TOXICITY OF COMPOST TEA FROM SPENT MUSHROOM SUBSTRATE AND SEVERAL FUNGICIDES TOWARDS *AGARICUS BISPORUS*

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ABSTRACT

The control methods used against fungal diseases in mushroom crops usually involve the application of fungicides and strict hygiene practices. The availability of fungicides within the mushroom industry is limited not only by strict regulations (Directive 91/414/ECC) but also by the fact that both pathogen and crop are fungi. The use of aqueous extracts (compost tea) made from agricultural wastes has been proposed as a potential biological control method to reduce the use of chemical products [1, 2].

In the present investigation, we evaluate the toxicity of several fungicides (carbendazim, iprodione, thiophanate-methyl, prochloraz and thiabendazole) used during mushroom production, and the resulting residue levels. The toxicity of spent mushroom compost tea, applied to the casing using different irrigation programmes, was also examined. A mushroom crop cycle was run and the yield and earliness of the crop were evaluated. All the fungicides, except thiabendazole, led to reductions in yields that varied from 4% (carbendazim) to 15% (iprodione). Higher MRL values than are permitted were detected with carbendazim, iprodione and thiophanate-methyl, while no such problem was observed with prochloraz and thiabendazole. Mushroom production fell by between 4% (one application) and 10% (three applications) when compost tea was used. Compost tea also slightly delayed the production - by one day when three applications were made. Regardless of this, the less pronounced decrease in yield and the absence of any residue-related problems suggest that compost tea is suitable for mushroom cultivation. Its effectiveness observed *in vitro* for controlling *L. fungicola* would also suggest that spent mushroom compost tea can be considered a suitable biocontrol substance for use against dry bubble disease.

Keywords: Toxicity; Fungicides; Compost tea; Biological control; *Agaricus bisporus*

INTRODUCTION

There are several reasons for using compost tea made from spent mushroom substrate (SMS) for the biological control of dry bubble disease, among them the promising results mentioned in the literature with water-based composts made from agricultural wastes, which have been proposed as an alternative to chemical products for the control of foliar pathogens [1, 3]. Favourable results have also been obtained *in vitro* with several compost teas made from agricultural wastes (SMS mixed with amended light peat, olive oil husk + cotton gin trash composted and mixed with rice husk, grape marc compost and cork compost) against the fungus *Lecanicillium fungicola* [2]. The ease with which such products can be obtained should also be taken into account as well as the revision process of active substances set in motion by the European Union,

which will inevitably lead to the withdrawal of several fungicides at present authorised for use with mushrooms.

Before using the compost tea made from SMS as biocontrol substance we evaluated its toxicity towards mushroom mycelium. A comparative analysis was also made with five fungicides, two authorised in Spain (iprodione and procloraz) for use in mushroom and three that are included in Annex I of Directive 91/414/EEC (carbendazim, thiophanate-methyl and thiabendazole). Lastly, an analysis was made of the residues found in harvested mushrooms to ascertain whether any of the fungicide treatments exceeded the permitted maximum residue limits (MRL).

MATERIALS AND METHODS

Phytotoxic effect of compost teas on mushroom mycelium. The compost tea used was made from SMS (60% *Agaricus* SMS and 40% *Pleurotus* SMS) treated with steam at 70 ºC for 12 hours to eliminate pathogenic organisms. The material was then re-composted for 57 days. Table 1 shows the physical, chemical and biological characteristics of the SMS after re-composting.

Table 1: Physical, chemical and biological characteristics of the SMS after re-composting

Parameter	Value
Bulk density $_{fresh}$ (g/ml)	0.609
Moisture (%)	50.4
pH 1:5 (w/v)	7.8
Electrical conductivity$_{25}$ 1:10 (w_{dry}/v) (µS/cm)	5,265
Nitrogen (%)	1.34
Ash (%)	64.62
Organic matter (%)	35.38
C/N ratio	15.3
Water-holding capacity (kg/kg)	1.95
Bulk density $_{dry}$ (g/ml)	0.302
Particle real density (g/ml)	2.118
Total porosity (%)	85.7
Acari	Predators
Nematodes	Saprophagous
Trichoderma	Absence

Non-aerated compost teas (NACT) and aerated compost teas (ACT) were used [1]. The ratio of SMS to water was 1:4 (w/v), while fermentation time was 1 day. The mixtures were incubated at 25 ºC with stirring (ACT) and without (NACT), and were finally filtered through two layers of muslin. The pH and electrical conductivity (EC_{25}) were measured in both compost teas with the following results: in the case of ACT, pH was 7.6, and EC_{25} was 5,190 µS/cm; for NACT, pH was 7.6, and EC_{25} was 5,245 µS/cm.

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 25 ºC 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). The compost tea was applied to the casing mixture at 100 ml per tray. Three different treatments were made with each compost tea (ACT and NACT) – 1R (one drench application on the same day as the casing material was applied, day 0); 2R (two drench applications, days 0 and 2) and 3R (three drench applications, days 0, 2 and 6). A control treatment was irrigated with water alone. A randomised complete block design with five replicates was used.

The phytotoxicity of the three tea treatments was evaluated during the first three flushes (F1, F2 y F3) by comparing the yield with that obtained in the control. In addition, the earliness of each treatment was assessed, and expressed as the number of days between casing and harvesting of the first flush.

Phytotoxic effect of different fungicides on mushroom mycelium. The phytotoxicity of the five fungicides was assessed in a crop cycle during which the chemicals were applied as shown in Table 2: (I) with the first irrigation water (day 0) or (II) with the second irrigation water (day 5), with 100 ml per tray, using the same volume of water in the control. The cultivation conditions were the same as those described above. A randomised complete block design with five replicates was used.

Table 2: Fungicides and doses used

Commercial name	Active substance	Dose
Bavisfor 50 (IQV)	Carbendazim 50% WP	0.1%
Rovral wp (AGRODAN)	Iprodione 50% WP	0.1%
Topsin 70 wg (BAYER)	Thiophanate-methyl 70% WG	0.1%
Sporgon (BASF)	Prochloraz 46% WP	0.05%
Textar 60t (TECNIDEX)	Thiabendazole 60% SC	0.1%

The phytotoxicity was evaluated as in the previous experiment, based on yield and earliness. Fungicide residues were also analysed in the mushrooms from the first two flushes: iprodione residues were determined by gas chromatography, and the other fungicides by liquid chromatography.

Statistical analysis. The data obtained were evaluated by analysis of variance using the statistical package Statgraphics Plus v. 4.1. A Tukey test was used to establish significant differences between means, Tukey-HSD (p=0.05).

RESULTS AND DISCUSSION

Phytotoxic effect of the compost teas on mushroom mycelium. Yield and earliness data for the different treatments are shown in Table 3. As can be seen, there was a small reduction in the yield (3-4%) in the trays which were irrigated once with compost teas. This decrease increased with the number of times the teas were applied, reaching 10% of the yield with three applications of aerated compost teas. This may be related with the high EC_{25} of the teas, which may increase

the conductivity of the casing layer, hindering mushroom fructification [4]. However, the statistical analysis of the data pointed to no significant differences between the treatments.

Table 3: Total mushroom yield, in kg/m^2 (mean value ± SD) and percentage compared with the control, and earliness (mean value ± SD) for each of the compost tea treatments

Treatment		Yield (kg/m^2)	%	Earliness (days)	
Control		20.00 ± 1.80	100	21.07 ± 0.34	a*
	1R	19.20 ± 2.55	96.00	21.86 ± 0.48	b
ACT	2R	18.60 ± 1.59	93.00	21.87 ± 0.58	b
	3R	17.97 ± 1.30	89.85	22.08 ± 0.64	bc
	1R	19.42 ± 2.26	97.10	22.41 ± 0.44	bc
NACT	2R	18.23 ± 1.87	91.15	22.46 ± 0.28	bc
	3R	18.87 ± 1.83	94.35	22.50 ± 0.22	c
		p = 0.4963		p = 0.0007	

*Different letters indicate significant differences ($p < 0.05$) between means.

As regards earliness, there was a slight delay in the harvest of the first flush compared with the control in all treatments using compost teas, regardless of the number of applications. This delay was slightly longer with the NACT extract (1.2-1.4 days) than with ACT (0.8-1 day).

Phytotoxic effect of the fungicides on mushroom mycelium; analysis of residues. Table 4 shows the yield and earliness data for the different fungicidal treatments carried out. Statistical analysis of the production data points to statistically significant differences between treatments.

Table 4: Total mushroom yield, in kg/m^2 (mean value ± SD) and percentage compared with the control, and earliness (mean value ± SD) for each of the fungicide treatments

Treatment	Yield (kg/m^2)		%	Earliness (days)	
Control	22.79 ± 1.41	bc*	100	20.81 ± 0.45	a*
Carbendazim – I	21.99 ± 0.96	abc	96.49	21.85 ± 0.76	bcd
Iprodione – I	19.25 ± 1.70	a	84.47	23.12 ± 0.83	f
Thiophanate-methyl – I	20.48 ± 1.68	ab	89.86	23.21 ± 0.48	f
Prochloraz – I	20.58 ± 2.13	ab	90.30	21.87 ± 0.71	bcd
Thiabendazole – I	23.91 ± 0.90	c	> 100	21.95 ± 0.74	bcd
Carbendazim – II	20.83 ± 7.91	abc	91.40	22.18 ± 0.66	cde
Iprodione – II	19.29 ± 1.63	a	84.64	22.61 ± 0.71	def
Thiophanate-methyl – II	20.48 ± 2.15	ab	89.86	22.85 ± 0.96	ef
Prochloraz – II	20.00 ± 1.44	ab	87.76	22.70 ± 0.87	def
Thiabendazole – II	22.94 ± 2.18	bc	> 100	21.68 ± 0.94	abc
	p = 0.0005			p = 0.0005	

* Different letters indicate significant differences ($p < 0.05$) between means.

Of particular note is the decreased yield obtained with iprodione for either time of application, with decreases around 15% in both cases. Decreases of around 10% were obtained with thiophanate-methyl and prochloraz, also regardless of the moment of application. Similar values were obtained with the trays treated with carbendazime at the second irrigation time, while the same substance led to a 5% decrease when applied with the first irrigation water. Lastly, the application of thiabendazole did not lead to production losses regardless of application time; in fact, the yields obtained were slightly higher than with the control for both application times.

Earliness showed a similar trend, with iprodione and thiophanate-methyl delaying the beginning of harvesting by up to 2.5 days, while thiabendazole led to a shorter delay (of hardly one day). Carbendazim and prochloraz led to an intermediate behaviour, the former leading to a delay of one day and the latter to a delay of 2 days.

The results obtained for the analysis of the different active substances in the mushrooms harvested from the first two flushes are shown in Table 5. It should be noted that thiophanate-methyl decomposes into carbendazim, so that an analysis of both active compounds was made in this case. The results show that the MRL was exceeded in all the treatments involving iprodione, carbendazim and thiophanate-methyl, especially when applied at the second irrigation time. However, the application of prochloraz and thiabendazole supposed no problem residue when applied at the times and doses specified herein.

Table 5: Fungicide residues (ppm) detected in mushrooms at the first two flushes

Treatment	MRL	First flush	Second flush
Carbendazim – I	0.10	0.42	0.14
Iprodione – I	0.02	0.03	0.06
Thiophanate-methyl – I*	0.10	< 0.10 (0.34)	< 0.10 (0.36)
Prochloraz – I	2.00	< 0.05	< 0.05
Thiabendazole – I	10.00	1.01	1.44
Carbendazim – II	0.10	0.88	0.84
Iprodione – II	0.02	0.18	0.28
Thiophanate-methyl – II*	0.10	< 0.10 (0.37)	< 0.10 (0.86)
Prochloraz – II	2.00	< 0.05	< 0.05
Thiabendazole – II	10.00	0.87	1.08

* Carbendazim residues were also analysed (in parentheses).

CONCLUSIONS

The results obtained in the analysis of residues rule out the application of the fungicides iprodione, thiophanate-methyl and carbendazim at the doses and application times specified in the text. In the case of iprodione and, to a lesser extent, thiophanate-methyl, this conclusion was lent weight by the fall in production recorded. The other two fungicides used, prochloraz and thiabendazole, showed no residue problems and, in the latter's case, no effect on production was noted, although it fell by 10% when prochloraz was used. In contrast, the application of compost teas led to falls in production of less than 10% regardless of the number of applications. However, there were no associated residue problems.

Therefore, the less pronounced decrease in yields recorded with compost teas, the absence of residue problems and the *in vitro* efficacy observed for the control of *L. fungicola* [2] suggest that they may be considered as a biological alternative for the integrated control of this disease.

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