



# Postharvest biological control of apple gray mold by *Trichoderma harzianum* Rifai formulated in an invert emulsion

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## Abstract

An invert emulsion (water-in-oil formulation) based on coconut and soybean oils provided the most stable emulsion layer (93% V/V) and lowest viscosity ( $27\pm 0.81$  cps), suitable for a formulation of *Trichoderma harzianum* conidia. Conidia remained viable for 36 months (shelf-life) with 50% reduction in viability (half-life) after 5.3 months at  $20\pm 1^\circ\text{C}$ ., compared with 2.7 and 0.7 months, respectively, for dry non-formulated conidia. Stability and viscosity of the formulation remained constant during the time period of viability study. Bio-assay tests on wounded apple fruit indicated the presence of significant preventive effect of formulated *Trichoderma* conidia against the causative organism of apple gray mold *Botrytis cinerea*. The diameter of *Botrytis* lesions was significantly reduced ( $P<0.05$ ) 5 days after inoculation and treatment with formulated *Trichoderma* conidia. *Botrytis* sporulation on the lesion surface was also inhibited 10 days after inoculation. These results were confirmed under simulated natural conditions by spraying a *Botrytis* conidial suspension on microwounded apple fruit following treatment by dipping fruit.

Treatment of healthy non-wounded apple fruits resulted in protection from *Botrytis* infections for >2 months following inoculation and treatment.

## Keywords

- *Trichoderma harzianum*;
- *Botrytis cinerea*;
- Invert emulsion;
- Apple fruit;
- Biological control;
- Bio-assay tests

Figures and tables from this article:

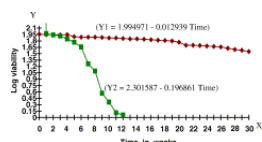


Fig. 1. Log viability versus time of formulated (Y1) and non-formulated (Y2) *T. harzianum* conidia (strain Th<sub>2</sub>) stored at 20±1°C and 30% ambient R.H. (equations indicated above were calculated from log linear regression analyses).

### [Figure options](#)

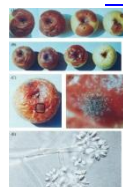


Fig. 2. Biocontrol of *Botrytis cinerea* on apple fruits using *Trichoderma harzianum* (strain Th<sub>2</sub>): (A) significant preventive effect of formulated *T. harzianum* conidia against localized infection with *B. cinerea* on wounded apple fruits compared to non-formulated *T. harzianum* conidia, control with sterile distilled water, and control with formulation without conidia (blank), respectively, from right to left for the four treatment types; (B) as in A, but apple fruits were microwounded with needle pricks, treated by fruit dipping in the four treatment types and then inoculated by spraying *B. cinerea* conidial suspension on the microwounded fruits; (C) sporulation of *B. cinerea* on the lesion surface after inoculation and treatment on wounded apple fruits; (D) enlarged square area in C (20×) showing topical view of aggregated *Botrytis* conidia borne on aerial conidiophores emerging from the lesion surface of inoculated fruits; and (E) typical *B. cinerea* conidiophores showing the main stem of conidiophores with four branches; each branch is terminating with a cluster of typical *Botrytis* conidia (magnification 400×).

### [Figure options](#)

Table 1. Screening for selection of formulation of invert emulsion for *Trichoderma harzianum* (strain Th<sub>2</sub>)



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Table 2. Viability of *T. harzianum* conidia (strain Th<sub>2</sub>) in dry non-formulated form and in formulated form using formulation 9<sup>a</sup>; the formulation contained the fungus and the fungal dry form at 20±1°C and 30% ambient RH



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Table 3. Development of *Botrytis* lesions on wounded apple fruit 5 days after localized infection with *B. cinerea* (strain: B20) and treatment with *T. harzianum* (strain Th<sub>2</sub>); incubation of fruit at 20±1°C



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Table 4. Evaluation of infection with *B. cinerea* (strain B20) after spraying *Botrytis* conidial suspension on pre-treated apple fruits with *T. harzianum* (strain Th<sub>2</sub>) by fruit dipping method; incubation of the fruit after inoculation and treatment at 20±1°C for different periods of time



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