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Control of postharvest diseases of fruit with an invert emulsion formulation of *Trichoderma harzianum* Rifai

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Abstract

Control of primary postharvest diseases caused by *Rhizopus stolonifer*, *Botrytis cinerea*, and *Penicillium expansum* on a variety of fresh fruit was evaluated with an invert emulsion formulation of *Trichoderma harzianum*. Diseases evaluated were quantified by the period of protection conferred by the antagonist and the diameter of decay lesions. Treatment of the various fruit species with formulated *T. harzianum* conidia in an invert emulsion significantly ($P \leq 0.05$) reduced the mean lesion diameters of *R. stolonifer* on apple, pear, peach and strawberry, *B. cinerea* on grape, pear, strawberry, and kiwifruit, and *P. expansum* on grape, pear, and kiwifruit in comparison with the control treatment. Significant differences ($P \leq 0.05$) were obtained in the mean percent reduction in lesion diameter caused by the same postharvest pathogens on the same fruit species due to the treatment with the formulated *T. harzianum* conidia relative to control treatment. The greatest mean percent reduction (86.7%) was obtained on apple fruit for the infection with *R. stolonifer*. Significant differences ($P \leq 0.05$) were also obtained in the mean durations of the minimum protection period due to treatment with the formulated *T. harzianum* against the infection with the same postharvest pathogens on the same fruit species. The longest mean duration of the minimum protection period (up to 59 days) was obtained for unwounded apple fruit against the infection with *R. stolonifer*. Overall, the results indicate that the treatment with the invert emulsion formulation of *T. harzianum* protected fruit from infection by the primary postharvest pathogens of the fruit tested for up to 2 months and reduced the diameters of decay lesion up to 86% and is a promising treatment to prolong the postharvest shelf-life of fresh fruit.

Keywords: Postharvest pathogens; *Trichoderma harzianum*; *Rhizopus stolonifer*; *Botrytis cinerea*; *Penicillium expansum*; Invert emulsion formulation

1. Introduction

The largest portion of postharvest losses in fruit and vegetable crops is due to rots caused by microorganisms, especially fungi, as the main causative agents of food spoilage. Fruit rots are also devastating agents in the spoilage of many fruit species after harvest and during cold storage.

Fungicides are the primary means of controlling postharvest rot pathogens on fruit (Eckert and Somer, 1967). It has been long established that fungicides applied to food during storage will prolong the shelf-life and reduce the food spoilage, but it is also true that these chemicals, besides their harmful effects on the human health and the possibility of posing oncogenic risks, are expensive. In addition, the lack of regulations in less regulated countries and the development of resistance to the most acceptable fungicides, allows the abuse of these chemicals. Promising approaches are available to us for developing new and potentially safer technologies instead of chemicals for postharvest disease control. Antagonistic microorganisms as an alternative approach to fungicides and as biocontrol agents have been used effectively, since 1983, in controlling postharvest diseases of fruit and vegetable crops (Wilson and Wisniewski, 1989; Janisiewicz and Korsten, 2002). A variety of antagonistic microorganisms, especially yeasts and bacteria, can control different rot pathogens of apple (Janisiewicz, 1987, 1988; Conway et al., 1999; Etebarian et al., 2005), plum and apri-

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cot (Pusey and Wilson, 1984), citrus (Chalutz et al., 1988a, 1988b; Singh and Deverall, 1984), pepper (Chalutz et al., 1988a), potato (Colyer and Mount, 1984), pear (Janisiewicz and Rotiman, 1988; Nunes et al., 2002), tomato (Chalutz et al., 1988a; Bora et al., 2000), and peach and nectarine (Pusey and Wilson, 1984; Fan et al., 2000; Karabulut and Baykal, 2003). However, the extent of control varies according to the pathogen causing the infection and the fruit species. In previous research, we have shown the biocontrol potential of the fungus *Trichoderma harzianum* (strain Th_2), especially in formulated form in an invert emulsion, against *Botrytis cinerea* on strawberry leaves (Batta, 1999) and on apple fruit (Batta, 2004a), *Alternaria alternata* on fig leaves (Batta, 2000) and on persimmon fruit (Batta, 2001), and *Penicillium expansum* on apple fruit (Batta, 2004b). The effectiveness of biocontrol with *Trichoderma* spp. has also been shown by other investigators against *Penicillium digitatum* on citrus fruit (Borras and Aguilar, 1990), *B. cinerea* on grape berries (Elad, 1994), *Monilinia fructigena* on stone fruit (Hong et al., 1998), *B. cinerea*, *M. fructigena* and *P. expansum* on apple (Falconi and Mendgen, 1994), and *B. cinerea* and *P. expansum* on yams (*Dioscorea* spp.) (Okigbo and Ikediugwu, 2000).

The objectives of the present research were: (i) to test the biocontrol effectiveness of formulated *T. harzianum* in an invert emulsion (water-in-oil type) against the following fungal postharvest pathogens of fruit: *Rhizopus stolonifer* on apple, pear, peach, and strawberry; *B. cinerea* on grape, pear, kiwifruit, and strawberry; *P. expansum* on grape, pear, and kiwifruit; (ii) to determine the duration of the minimum protection period from infection with the above-mentioned postharvest fruit-rot pathogens on the same fruit species due to treatment with the formulated *T. harzianum* conidia in an invert emulsion.

2. Materials and methods

2.1. Fruit species used

For a broad screen of biocontrol effectiveness of *T. harzianum* against the primary postharvest pathogens, the following fruit species were used: apple (*Malus domestica* Borkh., cv. Golden Delicious), pear (*Pyrus communis* L., cv. Spadona), peach (*Prunus persica* L. Batch, cv. Mohassan), strawberry (*Fragaria* × *ananassa* Duch., cv. Variety 43), grape (*Vitis vinifera* L., cv. Halawani), and kiwifruit (*Actinidia deliciosa* Planch., cv. Green kiwifruit: Triumph). The fruit used were mature and of excellent quality and were obtained from farms where fungicides had been applied no later than 4 weeks before harvest. All fruit, after harvesting, were washed with tap water then superficially disinfected with sodium hypochlorite (0.025%, v/v) before being rinsed three times with sterile distilled water, so that they become fit for inoculation with the postharvest pathogens mentioned above and for treatment with *T. harzianum*.

2.2. Fungal cultures used

All fungi used in the experiments were isolated by the laboratory of Plant Protection, Faculty of Agriculture, An-Najah National University, Nablus, Palestinian Authority. The isolated fungi were cultured on Potato Dextrose Agar (PDA) and were 10–14 days old when used in the experiment. *T. harzianum* (strain Th_2) was isolated from soil planted with various vegetable crops; *R. stolonifer* (strain RS1) was isolated from infected strawberry; *B. cinerea* (strain BC1) was isolated from infected apple fruit; and *P. expansum* (strain PE8) was isolated from infected pear fruit. The subculturing media for these fungi were: oat meal agar (OMA) for the strains Th_2, PE8, and BC1; and PDA for the strain RS1. A conidial suspension was prepared from each fungal culture for each strain by scraping the sporulating fungus growth formed on the plate surface of the culture using a sterile scalpel and then suspending the scraped conidia in sterile deionized water after sieving through a 75-μm mesh. The suspended conidia in sterile deionized water were then counted using a haemocytometer. The concentrations of the fungal conidial suspension used in the different experiments were: 9.6×10^8 conidia/ml for Th_2 in the unformulated form; 4.6×10^8 conidia/ml for Th_2 in the formulated form of invert emulsion; 2.4×10^6 conidia/ml for BC1; 2.2×10^6 conidia/ml for PE8; and 2.6×10^6 sporangiospores/ml for RS1.

2.3. Preparation of invert emulsion formulation and introduction of T. harzianum conidia into the formulation

Ingredients of the invert emulsion (water-in-oil type) that was used in the present research for formulation of *T. harzianum* conidia (strain Th_2) were the same as those used previously (Batta, 2004a). The emulsion contained the following ingredients (w/w): sterile deionized water (45.25%), glycerine (4.00%), water-soluble wax (Dehymuls K®, produced by Henkel Co., Dsseldorf, Germany) (0.75%), Tween 20 (2.50%), and a mixture of 19.00% coconut oil +28.50% soybean oil. *T. harzianum* conidia (strain Th_2) harvested from a 10-day-old culture of the fungus on OMA medium plates was introduced into the invert emulsion during its preparation according to the technique developed previously (Batta, 2004a). The concentration of the introduced *T. harzianum* conidia into the invert emulsion was 4.6×10^8 conidia/ml of the emulsion (viability and shelf-life of the fungus in the formulation have been described by Batta, 2004a).

2.4. Testing biocontrol effectiveness of T. harzianum on lesion development of postharvest pathogens of fruit

Fresh, mature and superficially disinfected fruit species (described in Section 2.1) were used in this test. The effectiveness of treatment with *T. harzianum* on lesion development of the three tested postharvest pathogens on the various fruit was tested by adding the biocontrol agent 1 h before

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inoculation of the postharvest pathogens on wounded fruit. In this test, a 25-μl droplet of formulated or unformulated *T. harzianum* conidia (original concentrations are indicated in Sections 2.2 and 2.3) was applied per fruit. A similar droplet size was also applied in the control treatments with sterile deionized water or a blank formulation of the invert emulsion. Inoculation of each one of the three pathogens on the fruit species was carried out by depositing a 25-μl droplet of conidial suspension (original concentration is indicated in Section 2.2) per wound. One wound per fruit was made on the surface (2-mm diameter by 2-mm deep) using a sterile cork borer of 2-mm diameter. The pathogen was added 1 h after the addition of the biocontrol agent to allow the absorption of the agent by the wound. The treatments were incubated for 3–4 days after inoculation and treatment at 20 ± 2 or $30 \pm 2\,^{\circ}C$ in closed plastic containers (one fruit per container) prior to rating. The reason for choosing these relatively high temperatures was that they are favorable for the development of these postharvest pathogens especially for *R. stolonifer*. The experimental design used in the tests was completely randomized with five replicates representing five fruit per treatment per postharvest pathogen and per fruit species in each experimental treatment. Experimental treatments used in the tests were: formulated *T. harzianum* conidia in an invert emulsion (composition of the emulsion and concentration of the introduced conidia are described in Section 2.3); unformulated *T. harzianum* conidia in the form of a conidial suspension in sterile deionized water (preparation and concentration are described in Section 2.2); blank formulation of invert emulsion as a control of the formulated *T. harzianum* treatment; and sterile deionized water as a control treatment. Each experiment was done twice and the data presented here are the average numbers.

2.5. Determination of the duration of the minimum protection period from infection with postharvest pathogens on treated fruit with T. harzianum

In order to simulate what happens under natural conditions, microwounds were made on the surface of healthy mature and superficially disinfected fruit. Unwounded fruit of the same species were used in the tests for comparison. The effect of treatment with *T. harzianum* on the protection period from infection with the three tested postharvest pathogens was determined when unwounded or microwounded fruit were treated with *T. harzianum* then inoculated with the pathogens. For this purpose, a fixed standardized volume of 2.0 ml of formulated *T. harzianum* conidia in an invert emulsion (original concentration of the fungus in the formulation is indicated in Section 2.3) was sprayed on each fruit using a small calibrated hand sprayer (1.5 l capacity). A similar standardized volume was also sprayed on each fruit in the control treatment with a blank formulation of the invert emulsion. Inoculation of each pathogen on the fruit was carried out by spraying a standardized volume of 1.0 ml of the conidial suspension (original concentration is indicated in Section 2.2) per fruit. Seven microwounds distributed on each fruit surface were made with a sterile needle pricks. The pathogen was added 1 h after the addition of the biocontrol agent. The treatments were incubated at $20 \pm 2\,^{\circ}C$ in closed plastic containers (one fruit per container) until the appearance of typical postharvest pathogen lesions prior to rating. A completely randomized design with four replicates representing four fruit per treatment per pathogen and per fruit species was used in each experimental treatment. Experimental treatments used in these tests were: formulated *T. harzianum* conidia in an invert emulsion (composition of the formulation and concentration of the introduced conidia are described in Section 2.3), and a control treatment with a blank formulation of invert emulsion. Each experiment was done twice and the data presented here are the average numbers. The duration of the minimum protection period from infection with each pathogen on each fruit species due to *T. harzianum* treatment was determined by calculating the difference between the minimum time required for the appearance of pathogen lesions on wounded or unwounded fruit species following the treatment with the formulated *T. harzianum* and that following the treatment with the blank formulation of invert emulsion as a control treatment. Standard error (S.E.) of the means as a measure of variation was calculated and added to the mean data.

2.6. Evaluation of T. harzianum effectiveness

The effectiveness was evaluated by measuring the pathogen-lesion diameter that formed around wounds made on the fruit surface 3–4 days after inoculation and treatment. The lesion diameter depends on the species of fruit tested and the postharvest pathogen studied. The mean lesion diameter was then calculated for each pathogen on each fruit species. The mean percent reduction in mean lesion diameter of each pathogen on each fruit species relative to the control (sterile deionized water treatment) was then calculated. Mean duration of the minimum protection period from infection with each postharvest pathogen on each fruit species due to treatment with the formulated *T. harzianum* conidia in an invert emulsion was also calculated and used in the comparison of effectiveness. Data obtained on both criteria of evaluation were statistically analyzed using ANOVA followed by Duncan's multiple range test (DMRT) to separate the means.

3. Results

3.1. Effect of treatment with T. harzianum on R. stolonifer lesion development on four fruit species

Significant differences ($P \leq 0.05$) were obtained in *R. stolonifer* lesion diameters on wounded fruit of apple, pear, peach, and strawberry (incubated at 20 ± 2 and $30 \pm 2\,^{\circ}C$) when they were treated with the formulated *T. harzianum* conidia in the invert emulsion and compared with the control

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Table 1
Development of typical *R. stolonifer* (strain RS1) lesions on mature fruit of apple, pear, peach and strawberry after inoculation and treatment with formulated and unformulated conidia of *T. harzianum* (strain Th_2)

Fruit type	Mean lesion diameter (in mm) of *R. stolonifer* on inoculated and treated fruit with[a]				Mean percent reduction in lesion diameter of *R. stolonifer* relative to control[b]		
	Formulated *T. harzianum* in invert emulsion	Unformulated *T. harzianum* in water	Sterile deionized water only	Blank formulation of invert emulsion	Formulated *T. harzianum* in invert emulsion	Unformulated *T. harzianum* in water	Blank formulation of invert emulsion
Apple (20 ± 2 °C)	7.7 a[c]	10.7 ab[c]	26.0 c[c]	19.7 bc[c]	70.2 C[d]	58.6 B[d]	24.0 A[d]
Apple (30 ± 2 °C)	9.7 a	49.5 b	73.2 c	75.7 c	86.7 C	32.4 B	0 A
Pear (20 ± 2 °C)	8.0 a	9.7 a	26.2 b	22.0 b	69.5 B	62.9 B	16.2 A
Pear (30 ± 2 °C)	4.0 a	4.5 a	7.0 a	6.7 a	–[e]	–[e]	–[e]
Peach (20 ± 2 °C)	36.7 a	40.0 ab	51.7 b	49.5 b	29.0 B	22.7 B	4.3 A
Peach (30 ± 2 °C)	0 a	0 a	10.5 a	6.7 a	–[e]	–[e]	–[e]
Strawberry (20 ± 2 °C)	23.7 a	30.0 b	36.7 c	35.3 c	35.4 C	18.2 B	3.6 A

[a] The lesion diameter typical of *R. stolonifer* was measured on each fruit 3 days after inoculation and treatment by deposition of 25 μl droplets of *R. stolonifer* conidial suspension and *T. harzianum* conidia (formulated or unformulated) in a wound made on each fruit surface.
[b] The control is the treatment with *R. stolonifer* and sterile deionized water only in comparison with other treatments with *R. stolonifer* and formulated *T. harzianum* in invert emulsion or unformulated *T. harzianum* in water or blank formulation of invert emulsion.
[c] Horizontally, means of *R. stolonifer* lesion diameter developed after treatment on each fruit type that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.
[d] Horizontally, means of percent reduction in lesion diameter of *R. stolonifer* relative to control obtained after treatment on each fruit species that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.
[e] Mean percent reduction in lesion diameter of *R. stolonifer* relative to control was not calculated because no significant differences were obtained in the means of lesion diameter.

(treatment with sterile deionized water or blank formulation of the invert emulsion) (Table 1). Therefore, the mean lesion diameters of *R. stolonifer* on wounded apple fruit (incubated at 30 ± 2 °C) significantly reduced ($P \leq 0.05$) from 73.2 mm in the control treatment to 9.7 mm in the treatment with the formulated *T. harzianum* conidia. This reduction was amounted to 86.7% (Table 1).

Similar reductions ($P \leq 0.05$) in *R. stolonifer* lesion diameters relative to the control were obtained for wounded pear, peach, and strawberry fruit (incubated at 20 ± 2 °C) when they were treated with the formulated *T. harzianum* and compared with the control (Table 1).

3.2. Effect of treatment with *T. harzianum* on *B. cinerea* lesion development on four fruit species

Significant differences ($P \leq 0.05$) were obtained in *B. cinerea* lesion diameters on wounded fruit of grape, pear, strawberry, and kiwifruit (incubated at 20 ± 2 °C) when they were treated with the formulated *T. harzianum* and compared with the controls (Table 2). Therefore, the mean lesion diameters of *B. cinerea* on wounded grape berries (incubated at 20 ± 2 °C) significantly reduced ($P \leq 0.05$) from 20.4 mm in the control treatment to 8.8 mm in the treatment with the formulated *T. harzianum*. This reduction was amounted to

Table 2
Development of typical *B. cinerea* (strain BC1) lesions on mature fruit of grape, pear, strawberry and kiwifruit after inoculation and treatment with formulated and unformulated conidia of *T. harzianum* (strain Th_2)

Fruit type	Mean lesion diameter (in mm) of *B. cinerea* on inoculated and treated fruit with[a]				Mean percent reduction in lesion diameter of *B. cinerea* relative to control[b]		
	Formulated *T. harzianum* in invert emulsion	Unformulated *T. harzianum* in water	Sterile deionized water only	Blank formulation of invert emulsion	Formulated *T. harzianum* in invert emulsion	Unformulated *T. harzianum* in water	Blank formulation of invert emulsion
Grape (20 ± 2 °C)	8.8 a[c]	12.2 a[c]	20.4 b[c]	19.6 b[c]	56.9 C[d]	40.2 B[d]	3.9 A[d]
Pear (20 ± 2 °C)	12.7 a	17.7 ab	23.0 b	23.7 b	44.9 C	5.3 B	0 A
Strawberry (20 ± 2 °C)	8.7 a	15.7 b	25.7 c	25.0 c	66.2 C	38.9 B	2.6 A
Kiwifruit (20 ± 2 °C)	8.6 a	11.8 a	18.6 b	17.6 b	53.8 C	36.6 B	5.4 A

[a] The lesion diameter typical of *B. cinerea* was measured on each fruit 4 days after inoculation and treatment by deposition of 25 μl droplets of *B. cinerea* conidial suspension and *T. harzianum* conidia (formulated or unformulated) in a wound made on each fruit surface.
[b] The control is the treatment with *B. cinerea* and sterile deionized water only in comparison with other treatments with *B. cinerea* and formulated *T. harzianum* in invert emulsion or unformulated *T. harzianum* in water or blank formulation of invert emulsion.
[c] Horizontally, means of *B. cinerea* lesion diameter developed after treatment on each fruit type that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.
[d] Horizontally, means of percent reduction in lesion diameter of *B. cinerea* relative to control obtained after treatment on each fruit species that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.

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Table 3
Development of typical *P. expansum* (strain PE8) lesions on mature fruit of grape, pear and kiwifruit after inoculation and treatment with formulated and unformulated conidia of *T. harzianum* (strain Th_2)

Fruit type	Mean lesion diameter (in mm) of *P. expansum* on inoculated and treated fruit with[a]				Mean percent reduction in lesion diameter of *P. expansum* relative to control[b]		
	Formulated *T. harzianum* in invert emulsion	Unformulated *T. harzianum* in water	Sterile deionized water only	Blank formulation of invert emulsion	Formulated *T. harzianum* in invert emulsion	Unformulated *T. harzianum* in water	Blank formulation of invert emulsion
Grape (20 ± 2 °C)	8.6 a[c]	12.0 ab[c]	17.0 b[c]	17.2 b[c]	49.4 C[d]	29.4 B[d]	0 A[d]
Pear (20 ± 2 °C)	8.7 a	14.0 b	21.0 c	19.7 c	58.7 C	33.3 B	6.3 A
Kiwifruit (20 ± 2 °C)	8.0 a	12.0 ab	18.2 b	18.8 b	56.0 C	34.0 B	0 A

[a] The lesion diameter typical of *P. expansum* was measured on each fruit 4 days after inoculation and treatment by deposition of 25 μl droplets of *P. expansum* conidial suspension and *T. harzianum* conidia (formulated or unformulated) in a wound made on each fruit surface.
[b] The control is the treatment with *P. expansum* and sterile deionized water only in comparison with other treatments with *P. expansum* and formulated *T. harzianum* in invert emulsion or unformulated *T. harzianum* in water or blank formulation of invert emulsion.
[c] Horizontally, means of *P. expansum* lesion diameter developed after treatment on each fruit type that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.
[d] Horizontally, means of percent reduction in lesion diameter of *P. expansum* relative to control obtained after treatment on each fruit species that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.

56.9% (Table 2). Similar reductions ($P \leq 0.05$) in *B. cinerea* lesion diameters relative to the control were obtained for wounded fruit of pear, strawberry, and kiwifruit when they were treated with the formulated *T. harzianum* and compared with the control (Table 2).

3.3. Effect of treatment with T. harzianum on P. expansum-lesion development on three fruit species

Significant differences ($P \leq 0.05$) were obtained in *P. expansum* lesion diameters on wounded fruit of grape, pear, and kiwifruit when they were treated with the formulated *T. harzianum* conidia in the invert emulsion and compared to the controls (Table 3). The mean lesion diameters of *P. expansum* on wounded grape berries significantly reduced ($P \leq 0.05$) to 49.4% in comparison with the control (Table 3). Similar reductions in lesion diameters of *P. expansum* relative to the control were obtained for wounded fruit of pear and kiwifruit when treated with the formulated *T. harzianum* and compared with the control (Table 3).

3.4. Effect of treatment with T. harzianum on the duration of the minimum protection period from infection with postharvest pathogens

The mean duration of the minimum protection period from infection with *R. stolonifer* due to the treatment with formulated *T. harzianum* conidia in an invert emulsion was the longest for apple fruit (59.0 days on unwounded fruit versus 20.0 days on microwounded fruit), and was the shortest

Table 4
Protection period from infection with *R. stolonifer* (strain RS1) on mature fruit of apple, pear, peach and strawberry due to fruit treatment with formulated conidia of *T. harzianum* (strain Th_2) in an invert emulsion (IE)

Fruit type	Mean of minimum time period in days needed for the development of typical *R. stolonifer* lesions[a]				Mean duration of minimum protection period in days from *R. stolonifer* infection due to the treatment[b]	
	On unwounded fruit		On microwounded fruit		On unwounded fruit	On micro-wounded fruit
	R. stolonifer + formulated *T. harzianum* in IE	*R. stolonifer* + blank formulation of IE	*R. stolonifer* + formulated *T. harzianum* in IE	*R. stolonifer* + blank formulation of IE		
Apple (20 ± 2 °C)	87.5 ± 3.7	28.5 ± 2.2	35.0 ± 3.2	15.0 ± 1.7	59.0 ± 2.2 b[c]	20.0 ± 1.7 a[c]
Pear (20 ± 2 °C)	19.8 ± 1.7	13.3 ± 1.5	9.0 ± 1.0	6.0 ± 0.7	6.5 ± 0.5 b	3.0 ± 0.2 a
Peach (20 ± 2 °C)	14.0 ± 1.2	11.0 ± 1.2	5.2 ± 0.5	3.0 ± 0.7	3.0 ± 0.2 a	2.2 ± 0.2 a
Strawberry (20 ± 2 °C)	12.6 ± 1.5	5.1 ± 0.7	5.4 ± 0.2	3.2 ± 1.2	7.5 ± 0.7 b	2.2 ± 0.2 a

[a] This minimum time period in each fruit species was extended from the time of inoculation and treatment by spraying 1.0 ml of *R. stolonifer* conidial suspension and 2.0 ml of formulated *T. harzianum* conidia per fruit until the appearance of first typical lesions to *R. stolonifer* on the surface of wounded (by needle pricks) and unwounded fruit of each type.
[b] Mean duration of minimum protection period in each fruit species was calculated as the difference between the mean of minimum time period for appearance of first typical lesions to *R. stolonifer* on the fruit surface (wounded or unwounded) treated with formulated *T. harzianum* conidia in invert emulsion and that period for appearance of the same typical lesion on fruit (wounded or unwounded) treated with blank formulation of invert emulsion. Standard error (S.E.) of the means was included in the table for each mean (mean ± S.E.).
[c] Horizontally, mean durations of minimum protection period from *R. stolonifer* infection due to treatment on each fruit species that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.

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Table 5
Protection period from infection with *B. cinerea* (strain BC1) on mature fruit of grape, pear, kiwi and strawberry due to fruit treatment with formulated conidia of *T. harzianum* (strain Th_2) in an invert emulsion (IE)

Fruit type	Mean of minimum time period in days needed for the development of typical *B. cinerea* lesions[a]				Mean duration of minimum protection period in days from *B. cinerea* infection due to the treatment[b]	
	On unwounded fruit		On microwounded fruit		On unwounded fruit	On micro-wounded fruit
	B. cinerea + formulated *T. harzianum* in IE	*B. cinerea* + blank formulation of IE	*B. cinerea* + formulated *T. harzianum* in IE	*B. cinerea* + blank formulation of IE		
Grape (20 ± 2 °C)	26.2 ± 2.7	11.3 ± 1.2	15.8 ± 1.7	7.7 ± 0.7	15.6 ± 1.2 b[c]	8.1 ± 1.2 a[c]
Pear (20 ± 2 °C)	19.1 ± 1.5	13.0 ± 1.2	7.3 ± 0.7	5.3 ± 0.5	6.1 ± 0.7 b	2.0 ± 0.5 a
Kiwifruit (20 ± 2 °C)	23.5 ± 2.2	11.5 ± 1.5	8.4 ± 0.5	6.3 ± 0.5	12.0 ± 1.2 b	2.1 ± 0.2 a
Strawberry (20 ± 2 °C)	14.5 ± 1.7	10.2 ± 1.2	9.0 ± 0.5	6.0 ± 0.7	4.3 ± 0.5 a	3.0 ± 0.2 a

[a] This minimum time period in each fruit species was extended from the time of inoculation and treatment by spraying 1.0 ml of *B. cinerea* conidial suspension and 2.0 ml of formulated *T. harzianum* conidia per fruit until the appearance of first typical lesions to *B. cinerea* on the surface of wounded (by needle pricks) and unwounded fruit of each type.

[b] Mean duration of minimum protection period in each fruit species was calculated as the difference between the mean of minimum time period for appearance of first typical lesions to *B. cinerea* on the fruit surface (wounded or unwounded) treated with formulated *T. harzianum* conidia in invert emulsion and that period for appearance of the same typical lesion on fruit (wounded or unwounded) treated with blank formulation of invert emulsion. Standard error (S.E.) of the means was included in the table for each mean (mean ± S.E.).

[c] Horizontally, mean durations of minimum protection period from *B. cinerea* infection due to treatment on each fruit species that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.

for peach fruit (3.0 days on unwounded fruit versus 2.2 days on microwounded fruit), and intermediate for the other fruit species (Table 4).

Similarly the longest mean duration of the minimum protection period from infection with *B. cinerea* was obtained for grape berries, shortest for strawberries, and intermediate for the other fruit species (Table 5). Also, the longest mean duration of the minimum protection period from infection with *P. expansum* was obtained for kiwifruit, shortest for pear, and intermediate for grape berries (Table 6).

Significant differences ($P \leq 0.05$) were obtained for the mean durations of the minimum period of protection on unwounded fruit compared to microwounded fruit of the following fruit species: apple, pear, and strawberry infected with *R. stolonifer*; grape, pear, and kiwifruit infected with *B. cinerea* or *P. expansum* (Tables 4–6).

4. Discussion

It is well-known that *T. harzianum* is an effective antagonistic fungus against many soil borne plant pathogenic fungi such as *Rhizoctonia*, *Sclerotinia*, *Pythium*, and *Fusarium*, and thus certain formulations of this antagonistic fungus, such as Trichodex, Trichoderma 2000, Soil gard, and Supresivit, were produced commercially for this purpose

Table 6
Protection period from infection with *P. expansum* (strain PE8) on mature fruit of grape, pear and kiwifruit due to fruit treatment with formulated conidia of *T. harzianum* (strain Th_2) in an invert emulsion (IE)

Fruit type	Mean of minimum time period in days needed for the development of typical *P. expansum* lesions[a]				Mean duration of minimum protection period in days from *P. expansum* infection due to the treatment[b]	
	On unwounded fruit		On microwounded fruit		On unwounded fruit	On micro-wounded fruit
	P. expansum + formulated *T. harzianum* in IE	*P. expansum* + blank formulation of IE	*P. expansum* + formulated *T. harzianum* in IE	*P. expansum* + blank formulation of IE		
Grape (20 ± 2 °C)	20.3 ± 1.7	10.2 ± 1.2	11.8 ± 1.0	6.5 ± 0.5	10.1 ± 1.2 b[c]	5.3 ± 0.7 a[c]
Pear (20 ± 2 °C)	17.8 ± 1.2	11.5 ± 1.5	9.0 ± 0.7	6.0 ± 0.7	6.3 ± 0.7 b	3.0 ± 0.2 a
Kiwifruit (20 ± 2 °C)	51.9 ± 5.2	14.3 ± 1.7	31.4 ± 3.2	7.2 ± 1.2	37.6 ± 3.7 b	24.2 ± 2.2 a

[a] This minimum time period in each fruit species was extended from the time of inoculation and treatment by spraying 1.0 ml of *P. expansum* conidial suspension and 2.0 ml of formulated *T. harzianum* conidia per fruit until the appearance of first typical lesions to *P. expansum* on the surface of wounded (by needle pricks) and unwounded fruit of each type.

[b] Mean duration of minimum protection period in each fruit species was calculated as the difference between the mean of minimum time period for appearance of first typical lesions to *P. expansum* on the fruit surface (wounded or unwounded) treated with formulated *T. harzianum* conidia in invert emulsion and that period for appearance of the same typical lesion on fruit (wounded or unwounded) treated with blank formulation of invert emulsion. Standard error (S.E.) of the means was included in the table for each mean (mean ± S.E.).

[c] Horizontally, mean durations of minimum protection period from *P. expansum* infection due to treatment on each fruit species that are followed by different letters are significantly different (at $P \leq 0.05$) using ANOVA and Duncan's multiple range test.

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(Harman and Kubicek, 1998; Adekunle et al., 2001; Monte, 2001). The interest in the application of *T. harzianum* for control of foliage phytopathogenic fungi is relatively recent (Falconi and Mendgen, 1994; Hong et al., 1998; Okigbo and Ikediugwu, 2000; Batta, 2004a, 2004b). Moreover, biocontrol of foliage diseases, especially of postharvest type, is usually done with other biocontrol agents such as the following bacterial species: *Pseudomonas putida*, *Pseudomonas cepacia*, *Bacillus subtilis*, *Pantoea agglomerans*, and *Enterobacter cloacae* (Colyer and Mount, 1984; Wilson et al., 1987; Bonaterra et al., 2003), and the following yeasts species: *Cryptococcus laurentii*, *Cryptococcus albidus*, *Pichia membranefaciens*, *Metschnikowia pulcherrima*, and *Candida oleophila* (Lima et al., 1997; Qing and Shiping, 2000; Spadaro et al., 2002; Zhang et al., 2005).

Although the antagonistic bacterial and yeast species used until the present for biocontrol of postharvest fruit rot pathogens have good biocontrol potential and their CFU/ml obtained during fermentation is high and the cost of their production during fermentation is relatively low, the antagonistic fungus *T. harzianum* (strain Th2) formulated in the invert emulsion has shown, in the present and previous research, to have good biocontrol efficacy against postharvest diseases (Batta, 2004a, 2004b). The formulation of *T. harzianum* in an invert emulsion (water-in-oil type) has shown the following characteristics in comparison with the above-mentioned biocontrol agents: (i) the fungus formulation has a good biocontrol efficacy against the tested postharvest fruit rot pathogens in reducing lesion diameters by up to 86.7% (average overall reduction was 66.4%) relative to the controls, depending on the pathogen and fruit species, (ii) the formulation has been shown to have a long duration of the protection period from infection with the tested pathogens, extending to 59 days for fruit species such as apple, (iii) the formulation could be applied directly on fresh fruit without being diluted in water, either through spraying or dipping and no adverse appearance or phytotoxic effect was observed on the treated fruit, since the deposit of the formulation on treated fruit could be easily removed by washing with water, (iv) the fungus in the formulation has a long shelf-life (up to 36 months) with a 50% reduction of the fungus conidial viability (half-life) after 5.3 months of storage at 20 ± 1 °C (Batta, 2004a). In addition, the deposit of the formulation containing the fungus on treated fruit is of a white milky appearance (the emulsion color). In addition, the formulation has no adverse side-effects either on consumer health or on the environment, since the formulation ingredients are usually used in food additives or in manufacturing of cosmetics. The formulation could be used as a fruit treatment at the postharvest stage under dry storage conditions since the nature of the formulation (water-in-oil emulsion) can secure the required water content for conidial germination and development during the application. It is important to note that the most probable mode of action of *T. harzianum* as a biocontrol agent of postharvest pathogens of fruit is direct parasitism by interference with the development and growth of these pathogens after conidial germination by a coiling of mycelium over the pathogen mycelium or by disruption of the host-fungus cell wall and consequent death of the pathogen (Goldman and Goldman, 1998; Monte, 2001).

It is important to mention that the bacterial and yeast preparations that are present in the market and used for biocontrol of postharvest fruit rot pathogens are mostly in dried or powdered forms and should be dissolved in water at the time of application. Therefore, the dry conditions of fruit storage causing rapid water loss are considered limiting factors in the success of these biocontrol agents when used against these pathogens. In contrast, the present formulation of Th2 in the invert emulsion has shown, in the previous research, its efficacy against many phytopathogenic fungi when applied under dry conditions (Batta, 1999, 2004a, 2004b). Moreover, in the present research, a high biocontrol potential reaching up to 86% was obtained on apple fruit incubated at 30 ± 2 °C against *R. stolonifer* as a result of the treatment with the formulation.

Overall, the results indicate a significant biocontrol potential for formulated *T. harzianum* (Th2) in the invert emulsion against the major postharvest pathogens of fruit. High reductions in the disease intensity caused by these pathogens in comparison with the control has been obtained on the fruit species tested. In addition, a long protection period from infection with these pathogens has been obtained by the treatment of the same fruit species with the fungus formulation, thus the decay of these fruit could be prevented or, at least, delayed until reaching the consumer. Similar results have been obtained in previous research on other fruit species when the same formulation was used for the same strain of *T. harzianum*. Significant reductions in the lesion development of *B. cinerea* and *P. expansum* was shown on harvested apple fruit (Batta, 2004a, 2004b).

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