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Biological control of tomato grey mould with compost water extracts, *Trichoderma* sp., and *Gliocladium* sp.

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Summary. Compost water extracts prepared from animal sources (horse, sheep, and cattle) and a plant source (olive) were tested for their control of leaf grey mould on tomato. Disease was reduced by 27–66% and by 41–70% by extracts after 7 days and 15 days of fermentation respectively. No effect was noted with extracts after only 10 hours of fermentation. Conidial suspensions of *Trichoderma harzianum*, of *Gliocladium* sp. and compost extracts derived from horses achieved similar and significant ($P<0.05$) reductions in grey mould as compared to the water control. Dilution of extracts did not impair extract effectiveness if they were derived from horse and ovine compost. Pasteurization of extracts significantly reduced their effectiveness, particularly in the case of olive, but compost extracts from other sources achieved significant grey mould reduction despite pasteurization.

Key word: biocontrol, *Botrytis cinerea*, compost, *Gliocladium* sp., *Trichoderma harzianum*.

Introduction

Grey mould is an important disease caused by *Botrytis cinerea*. It causes great damages in greenhouses crops grown under conditions of high humidity. Despite efforts to control grey mould, it continues to be a threat to farmers. Chemical fungicides are still the most important means of control in North Africa but the rapid emergence of resistant strains of *B. cinerea* has reduced fungicide effectiveness and consequently their constant use (Hmouni *et al.,* 1996; Hmouni *et al.,* 2003). The discovery that some micro-organisms, such as *Trichoderma* spp. and *Gliocladium* spp. have an antagonistic potential has opened the way to other means of control more respectful of nature. It may be possible to apply biocontrol agents against many pathogenic fungi and especially against *B. cinerea* (Cook and Baker, 1983; Elad *et al.*, 1996). Nevertheless, only a few studies have so far examined the utilization of biological control alternatives against *B. cinerea* under Moroccan conditions (Hmouni *et al.,* 1996; Hajlaoui *et al.,* 1997; Hmouni *et al*, 1999). In addition, the disease-suppressive characteristics of composts has recently suggested that they too may be used as a crop protection tool suitable for both organic and conventional producers. Attempts have been made to control grey mould and other pathogens with compost water extracts from animal (horse, chicken, cattle, etc.) and plant sources (Weltzien and Ketterer, 1986; Trankner, 1992; Weltzien, 1992; Elad and Shtienberg, 1994; Malathrakis *et al.,* 1995; Al-Dahmani *et al.,* 2003).

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The objective of the present work was to study the effect of compost water extracts of animal or plant origin on tomato grey mould caused by *B. cinerea*, and to compare them with the biocontrol activity of *Trichoderma harzianum* and *Gliocladium* sp.

Materials and methods

Organisms and growth conditions

Botrytis cinerea (B13) was isolated from naturally infected tomato leaves obtained from the region of Fouarat (Kénitra) and was maintained on potato dextrose agar (PDA) in darkness at 22°C.

The antagonistic fungi, previously tested against *B. cinerea,* were maintained in the same culture conditions as the pathogen. Conidial suspensions were prepared by washing 5- day-old cultures, and were adjusted to 10^6 spores ml^{-1}. The *Trichoderma harzianum* isolate is referred to as TH20 and the *Gliocladium* sp. isolate as G1 (Hajlaoui *et al.,* 1997; Hmouni *et al*, 1999)

The tomato plants (*Lycopersicon esculentum* Mill. Campbell) in the test were 5 weeks old (3 to 4 leaves). Plants were maintained in a plastic greenhouse.

Preparation of composts water extracts

Composts were prepared from sheep, cattle or horse manure and are designated as Cpovi, Cpbov and Cpequ, respectively. The compost extract of plant origin was prepared from solid manure obtained after extraction of olive oil and are designated as Cpoli. The compost was mixed with water in a ratio of 1:5 (v:v) in open polyethylene containers. The compost mixtures were stirred, homogenized and incubated at 20±2°C for the desired fermentation time (Elad and Shtienberg, 1994). Unless otherwise indicated, the time of fermentation was two weeks. Immediately before use, compost extracts were filtered two times through cheesecloth.

Compost extracts were compared with the biocontrol activity of TH20 and G1 previously tested *in vitro* against *B. cinerea* (Hajlaoui *et al.,* 1997; Hmouni *et al.*, 1999).

To evaluate the effect of fermentation on extract activity against *B. cinerea*, we tested 3 incubation times: 10 hours, 7 days and 15 days. In order to increase the volume of extracts, dilutions with tap water to 1:2, 1:4 and 1:10 were done before spraying.

For other tests, extracts were pasteurized by heating for 1 h at 100°C on two consecutive days, and were then sprayed on tomato leaves (Elad and Shtienberg, 1994).

Inoculation of tomato plants

Plants sprayed with compost extracts or with suspensions of antagonistic isolates (10^6 spores ml^{-1}), were inoculated 24 hours later with a conidial suspension of *B. cinerea* (10^5 conidia ml^{-1}, from two-week-old cultures) supplemented with 0.02 M glucose and 0.04 M KH_2PO_4 (Leone and Tonneijck, 1990). Controls were sprayed with tap water, or with tested extracts alone, or with conidial suspensions of the antagonistic fungi tested.

Inoculated plants were placed under plastic bags or in a dew chamber to promote plant infection by *B. cinerea*. Ten days later, disease severity was evaluated on a 5 point scale where 0=healthy plant; 1=1–5% of plant covered by rot; 2=6–15% ; 3=16–50% ; 4=51–95% and 5=host tissue completely destroyed (Elad and Shtienberg, 1994).

Plants were arranged in a randomized block design. Each treatment consisted of at least six replicates. Each experiment was repeated at least twice. Results were analyzed statistically and means separated *(P*=0.05) according to the Newman Keuls test.

Results

Effect of fermentation time on effectiveness of compost extracts

After 10 hours of fermentation, the water extracts sprays did not significantly protect plants from *B. cinerea* infection (Fig. 1). The infection index after 10 hours ranged from 3.17 with Cpequ extract to 3.92 for the control. After 7 days of fermentation a significant reduction in disease severity was obtained with all extracts, the grey mould index ranging from 1.33 for Cpequ and to 2.83 for Cpoli.

Compared with extracts fermented for seven days, extracts fermented for 15 days further reduced significantly the severity of grey mould as compared with the control. Cpbov was however the only extract in which this further reduction became significant. We therefore decided to use

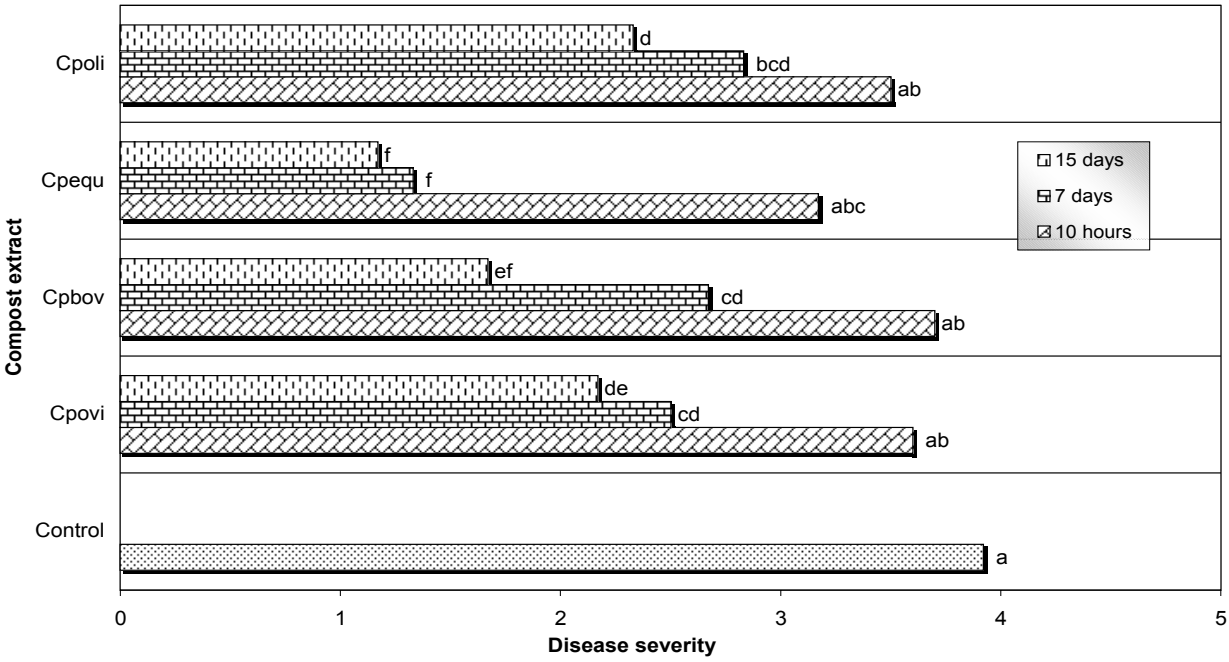

Fig. 1. Effect of fermentation time on compost extract effectiveness against *Botrytis cinerea* on tomato plants. Controls were sprayed with tap water before inoculation with *B. cinerea*. Grey mould severity was evaluated according to a 5 point index where 0, healthy and 5, completely rotted leaves. Treatments with different letters differed significantly (P=0.05) according to the Newman Keuls test.

only extracts fermented for 15 days in the remaining tests.

Comparison of the effectiveness of compost extracts, *Trichoderma* and *Gliocladium*

Cpequ showed the greatest control activity, with a disease index of 1.17, compared to 3.92 for the control (Fig. 2). *Trichoderma* and *Gliocladium* showed disease index values in between, but which were also significantly different from the control.

Effect of dilution on composts extracts activity

Control effectiveness decreased with increasing dilution of the compost extracts (Fig. 3). The reduction level however also depended on the extract tested. Cpoli was effective only at full concentration, but Cpequ had control effectiveness even after a 1:10 dilution. Cpbov extract diluted to 1:4 did not lose its effectiveness but it did when diluted to 1:10. Similar results were obtained with Cpovi, except that it was still efficient even at a dilution of 1:10.

Effect of pasteurization on extracts effectiveness

Pasteurization of compost extracts (100°C for 1 h on two successive days) significantly reduced effectiveness (Fig. 4). However, the reduction in control effectiveness was not total and the level of inhibition remained significant.

It should be noted that the tomato plants sprayed with compost extracts did not show any symptoms of stress or damage.

Discussion

That compost extracts could control foliar diseases was suggested by a number of authors and composts have been tested on various host-pathogen systems such as: *Uncinula necator* and *Pseudopeziza tracheiphila* on grapevine; *Erysiphe graminis* on barley and *E. betae* on beet; *Sphaerotheca fuliginea* on cucumber; *Phytophthora infestans* on potato and tomato; *B. cinerea* on strawberries and on bean; and *Fusarium* spp. on oats (Weltzien and Ketterer, 1986; Achimu and Schlöss-

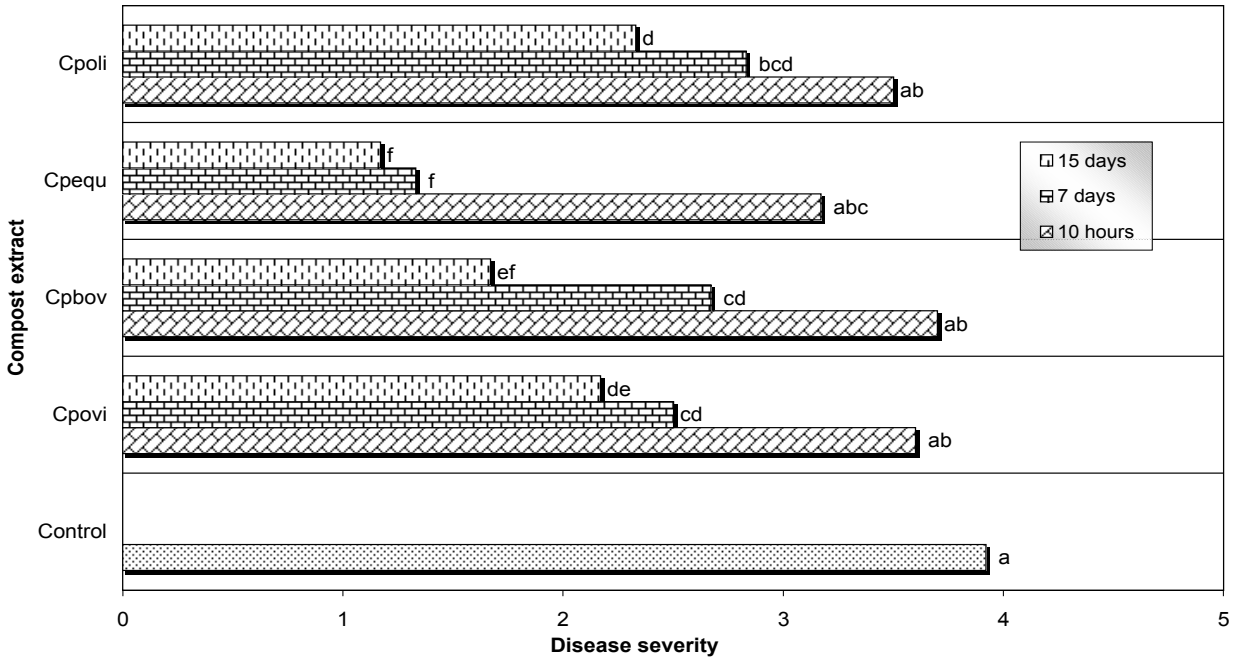

Fig. 2. Control of tomato grey mould by *Trichoderma harzianum* (TH20), *Gliocladium* (G1), and compost extracts (15 days of fermentation). Controls were sprayed with tap water before inoculation with *Botrytis cinerea*. For disease scale and statistical analysis of data see caption in Fig. 1.

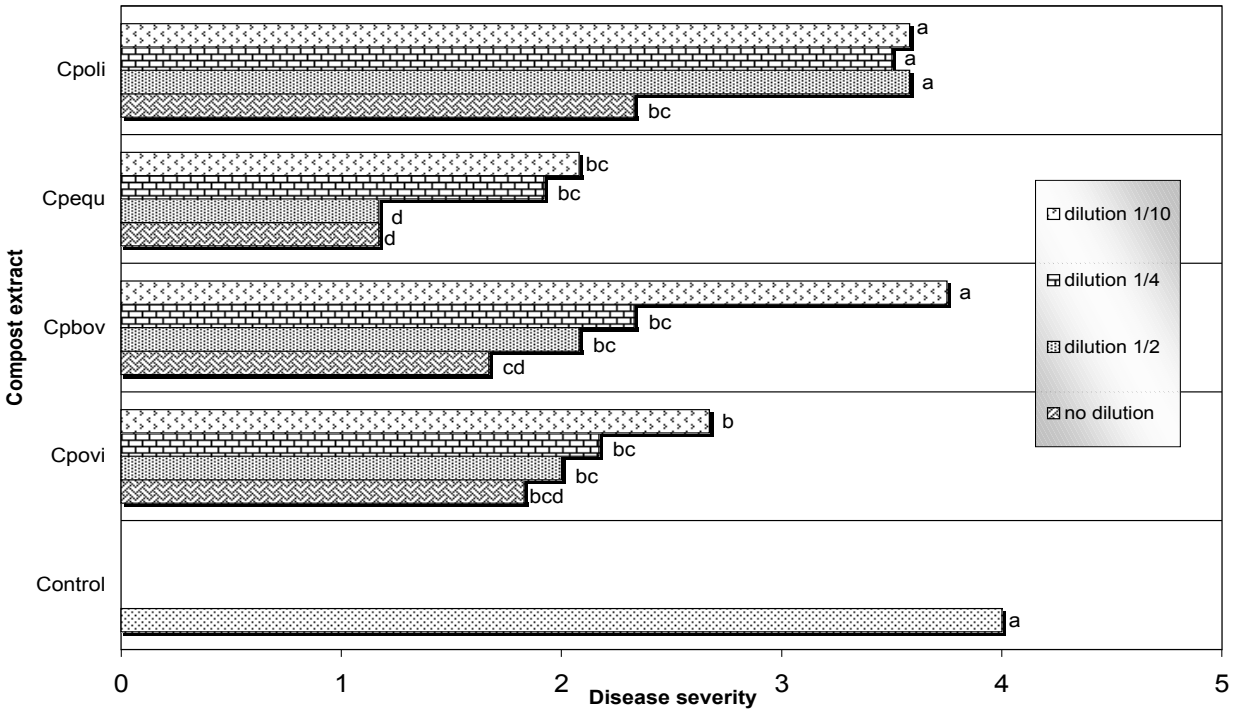

Fig. 3. Effect of dilution on compost extract effectiveness against *Botrytis cinerea* on tomato plants. Controls were sprayed with tap water before inoculation with *B. cinerea*. For disease scale and statistical analysis of data see caption in Fig. 1.

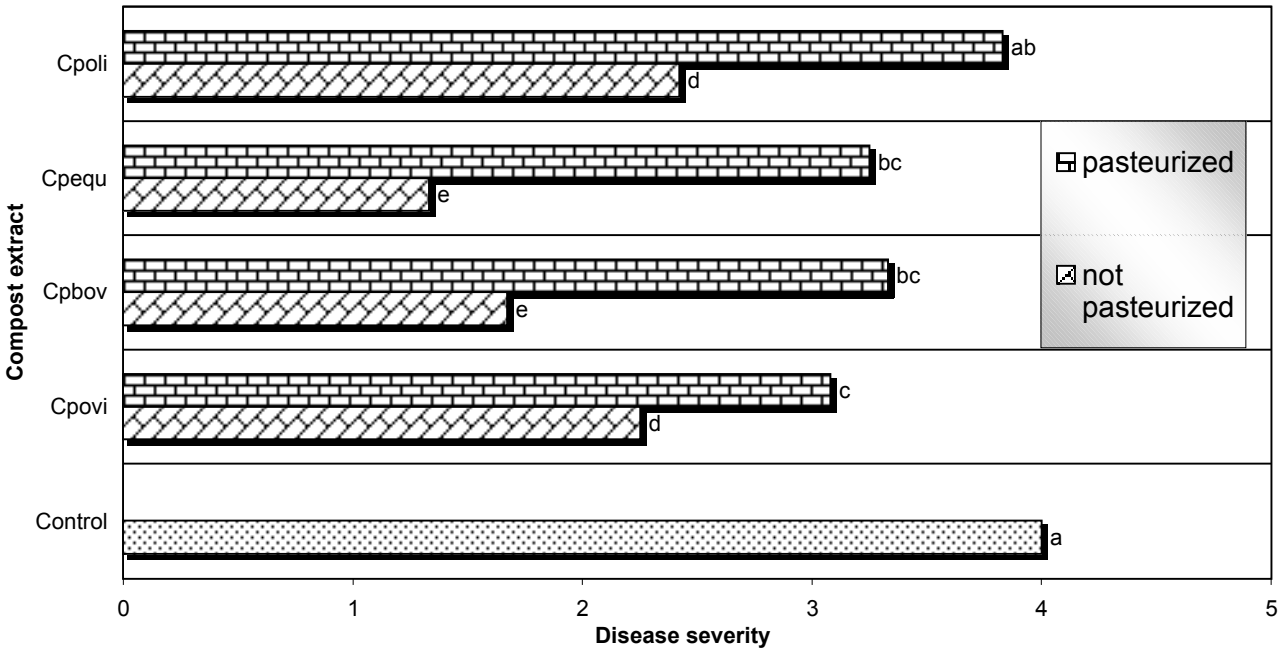

Fig. 4. Effect of pasteurization of compost extracts on their control of grey mould. Controls were sprayed with tap water before inoculation with *Botrytis cinerea*. For disease scale and statistical analysis of data see caption in Fig. 1.

er, 1992; Trankner, 1992; Elad, 1993; Elad *et al*., 1996; Boyd-Wilson and Walter, 2002).

All the extracts in our study significantly reduced the severity of gray mould on tomato plants though the degree of reduction varied with the source of the extract, and with the time of fermentation. The greatest protection was achieved with a fermentation time of 7 days and still more by fermentation for 14 days. These durations agreed with those reported by Elad and Shtienberg (1994) who stated that the optimal fermentation time was longer than 10 days. These authors also found that certain compost extracts became effective for control purposes after only 4 hours of fermentation, but in our study compost extracts fermented for 10 hours were ineffective.

Pasteurization of extracts did not destroy their capacity to control grey mould except in the case of olive compost extract. Elad and Shtienberg (1994) reported similar results for *B. cinerea* on tomato. By contrast, McQuilken *et al*. (1994) found that only if manure extracts were sterilized twice did they lose their activity against *B. cinerea*. Weltzien (1992) noted that sterilized extracts of a short fermentation time lost their effectiveness against *B. cinerea*, while those sterilized filtrates extracts that had been fermented for 16 days remained as active as the non-filtered controls. The inhibitory activity of compost extracts is affected though not destroyed completely by heat treatment when they are tested against many pathogens (Nelson and Hoitink; 1983; Craft and Nelson, 1996; Zhang *et al.,* 1998; Aryantha *et al*., 2000; Reuveni *et al*., 2002; Rose and Parker, 2003). These findings suggest that the extracts contain heat-stable inhibiting compounds that accumulate during fermentation via the action of micro-organisms present in the extracts (Zhang *et al.,* 1998; Boyd-Wilson *et al*., 2000; Boyd-Wilson and Walter, 2002).

That composts can be used as a source of antagonistic organisms was an interesting suggestion of Elad and Shtienberg (1994) and Malathrakis *et al*. (1995), who isolated bacterial strains from composts that were effective against *B. cinerea* and other pathogens. The amendment of composts with specific antagonists may be a valuable means to enhance their effectiveness against plant diseases suppression (Pharand *et al*., 2002; Krause *et al.,*

2003). In order to profit from this postulated microbial activity, nutritional supplements have been added to test composts during fermentation. The results obtained were however controversial (Urban and Trankner, 1993; Elad and Shtienberg, 1994; Malathrakis *et al*., 1995). Greenhouse tests of extract effectiveness against grey mould, revealed that they significantly reduced the disease, but were not as effective as vinchlozoline (Elad and Shtienberg, 1994). However, composts suppressed dollar spot (*Sclerotinia homoecarpa*) of turfgrass to levels not significantly different from those achieved by the fungicide-treated controls (Boulter *et al*., 2002).

Multiple applications of composts may reduce the incidence and severity of grey mould to levels where chemical control can be reduced or eliminated (Mills *et al.,* 2002). In addition, Elad *et al*. (1996) noted in tests applying extracts against *B. cinerea* that these extracts also reduced an attack of powdery mildew (*Leveillula taurica*) on tomato. However, under a severe epidemic of powdery mildew, extract treatment only delayed but did not prevent the onset of powdery mildew.

Besides their utilization as a source of antagonistic organisms, extracts can also conceivably be combined with other methods such as biocontrol preparations. Alternating fungicides with biocontrol preparations can reduce a dependency on chemical control. Such a combination may also reduce toxic residues and the exposure of *B. cinerea* to fungicides.

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