Killing the green mould menace with disinfectants

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Green moulds caused by *Trichoderma* species are capable of causing severe or complete mushroom crop loss if they get into the cropping substrates or growing room, even at low levels that are close to their detection limit. Farm hygiene is therefore of paramount importance in keeping green moulds out of mushroom crops, and the use of effective disinfectants is part of this strategy. This project examined the efficacy of a range of disinfectants available in the UK in killing inoculum of green moulds.

Trichoderma aggressivum f. europaeum (previously called *T. harzianum* type Th2) is an aggressive compost green mould that predominantly infects Phase II Agaricus compost at spawning. As the crop develops, the mushroom mycelium is rapidly out-competed and green spores can be seen in the compost and on the casing surface. Outbreaks are often accompanied by swarms of red pepper mites which feed on the green mould. *Trichoderma harzianum* (previously called type Th1) is less aggressive in colonising spawned Agaricus compost than *T. aggressivum* but can also be found as green patches of spores on the casing surface where it reduces yield and causes spotting of caps. It is also a serious competitor in shiitake and oyster mushroom substrates.

Fig.1 Green mould (*Trichoderma aggressivum*) on *Agaricus* mushroom culture

Fig. 2 Shiitake blocks heavily contaminated with green mould

Table 1. Disinfectants used in the tests.

Disinfectant	**Active ingredient**	**Recommended dilution rate**	**Manufacturer/ UK Supplier**
Bleach	sodium hypochlorite available chlorine	n/a	Staples Disposables Ltd
Disolite	2-phenylphenol benzyl-chlorophenol propan-2-ol	1:250 to 1:50	Progress Products
Environ	o-phenylphenol o-benzyl-p-chlorophenol isopropanol	1:250 to 1:100	Steris Ltd
Jet 5	peroxyacetic acid	1:500 to 1:125	Certis
Omnicide M	glutaraldhehyde cocobenzyldimethyl ammonium chloride	1:150 to 1:50	Progress Products
Prophyl	4-chloro-3-methyl phenol 2-benzyl-4-chlorophenol	1:250	J.F. McKenna Ltd
Purogene, activated	chlorine dioxide	1:200 to 1:20	Tristel Technologies Ltd
Sporekill	potassium salts of fatty acids	1:1000 to 1:100	Nutrigain Ltd

The withdrawal of formaldehyde as a gaseous disinfectant and fungicide tray dips has been a particular problem for farms without the facility to cook-out. Phenolic disinfectants including Disolite, Environ and Prophyl have been used in the mushroom industry for several years and there are several other disinfectants that are marketed in the mushroom industry (Table 1). The efficacy of disinfectants at different concentrations in killing the mycelium and spores of green moulds was not previously established.

Testing disinfectants against *Trichoderma aggressivum* spores

An aggressive isolate of *Trichoderma aggressivum* f. *europaeum* (23443B) obtained from Dr Charles Lane, Fera, was used for the tests. Suspensions of *T. aggressivum* spores and mycelial fragments were prepared by washing agar plate cultures with sterile water and counting the numbers of spores and colony forming units (cfu, viable spores + mycelial fragments) in the suspension. Suspensions containing 2.3×10^7 spores and 5.2×10^8 cfu/ml (low rate) or 2.2×10^8 spores and 1.7×10^9 cfu/ml (high rate) were obtained by appropriate dilution. The spores and mycelial fragments in the suspension were then exposed to known concentrations of the disinfectants for controlled time periods using a series of centrifuging and resuspending procedures described in the report of M 57. Disolite, Environ, Jet 5, Omnicide M, Prophyl and Sporekill were tested in solutions with sterile water at 1:100 and 1:250 dilution rates; Purogene was tested at 1:20 and 1:100 and bleach was tested at 1:5 and 1:9 rates. Sterile water was used as a control. Exposure of both concentrations of Trichoderma suspension (low or high) to each disinfectant and concentration was tested for 0.5, 2, 8 and 14 minutes. The viability of the treated spores and mycelial fragments was then tested by plating on to plates of potato dextrose yeast agar. The plates were incubated and the numbers of colony forming units of Trichoderma were recorded. Absence of any Trichoderma colonies indicated that the disinfectant treatment had reduced the inoculum to below the detectable limit (about 0.001% of the original Trichoderma inoculum).

The control spores remained viable at high numbers after immersion in water (Table 2). Sporekill and activated Purogene were not effective in killing spores at dilutions of 1:100 and 1:20 respectively. Making the other disinfectant more dilute and/or increasing the initial concentration of spores, either increased the length of the exposure time needed to kill all the spores or made the disinfectant ineffective. Jet 5 only eradicated the lower concentration of spores, and only if it was used at the higher concentration (1:100) for the longest time period tested (14 minutes). Disolite and Omnicide M were more effective than Environ and Prophyl at the same dilutions in killing Trichoderma spores, with Disolite being the most effective. Bleach also showed efficacy in killing spores, but at a dilution rate of 1:9, 14 minutes exposure were needed to eradicate the higher concentration of spores. Results in project M 57 also

showed that the vapours from a Disolite solution at 1:250, Prophyl at 1:100 or Purogene at 1:33 were effective in killing *Trichoderma aggressivum* spores exposed to them for a 17 hour period.

Table 2. Effect of disinfectants on Trichoderma spore suspensions and mycelial growth rates on agar; treatments that eradicated the Trichoderma inoculum within the test periods (14 minutes or 20 days respectively) are indicated by pink shading. Each value is based on three replicate samples.

Disinfectant	**Dilution rate**	**Time to kill *T. aggressivum* spores, minutes**		**Mycelial growth rate, mm/day**	
		Low spores	**High spores**	***T. aggressivum***	***T. harzianum***
Bleach	1:5	2	8	0	0
	1:9	2	14	5.7	4.8
Disolite	1:100	2	8	0	0
	1:250	14	+ve	0	0
Environ	1:100	14	+ve	0	0
	1:250	+ve	+ve	0	0
Jet five	1:100	14	+ve	0	0
	1:250	+ve	+ve	6.3	3.2
Omnicide M	1:100	8	8	0	0
	1:250	+ve	+ve	1.0	1.2
Prophyl	1:100	14	14	0	0
	1:250	+ve	+ve	0	0
Purogene activ.	1:20	+ve	+ve	7.6	2.8
	1:100	+ve	+ve	12.0	5.6
Sporekill	1:100	+ve	+ve	0	0
	1:250	+ve	+ve	0	0
Water control	-	+ve	+ve	11.1	11.6

Low spores =2.3 x 10^7 spores and 5.2 x 10^8 cfu/ml

High spores = 2.2 x 10^8 spores and 1.7 x 10^9 cfu/ml

Testing disinfectants against Trichoderma mycelial growth

Potato dextrose agar containing the following disinfectants at 1:150 (i.e. most concentrated), 1:250, 1:500 and 1:750 (i.e. most dilute) was prepared: Disolite, Environ, Jet 5, Omnicide M, Prophyl, activated Purogene and Sporekill. Jet 5 was also incorporated at 1:100 and activated Purogene at 1:20, 1:50 and 1:100. Bleach was incorporated into agar at 1:5, 1:10, 1:20 and 1:50. Agar plates prepared only with water, without disinfectant, were used as a control. Plates of each disinfectant treatment were prepared and inoculated in the centre with a 5 mm plug of a sporulating culture of either *Trichoderma aggressivum* or *Trichoderma harzianum* obtained from the casing of a commercial mushroom crop. The radial growth of the mycelium on the plates was recorded at daily intervals for up to 20 days to obtain a radial growth rate.

When Disolite, Environ or Prophyl were added in dilutions of 1:750 (or more concentrated) to the agar medium, no mycelial growth of either *T. aggressivum* or *T. harzianum* occurred. Bleach totally suppressed mycelial growth when added to agar at a 1:5 dilution (Table 2). Activated Purogene only reduced mycelial growth of *T. aggressivum* if it was not diluted by more than 1:50 although it was more inhibitory to the growth of *T. harzianum*. Sporekill totally suppressed mycelial growth of both *Trichoderma* species at a dilution of 1:250, whereas Omnicide M required a higher concentration (1:150) to achieve the same effect. Jet 5 was more inhibitory to the growth of *T. harzianum* than to that of *T. aggressivum,* which required a concentration of 1:100 to completely suppress growth.

Testing disinfectants against Trichoderma in infected mushroom compost

Plastic mesh bags containing spawn-run mushroom compost infected with *T. aggressivum* were immersed in each of the following disinfectant solutions for 14 minutes: Disolite, Environ, Jet 5, Prophyl and Sporekill at 1:100 dilution and activated Purogene at 1:20. Sterile water was used as a control. After rinsing, the compost from each bag was tested for Trichoderma by plating a suspension on to a selective agar medium.

None of the disinfectants used at a dilution of 1:100 eradicated *T. aggressivum* from compost inoculum: Disolite and Environ reduced the number of surviving Trichoderma propagules by x100, Jet 5 and Prophyl reduced it by x10, and Sporekill had no effect. Activated Purogene at 1:20 was also ineffective sanitising *T. aggressivum* infected compost.

Conclusions

Phenolic disinfectants are the most suppressive to the growth of Trichoderma mycelium, and of these, Disolite is more effective in killing Trichoderma spores than Environ or Prophyl at the same dilution rate. However, phenolic disinfectants should not be used where they can come into contact with the crop since they are detectable at very low levels. The EU minimum residue level for 2-phenylphenol in mushrooms is 0.05 mg/kg and the level for benzyl-

chlorophenol and chloro-methylphenol is only 0.01 mg/kg. They can be used on floors, in foot dips and for washing parts of machinery and vehicles that do not come in contact with the crop or substrates. However, they cannot be used on organic farms.

Of the non-phenolic disinfectants tested, Omnicide M was the most effective in killing Trichoderma spores and Sporekill was the most suppressive to mycelial growth. Omnicide M can be used for disinfecting trays and shelves, which should then be washed down, whereas Sporekill may be suitable for preventing Trichoderma mycelium growth on wood surfaces but this was not tested in this project. The effect of disinfectants on other mushroom pathogens and pests, and the possibility of resistance must also be considered. In the event of a Trichoderma outbreak, the use of concentrated bleach solution and fogging rooms with activated Purogene (chlorine dioxide) should also be considered, although they are corrosive materials.

Disinfectants are not completely effective in sanitising compost residues, which form a protective barrier for green mould inoculum. This demonstrates the importance of thoroughly washing off all organic material before disinfectants are used.

Further Developments

Green mould, particularly *Trichoderma harzianum* is a serious problem in casing where peat has been partially or completely replaced by organic materials such as bark and coir (Figure 3). The cellulose and hemi-cellulose in these undegraded materials is a nutrient source for green moulds. A current project funded by AHDB Horticulture and conducted in conjunction with Fera Science is examining the development of *Trichoderma* species populations is casing materials using qPCR assays for *T. harzianum* and *T. aggressivum*

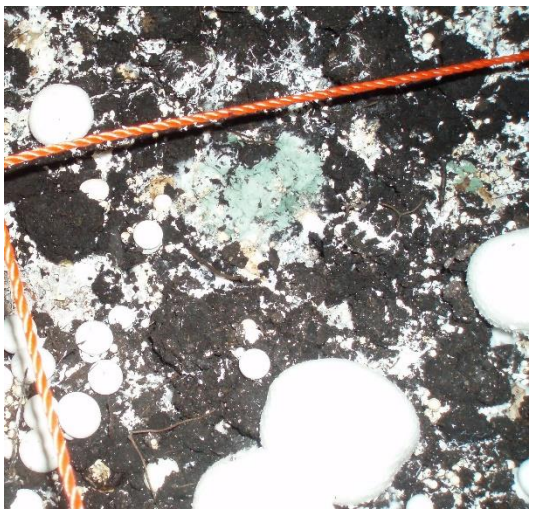

Fig. 4 *Trichoderma harzianum* growing on peat casing containing a proportion of bark in New Zealand