strain (Th Co) was the next most effective, suppressing 82.3% of *A. carbonarius* mycelia growth. None of the yeast isolates was significantly effective against this pathogen. *Bacillus subtilis* (EPCO-16) showed the highest mycelial growth inhibition of *P. expansum* (88.1%), followed by *T. harzianum* (Th Co) (85.2%), and *B. subtilis* (EPCO-8) (47.9%). None of the yeast isolates was significantly effective against *P. expansum*. *Trichoderma viride* strain (Tv Tvm) had the greatest inhibitory effect (88.8%) on *F. moniliforme* mycelial growth followed by *B. subtilis* strain EPCO-16 (79.3%). Isolates CY1 of *C. albicans* and *SY1* of *Saccharomyces* were significantly effective against *F. moniliforme*. When tested *in vitro,* the biological antagonists effectively managed postharvest pathogens of grapes maintained under *in vivo* conditions.

Effect of pre inoculation of antagonists on disease incidence

Pre inoculated *B. subtilis* (EPC-8) reduced post-

Table 1. Efficacy of biological agents on postharvest pathogens of grapes in vitro.

Biocontrol agent	*Aspergillus carbonarius*[a,b,c]		*Penicillium expansum*[a,b,c]		*Fusarium moniliforme*[a,b,c]	
	Radial growth of pathogen (mm)	Inhibition over control (%)	Radial growth of pathogen (mm)	Inhibition over control (%)	Radial growth of pathogen (mm)	Inhibition over control (%)
Trichoderma viride (Tv Tvm)	80.8 (64.1)d	10.1	65.9 (54.3)c	26.8	10.1 (18.5)a	88.8
T. harzianum (Th Co)	15.9 (23.5)b	82.3	13.3 (21.4)a	85.2	20.7 (27.0)b	77.0
Pseudomonas fluorescens (Pf1)	85.2 (67.5)de	5.3	87.9 (69.8)e	2.4	42.7 (40.8)d	52.6
Bacillus subtilis (EPCO-16)	32.7 (34.9)c	63.7	10.7 (19.2)a	88.1	18.7 (25.6)b	79.2
B. subtilis (EPC-8)	10.1 (18.6)a	88.7	46.9 (43.2)b	47.90	30.3 (33.4)c	66.3
Candida albicans (CY1)	86.6 (68.5)de	3.8	81.7 (64.7)d	9.27	41.7 (40.2)d	53.7
C. albicans (CY2)	88.5 (70.34)e	1.69	67.6 (55.29)c	24.9	45.35 (42.3)d	49.6
Saccharomyces sp. (SY1)	89.1 (70.88)e	0.98	67.1 (55.02)c	25.4	41.79 (40.3)d	53.6
Saccharomyces sp. (SY2)	86.3 (68.38)de	4.11	82.2 (65.14)d	8.6	43.89 (41.5)d	51.2
Control	90.0 (71.76)e		90.0 (71.76)e		90.00 (71.8)e	

[a] Mean of three replications.
[b] Values in parentheses are arcsine transformed values.
[c] Values followed by the same letter are not significantly different at the 5% level by DMRT.

harvest rot from *A. carbonarius* by 57.8% followed by *T. harzianum* (Th Co) with 46.3% after 10 days at room temperature when compared with the control. Pre inoculation of *T. harzianum* (Th Co) reduced postharvest rot caused by *P. expansum* by 57.1% followed by *B. subtilis* (EPCO-16) with 48.4%. Pre inoculation of *T. viride* (Tv Tvm) reduced postharvest rot of *F. moniliforme* by 70.1% followed by *B. subtilis* (EPCO-16) with 65.4% (Table 2; Figure 1a, b, c).

Effect of combined inoculation of antagonists and pathogens on disease incidence

Bacillus subtilis (EPC-8) in combined antagonists + pathogens inoculations reduced the growth of *A. carbonarius* by 67.6% compared with the control. *T. harzianum* (Th Co) reduced rot incidence from *P. expansum* by 65.1%, followed by *B. subtilis* (EPCO-16) with about 48.4%. Similarly, *T. viride* (Tv Tvm) reduced the postharvest rot of *F. moniliforme* by 70.1 % over the control (Table 3; Figure 1a, b, c).

Effect of post inoculation of antagonists on disease incidence in grapes

The effect of post- inoculation of antagonists on mould rot of grapes is shown in Table 4. Post-inoculation of *B. subtilis* (EPC-8) on grapes was significantly effective, reducing the incidence of rot caused by *A. carbonarius* by 47.3%. *T. harzianum* (Th Co) applied after *P. expansum* led to the lowest incidence of postharvest rot, from this pathogen, with a reduction of 43.1%. Similarly, *T. viride* (Tv Tvm) reduced grape rot from *F. moniliforme* by 51.2% over the control (Figure 1a, b, c).

Table 2. Effect of pre-inoculation of antagonists on the incidence of postharvest rot disease in grapes.

Pathogen	Treatment	Percentage disease index (PDI)[a,b,c]					Mean	Reduction over control on 10th day (%)
		Days after treatment						
		2	4	6	8	10		
Aspergillus carbonarius	*Trichoderma harzianum* (Th Co)	5.87 (14.02)b	10.74 (19.13)b	16.29 (23.80)b	21.48 (27.61)b	28.29 (32.13)b	16.53	46.28
	Bacillus subtilis (EPC-8)	3. 66 (11.03)a	8.14 (16.57)a	12.59 (20.78)a	15.56 (23.23)a	22.22 (28.12)a	14.63	57.80
	Control	8.14 (16.58)c	17.04 (24.38)c	28.96 (32.55)c	36.37 (37.09)c	52.66 (46.53)c	28.63	
Penicillium expansum	*T. harzianum* (Th Co)	3.33 (10.51)a	7.04 (15.38)a	11.85 (20.13)a	15.56 (23.23)a	20.00 (26.56)a	11.56	57.15
	B. subtilis (EPCO-16)	4.44 (12.16)b	9.25 (17.71)b	14.07 (22.03)b	20.37 (26.83)b	24.07 (29.38)b	14.44	48.43
	Control	8.52 (16.97)c	18.52 (25.49)c	24.81 (29.87)[c]	34.44 (35.93)[c]	46.67 (43.09)c	26.59	
Fusarium moniliforme	*T. viride* (Tv Tvm)	1.85 (7.82)a	4.44 (12.16)a	9.26 (17.71)a	11.48 (19.80)a	14.07 (22.03)a	8.22	70.09
	B. subtilis (EPCO-16)	3.33 (10.51)b	7.04 (15.39)b	9.63 (18.08)a	12.96 (21.10)b	16.29 (23.80)b	9.85	65.37
	Control	6.67 (14.97)c	14.07 (22.03)c	28.67 (32.37)b	39.78 (39.10)c	47.04 (43.30)c		27.25

[a,b,c] See Table 1.

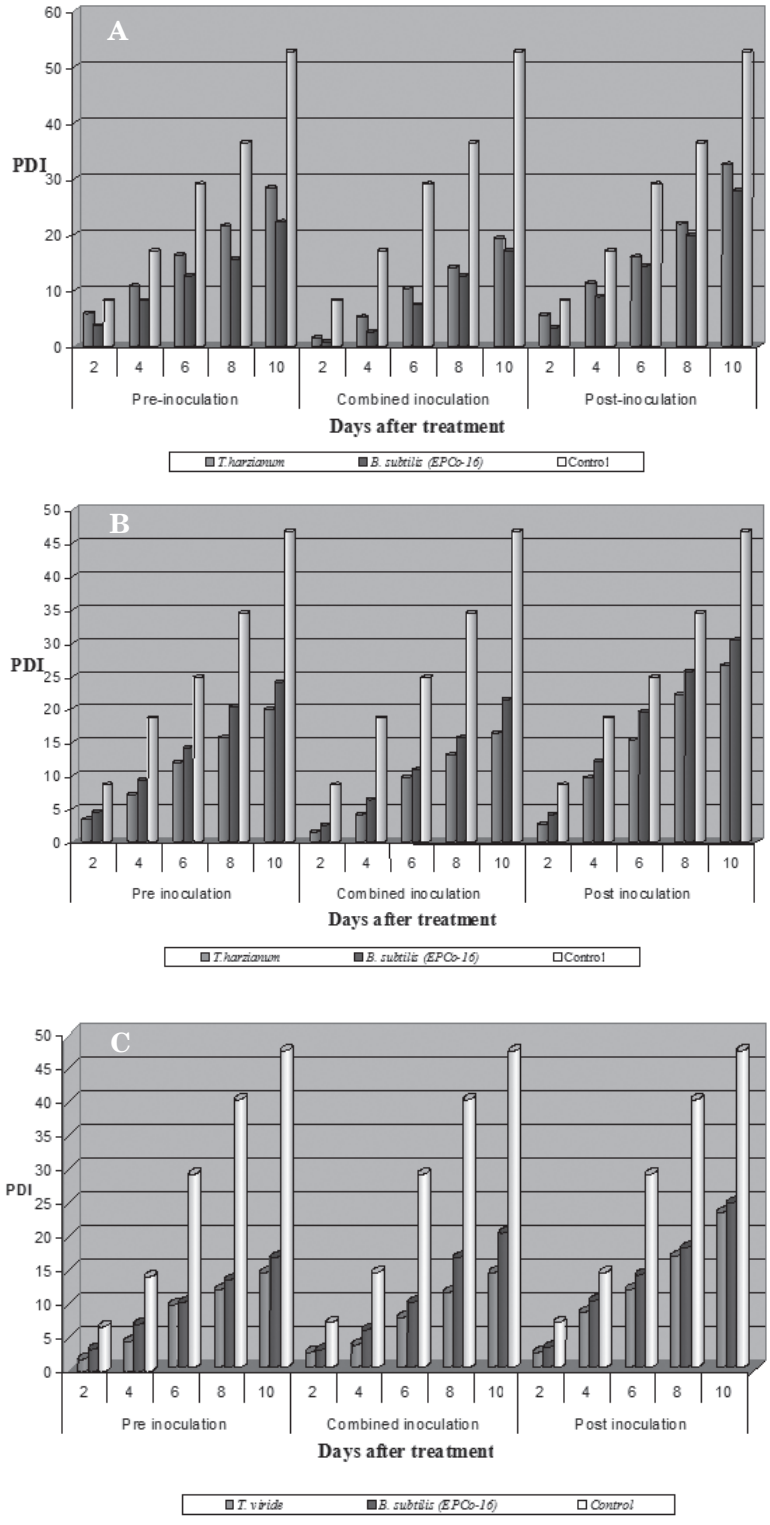

Figure 1. Effect of pre-, combined and post- inoculation of antagonists on the incidence (percent disease index, PDI) of rot caused by: A) *Aspergillus carbonarius*, B) *Penicillium expansum* and C) *Fusarium moniliforme* in grapes.

Table 3. Effect of combined inoculation of antagonists and pathogen on the incidence of postharvest rot disease in grapes.

Pathogen	Treatment	Percentage disease index (PDI) [a,b,c]						Reduction over control after 10 days (%)
		Days after treatment					Mean	
		2	4	6	8	10		
Aspergillus carbonarius	*Trichoderma harzianum* (Th Co)	1.48 (6.99)b	5.19 (13.16)b	10.37 (18.78)b	14.07 (22.03)b	19.25 (26.02)b	10.07	63.44
	Bacillus subtilis (EPC-8)	0.77 (5.04)a	2.59 (9.26)a	7.4 (15.80)a	12.59 (20.78)a	17.04 (24.38)a	7.93	67.64
	Control	8.14 (16.58)c	17.04 (24.38)c	28.96 (32.55)c	36.37 (37.09)c	52.66 (46.53)c	28.63	
Penicillium expansum	*T. harzianum* (Th Co)	1.48 (6.99)a	4.07 (11.64)a	9.63 (18.08)a	12.96 (21.10)a	16.29 (23.80)a	8.886	65.10
	B. subtilis (EPCO-16)	2.48 (9.06)b	6.29 (14.53)b	10.74 (19.13)b	15.55 (23.22)b	21.48 (27.61)b	11.108	53.97
	Control	8.52 (16.97)c	18.52 (25.49)c	24.81 (29.87)[c]	34.44 (35.93)c	46.67 (43.09)c	26.59	
Fusarium moniliforme	*T. viride* (Tv Tvm)	2.24 (8.61)[a]	3.33 (10.51)[a]	7.41 (15.79)[a]	11.11 (19.47)[a]	14.09 (22.04)[a]	7.19	70.06
	B. subtilis (EPCO-16)	2.59 (9.27)b	5.56 (13.64)b	9.63 (18.08)b	16.29 (23.70)b	20.00 (26.56)b	10.81	57.48
	Control	6.67 14.97)c	14.07 (22.03)c	28.67 (32.37)c	39.78 (39.05)c	47.04 (43.30)c	27.25	

[a,b,c] See Table 1.

Discussion and conclusions

Grapev ine (*Vitis vinifera* L.) is a non-climacteric fruit that has a relatively low physiological activity, and that loses a considerable amount of water following harvest. In the developing countries, post-harvest losses are much higher because operations to protect grapes from mechanical damage during harvest and post-harvest are very poor or altogether lacking. Worldwide, postharvest losses for most perishable crops are estimated at between 10 and 30%. Snowdon (1990) reported that, on grapes, *A. carbonarius* and *A. niger* cause black mould, that *P. expansum* causes blue mould, that *Botryotinia fuckeliana* (conidial state: *B. cinerea*) causes grey mould, that *F. moniliforme* causes dry rot, and that *R. stolonifer* causes *Rhizopus* rot. Currently, biological control is considered a very promising alternative to synthetic fungicide in the control of postharvest decay of fruits and vegetables (Wisniewski and Wilson, 1992). Plants benefit extensively by harbouring these endophytic microbes, which promote plant growth (Compant *et al.,* 2005) and increase resistance to various pathogens (Arnold *et al.,* 2003) by producing antibiotics (Ezra *et al.,* 2004). Endophytic bacteria penetrate and become systemically disseminated in the host plant, actively colonizing the apoplast (Quadt-Hallmann *et al.,* 1997), the conducting vessels (Hallmann *et al.,* 1997), and occasionally the intracellular spaces (Quadt-Hallmann *et al.,* 1997). Several mechanisms may control this suppression, either directly inside the plant, by antibiosis against the pathogen (Sturz *et al.,* 1998) and by the

Table 4. Effect of post inoculation of antagonists on the incidence of postharvest rot disease in grapes.

Pathogen	Treatment	Percentage disease index (PDI) [a,b,c]						Reduction over control after 10 days (%)
		Days after treatment					Mean	
		2	4	6	8	10		
Aspergillus carbonarius	*Trichoderma harzianum* (Th Co)	5.56 (13.64)b	11.26 (19.61)b	15.96 (23.55)b	21.92 (27.92)b	32.48 (34.74)b	17.436	38.32
	Bacillus subtilis (EPC-8)	3.33 (10.51)a	8.89 (17.34)a	14.37 (22.27)a	19.76 (26.12)a	27.77 (31.79)a	15.316	47.27
	Control	8.14 (16.58)c	17.04 (24.38)c	28.96 (32.55)c	36.37 (37.09)c	52.66 (46.53)c	28.63	
Penicillium expansum	*T. harzianum* (Th Co)	2.59 (9.26)a	9.56 (18.01)a	15.26 (22.99)a	22.22 (28.12)a	26.56 (31.02)a	15.238	43.09
	B. subtilis (EPCO-16)	4.07 (11.64)b	12.03 (20.29)b	19.59 (26.27)b	25.66 (30.43)b	30.37 (33.44)b	18.344	34.93
	Control	8.52 (16.97)c	18.52 (25.49)c	24.81 (29.87)c	34.44 (35.93)c	46.67 43.09)c	26.59	
Fusarium moniliforme	*T. viride* (Tv Tvm)	2.22 (8.57)a	8.18 (16.62)a	11.52 (19.84)a	16.48 23.95)a	22.96 (28.63)a	12.672	51.19
	B. subtilis (EPCO-16)	2.96 (9.91)b	9.92 (18.36)b	13.74 (21.75)b	17.78 (24.94)b	24.40 (29.60)b	13.76	48.13
	Control	6.67 (14.97)c	14.07 22.03)c	28.67 (32.37)c	39.78 (39.10)c	47.04 (43.30)c	27.25	

[a,b,c] See Table 1.

competition for nutrients (Mari *et al.,* 1996), or indirectly by inducing a resistance response in the plant (M'Piga *et al.,* 1997). Of the bioagents tested, *B. subtilis* strain EPC-8 had the highest level of suppression against *A. carbonarius*. A *Bacillus* sp. was also reported to reduce mycelial growth of *B. cinerea* in grapes (Elad, 1994). The *B. subtilis* antagonist strain EPCO-16 significantly reduced the growth of *P. expansum*. *B. subtilis* had an antagonistic effect on the papaya anthracnose fungus *Colletotrichum gloeosporioides* (Linn, 1987). Similarly, *B. subtilis,* isolated from the citrus fruit surface, controlled citrus green and blue moulds caused by *Penicillium digitatum* and *P. italicum* respectively (Obagwu and Korsten, 2003). *B. subtilis* produces iturin, a powerful antifungal peptide (Gueldner *et al*., 1988), as well as gramicidin S (Edwards and Seddon, 2001), the only known substances that control infections by *B. cinerea* and *P. expansum* on pome fruit (Janisiewicz *et al.,* 1991). The fungal antagonist *T. viride* had the greatest inhibitory effect on *F. moniliforme in vitro*. Similarly, *T. viride* and *Gliocladium roseum* were antagonistic to *Fusarium oxysporum in vitro* and *in vivo* (Rod, 1984). Muthuraman and Sekar (1993) found that *T. viride* and *T. harzianum* inhibited the onion wilt pathogen *Fusarium oxysporum* f. sp. *cepae in vitro*. In our study, none of the yeast isolates was effective against *A. carbonarius* or *P. expansum* rot in grapes, but Zhang *et al*. (2005) reported that *Candida laurentii* was a potential biocontrol agent against postharvest gray mold rot, blue mold rot, and *Rhizopus* rot, caused by *B. cinerea*, *P. expansum*, and *R. stolonifer* respectively. The antagonistic yeast strains *Candida saitoana* (El-Ghaouth *et al.,* 1998) and *C. sake* (Teixido *et al.,*

1998) were effective on postharvest grapes pathogens. In the present study, pre-, post-, and combined treatment with *B. subtilis* (EPC-8) reduced the incidence of postharvest rot of grapes caused by *A. carbonarius*. Similarly, *B. subtilis* is an antagonist against the major postharvest pathogens of stone fruits (Pusey *et al*., 1988), pome fruits (Wilson *et al.,* 1993) and citrus fruits (Smilanick and Denis-Arrue, 1992). Postharvest application of *B. subtilis* also controlled postharvest avocado diseases (Korsten *et al.,* 1995). *T. harzianum* (Th Co) reduced *P. expansum* disease incidence in the pre-, post- and combined treatment of grape berries. It has also been reported that combined inoculations of *T. harzianum* and *B. cinerea* conidia, or inoculation of *T. harzianum* conidia only 8 hrs before inoculation with *B. cinerea* prevented wounded grape berries from becoming infected (O'Neill *et al.,* 1996). In other studies, antagonistic *T. harzianum* strain Th2 was highly effective against *B. cinerea* on apple fruit (Batta, 2004a), against *Alternaria alternata* on fig leaves (Batta, 2000) and persimmon fruit (Batta, 2001), and against *P. expansum* on apple fruit (Batta, 2004b). The pre-, post- and combined treatment of grapes with *T. viride* strain Tv Tvm reduced disease incidence from *F. moniliforme*. D'Ercole and Nipoti (1986) found that *T. viride, T. harzianum* and *T. koningii* were antagonistic to *Fusarium oxysporum* f. sp. *lycopersici* affecting tomato. At a temperature of 25°C *T. viride* suppressed germination of *B. cinerea* on lettuce when it was co-inoculated with the pathogen, but at 15°C it was effective only if it was inoculated 2 days before the pathogen (Wood, 1951). *T. viride* has been effectively used against several plants pathogenic fungi, including *Botrytis, Rhizoctonia, Sclerotinia, Pythium* and *Fusarium* in different crops (Batta, 2004a). Therefore, this bacterium is potentially an ecofriendly biological fungicide on grape berries, after long term and wide ranging trials for the management of postharvest pathogens of grapes. It is concluded that for pre and combined inoculation of biological agents, it would be more effective to apply those agents that have a synergistic action for broad spectrum activity against post harvest pathogens of grapes. In this study we used mainly those biological agents that were effective against black mold caused by *A. carbonarius*, blue mold caused by *P. expansum* and dry rot caused by *F. moniliforme* on grape.

In the whole, the trials here reported showed that the bacterial antagonist *B. subtilis* strain EPC-8 showed a high level of mycelial growth suppression of *A. carbonarius*. *B. subtilis* strain EPCO-16 was effective in suppressing mycelial growth *P. expansum*. The fungal antagonist *T. viride* strain Tv Tvm had the greatest inhibitory effect on *F. moniliforme,* reducing mycelial growth by 88.8%. Pre-, post-, and combined inoculation of those antagonists significantly reduced the postharvest rots caused by *A. carbonarius, P. expansum* and *F. moniliforme* in grape berries.

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