EFFECTIVENESS OF COMPOST TEA FROM SPENT MUSHROOM SUBSTRATE ON DRY BUBBLE (*LECANICILLIUM FUNGICOLA*)

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ABSTRACT

Dry bubble, caused by the fungus *Lecanicillium fungicola* (Preuss) Zare & W. Gams, is a serious and common disease of white button mushroom. The most widely used control method against this disease is strict hygiene in the growing installation accompanied by the application of prochloraz. However, the gradual decrease in the sensitivity of *Lecanicillium fungicola* to prochloraz [1] and the relatively short persistence of the fungicide [2] mean that alternative methods of control need to be found. Among biocontrol methods considered as an alternative to chemical products is the use of aqueous extracts (compost tea) made from agricultural wastes. Indeed, the results obtained with this method to control dry bubble have proved encouraging in *in vitro* trials [3].

Here, we study the possibility of using compost tea made with spent mushroom substrate as a biocontrol method against the disease. Its effectiveness was evaluated in a mushroom growth cycle artificially infected with *L. fungicola*. Two compost teas were tested, one obtained from spent mushroom substrate with mineral soil as casing layer and the other with peat. One, two or three drench applications with these compost teas were carried out. Three controls were used: one pure control, one inoculated with *L. fungicola*, and the other inoculated and treated with prochloraz. In all treatments the yield of mushrooms, with and without *L. fungicola*, was calculated. The application of the compost tea made from substrates using peat was the most effective. In this treatment, infected mushrooms represented 14.6 to 26.2% of the total crop, while in the control to which prochloraz was applied 35.6% of mushrooms showed signs of the disease. The best results were obtained when the compost teas were applied close to the beginning of harvesting.

Keywords: Biological control; Compost tea; Spent mushroom substrate; *Lecanicillium fungicola*; *Agaricus bisporus.*

INTRODUCTION

Verticillium fungicola (Preuss) Hassebrauk, recently classified as *Lecanicillium fungicola* (Preuss) Zare & W. Gams [4], is the causal agent of dry bubble disease and the principal micoparasitic fungus of white button mushroom [*Agaricus bisporus* (Lange) Imbach] in Spain [5]. Methods of control include the application of fungicides (prochloraz) and the observation of strict measures of hygiene. However, some data confirm that the sensitivity of *L. fungicola* to prochloraz is gradually diminishing [1], and that its persistence in the casing layer falls considerably at the end of the second flush [2]. Taken together, these observations suggest that protection against dry bubble is less than desirable. It must also be borne in mind that the voluntary withdrawal of prochloraz has been suggested during the evaluation of active substances set in motion by the EU (Directive 91/414/ECC). This implies that in the near future mushroom crops will be left without protection against dry bubble, so that present day management strategies should include reinforcing hygiene practices accompanied by a search for new control methods.

The use of water-based composts of agricultural wastes has been proposed as an alternative biocontrol to the use of chemical products in the control of foliar pathogens [6, 7]. The bibliography consulted in this respect includes mention of the use of compost teas in the fight against *L. fungicola*. For example, Dianez *et al*. (2006), obtained good results in controlling nine pathogens, among them the mycopathogen *L. fungicola* using several grape marc aerated compost teas. Gea *et al*. (2009*)* analysed the *in vitro* efficacy of several non-aerated compost teas (NACT) obtained from agricultural wasted for controlling *L. fungicola*. The results obtained provide hope that dry bubble disease can be controlled by the use of compost teas made from agricultural wasted with no pre-sterilisation step.

In this work, we present the results obtained concerning the *in vitro* efficacy of compost tea made from spent edible mushroom substrate against *L. fungicola* and concerning its use for controlling dry bubble disease in mushroom crops artificially infected with *L. fungicola*.

MATERIALS AND METHODS

***In vitro* efficacy of compost tea against *L. fungicola*.** Spent mushroom substrate (SMS) mixed with peat moss in a 1:1 (v/v) proportion was used to prepare the compost tea. The ratios of compost to water were 1:4 and 1:8 (w/v), while fermentation times were 1, 7 and 14 days. The mixtures were incubated at 20ºC without stirring and were finally filtered through two layers of muslin. Three different culture media were prepared with each of the six compost teas obtained, using 1.5% agar-water as basic medium (1:1, v/v). The first of these media was elaborated by mixing the basic medium with compost tea with no treatment (CT). The second was prepared by mixing the basic medium with the compost tea, previously autoclaved for 20 minutes at 121ºC (AT). The third medium was elaborated from the basic medium mixed with microfiltered compost tea (MT), first at 25 μm and then through Millex® 0.22 μm filters. A control with 1.5% agar-water and sterile water (1:1, v/v) and a positive control (FC) with the same agar and the fungicide prochloraz-manganese 46% WP (1:1, v/v) (Sporgon®, AgrEvo, Valencia, Spain), giving a final concentration of 50 ppm of active ingredient (a.i.), were also prepared. For each compost tea treatment and control, five Petri dishes were inoculated centrally with a 5 mm diameter mycelial disc of *L. fungicola* [7, 8]. Three different isolates of *L. fungicola* per treatment and control were used. Two perpendicular colony diameters were measured on each dish after incubation in the dark at 20ºC for 12 days. The experiments were carried out in duplicate.

The results are expressed as percentage inhibition of mycelium growth of the *L. fungicola* isolates for each of the treatments assayed with respect to mycelium growth obtained with the sterile water control. The mean values were examined using analysis of variance (ANOVA) after transformation. Significance of treatments was determined using the Tukey-HSD test ($P = 0.05$). Data were analyzed using Statgraphics® Plus v. 5.1 (Statistical Graphics Corp., Princeton, NJ).

Effectiveness of compost tea in a mushroom crop artificially infected with *Lecanicillium fungicola*. The compost tea used was made from SMS (60% *Agaricus* SMS and 40% *Pleurotus* SMS) treated with steam at 70ºC for 12 hours to eliminate pathogenic organisms. The material was then re-composted for 57 days. The ratio of compost to water was 1:4 (w/v), while fermentation time was one day. The mixture was incubated at 20ºC without stirring and was finally filtered through two layers of muslin.

A cropping trial was set up in an experimental mushroom growing room, according to standard practices used in mushroom farms in Spain. *A. bisporus* was cultivated in experimental trays (16 l in volume, 870 cm^2 in area) filled with 6 kg of commercial mushroom compost spawned at 1% (Gurelan 45 strain, Gurelan S. Coop., Huarte, Pamplona, Spain). Spawn-run took place for 15 days in a cropping room set at 25ºC and 95% relative humidity (RH). On day 0 of

the cropping cycle, trays of spawn-run compost were cased with a 30 mm layer of a casing soil made with mineral soil + *Sphagnum* peat 4:1 (v/v). One day after casing (day 1), a spore suspension of *L. fungicola* (10^6 spores ml^{-1}) was sprayed onto the surface of the casing layer (120 ml per m^2).

Two different compost teas were used: one obtained from an SMS in which mineral soil + sphagnum peat 4:1 (v/v), denominated "*mineral soil*" was used as casing layer , while the other was obtained from a substrate with a casing based on Topterra®, type peat, which we shall refer to as "*peat*". The compost teas were applied at a rate of 1.2 litres per m^2 at the following times: the first (R1) was applied with the irrigation water on the same day as casing (day 0), the second (R2) three days after casing (day 3), and the third (R3) seven days after casing (day 7). Three controls were used: one inoculated with spores of *L. fungicola*, in which irrigation was carried out with water alone (CI); another, also inoculated, but including the fungicide prochloraz at the third irrigation (P); and a pure control (C), consisting of water and no spores. A randomised complete block design with six replicates was used.

The effectiveness of the compost against *L. fungicola* was tested during the first three flushes (F1, F2 y F3), by comparing the productions of healthy mushrooms and those infected with *L. fungicola* obtained in each treatment, with the production obtained for the controls.

RESULTS AND DISCUSSION

In vitro* efficacy of compost tea against *L. fungicola. Table 1 depicts the mean percentage of mycelial growth inhibition for the three isolates of *L. fungicola* treated with compost teas and the fungicide prochloraz. The grouped data show the behaviour of the *L. fungicola* isolates in each of the three culture media prepared with compost tea (CT, AT and MT) and with the fungicide prochloraz (FC). Table 1 also shows the effect of the extraction times used (1, 7 and 14 days) to obtain the compost teas and the effect of the dilutions of compost and water on *L. fungicola* mycelium growth.

Table 1: Effects of treatments with compost teas and the fungicide prochloraz, extraction times and dilution on growth of mycelium of three isolates of *L. fungicola*.

		n	Mean ± Standard deviation[2]
Effect of treatment with compost tea and fungicide on growth of *L. fungicola*	CT[1]	360	95.74 ± 9.79 c
	AT[1]	360	11.51 ± 8.50 b
	MT[1]	358	6.99 ± 8.33 a
	FC[1]	360	95.34 ± 6.78 c
Effect of extraction times (days) on *L. fungicola* mycelium growth	1	120	100.00 ± 0.0 b
	7	120	98.94 ± 16.19 b
	14	120	88.39 ± 13.81 a
Effect of dilution on *L. fungicola* mycelium growth	1:4	180	96.12 ± 10.25
	1:8	180	95.37 ± 9.57

[1]CT: culture medium prepared with 1.5% agar-water and filtered compost tea (1:1, v/v); AT: culture medium prepared with 1.5% agar-water and compost tea autoclaved for 20' at 121ºC (1:1, v/v); MT: culture medium prepared with 1.5% agar-water and microfiltered compost tea (1:1, v/v); FC: culture medium prepared with 1.5% agar-water and prochloraz 46% in manganese complex (1:1, v/v).
[2]Meand followed by the same letter do not differ significantly ($P \leq 0.05$) according to Tukey-HSD test.

The percentage of inhibition was lowest in the microfiltered (MT) and autoclaved (AT) compost teas (7 and 15%, respectively), while the inhibition attained with filtered tea (CT) was 96%, which exceeds that obtained with the fungicide prochloraz (FC). This shows that the compost

teas obtained by autoclaving and microfiltration lose a substantial part of their activity, hence their little effect on mycelium growth, suggesting that the inhibition is produced by the presence of microorganisms in the aqueous extracts which would compete for the nutrients and space [7]. Therefore, processes like microfiltration and heat sterilisation eliminate these microorganisms from the teas, diminishing their ability to suppress the disease [6]. In contrast, Yohalem *et al*. (1994) found that aqueous extracts of spent mushroom composts fermented anaerobically maintained their inhibitory properties after autoclaving and microfiltration, even maintaining their effect on the germination of *Venturia inaequalis* conidia for at least four months when stored at a -20 ºC, at 4 ºC and at room temperature. Diánez *et al*. (2006) obtained up to 60% inhibition of *L. fungicola* using a compost tea of microfiltered grape marc incubated for one day.

As can be seen in Table 1, the best results were obtained with extraction times of 1 and 7 days, whereas Diánez *et al*. (2006) obtained 100% growth inhibition of *L. fungicola* mycelium after two week extraction. Several studies have indicated that suppression of disease varies widely with the fermentation time, increasing with fermentation times up to a maximum level, after which it decreases [6]. In this sense, Weltzien (1991) suggested that a 5-16 day extraction period is necessary to reach a given level of control over the disease, although the ideal fermentation time must be established for each host-pathogen-type compost used [6].

As regards the dilutions studied, no statistically differences were found, and in both cases high inhibition percentages were obtained. Scheuerell & Mahaffee (2002) maintained that it was unclear how the compost/water ratio influences disease suppression, and suggested a maximum dilution level of 1:10.

Effectiveness of compost tea in a mushroom crop artificially infected with *Lecanicillium fungicola*. The healthy mushroom yields obtained with the different treatments are shown in Fig. 1. As expected, the lowest yields (9.36 kg/m^2) were obtained with the control inoculated with *L. fungicola* (CI), and the highest (16.86 kg/m^2) with the pure control (C). In general it can be seen that the treatments involving compost teas based on peat provided better results (12.71-14.35 kg/m^2) than those based on mineral soil (9.77-12.32 kg/m^2). It should also be noted that the yields obtained with all the compost tea treatments based on peat and treatment 23 using mineral soil-based compost tea were higher than those obtained with the prochloraz control (11.19 kg/m^2).

Fig. 2 shows the yields of mushrooms with *L. fungicola* obtained with the different treatments. This time, the control inoculated with *L. fungicola* (CI) provided the best results (8.26 kg/m^2), while dry bubble hardly appeared in the pure control (0.1 kg/m^2). The yields of mushrooms with *L. fungicola* harvested in the treatments with peat-based compost tea (2.45-4.51 kg/m^2) were lower than those obtained with the mineral soil-based compost teas (3.80-6.18 kg/m^2). In both cases the figures are lower than those for the inoculated control. A comparison of the results obtained with compost teas and those obtained with the prochloraz treatment (P) (6.09 kg/m^2) shows that this last treatment led to a higher production of mushrooms with *L. fungicola*, with the exception of the mineral-soil based treatment R1. That is, the application of irrigation containing compost teas, especially those based on peat, controls dry bubble better than the application of the fungicide prochloraz. It can also be seen that irrigation with compost tea applied on days close to harvest (irrigations 2 and 3) favour disease control even further.

Taking into account that the phytotoxic effect of the compost teas was not very pronounced, and that the control of *L. fungicola* obtained with them was even better than that obtained using the fungicide prochloraz, such compost teas made with the substrates of edible mushrooms can be recommended as an alternative means of dry bubble disease control.

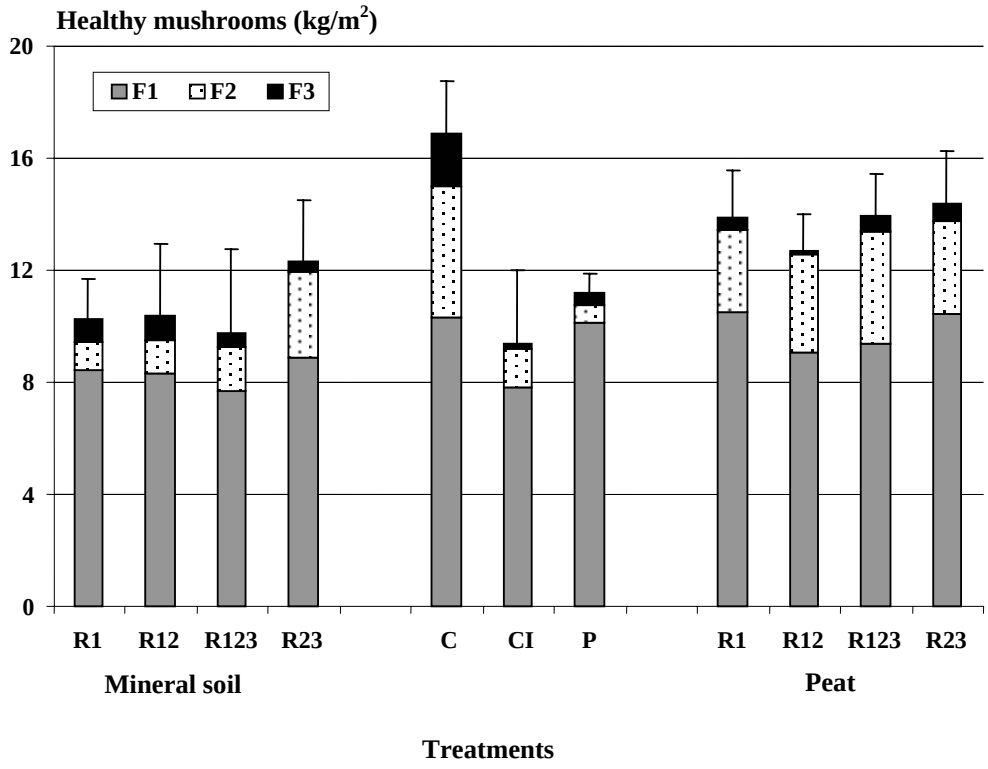

Figure 1: Healthy mushroom production for each of the treatments

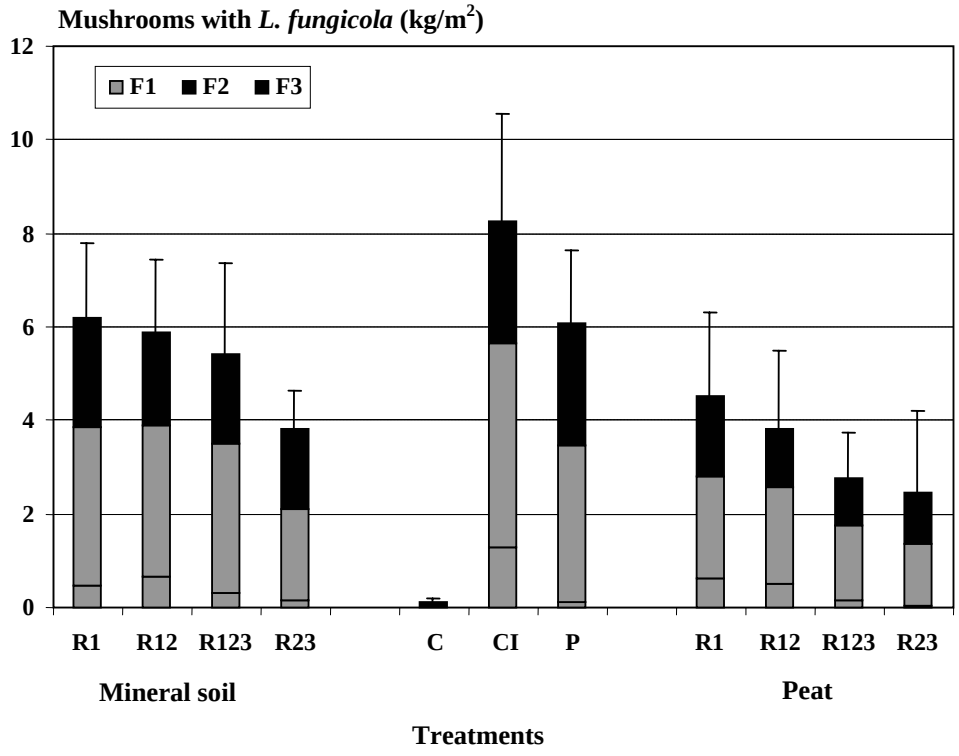

Figure 2: Production of mushrooms with *Lecanicillium fungicola* for each treatment

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