Mushroom Advice and Analysis

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I present the results of the trials that we conducted in the laboratory.

We conducted a series of preliminary trials on samples that you sent us to establish the efficacy of the product and determine whether or not more detailed trials should be considered. The results from the preliminary tests indicated that the product was effective at inhibiting spore germination on the major mushroom pathogens.

It did however also highlight that at low concentrations the active ingredient had a tendency to precipitate and come out of solution. You addressed this problem and by the addition of a simple buffering solution the problem was solved.

The Laboratory was then supplied with the modified solution and further more discerning tests were conducted using that solution. The results are presented below:

Trial: To determine the effect of Sporekill on the germination of some common mushroom pathogens

In vitro tests were carried out in the laboratory, using the solution supplied by IPP Ltd and a range of the common mushroom pathogens.

Method:

The solution under test

A dilution series of the Sporekill solution supplied for testing was prepared over a concentrations range from 2.4%– 0.2% these were twice the final concentration required as an equal concentration of spores and test solution are mixed together in the assay.

The pathogens tested

Spores from pure cultures grown on Malt Agar plates of: Cladobotryum dendriodes (Cobweb), Verticillium fungicola(Dry Bubble), Mycogone periciosa (Wet Bubble) and Trichoderma harzianum (Green Mould) were prepared by washing the colonies with distilled water and agitating them with a sterile glass "hockey stick".

With all cultures it was asexual spores that were experimented on. The number of spores collected from each species was measured and the numbers for each of the pathogens equated to around 10^4 spores per ml. then an equal solution of

sterile mushroom extract was added to each spore suspension to act as a germination stimulant and isotonic modifier.

The testing protocol

Sterile filter papers were placed in the base of large 140 mm Petri dishes and moistened with sterile distilled water, sterile microscope slides were placed on glass hockey sticks positioned on the filter paper. Each Petri dish contained three microscope slides and on each slide two replicates were positioned at either end of the slides.

Onto each replicate position using a micro pipette 1ml of the concentration of the solution under test was placed, then using a separate micro pipette and sterile tips, another 1ml of each pathogen under test was placed in the same location and mixed together.

The lids were placed on the Petri dishes before they were placed in an incubator maintained at 24^{o}C for a period of 48 hrs, after that time the plates were removed and the slides were examined under the microscope.

The percentage germination in each replicate was recorded separately and the means of each concentration tested are presented in table 1. The data within each pathogen treatment has been corrected for the spores that were non viable and did not germinate in the controls.

Final Concentration of Sporekill in %	Verticillium fungicola	Mycogone perniciosa	Cladobotryum dendriodes	Trichoderma harzianum
1.2	0	0	0	0
0.6	0	0	0	0
0.5	0	0	0	0
0.4	3.64	1.03	2.49	1.92
0.3	8.84	10.60	5.52	5.58
0.2	48.18	41.20	18.68	27.05
0.1	99.65	96.92	85.94	97.21
0.0	100	100	100	100

Although the results indicate a small reduction in germination at 0.1%, the actual growth of the germ tubes was significantly reduced.

It was clear that the vigour and growth of the germ tube in all of the pathogens tested was markedly reduced even at the lowest concentration tested.

In all treatments the vigour of the germination recorded was markedly and increasingly reduced as the concentration of the test solution increased.

Above 0.4% no germination was recorded in any of the treatments in the 48h period tested.

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