CASING LAYER DISINFECTION BY COLLOIDAL SILVER AND ACTIVE OXYGEN, EFFECTS ON YIELD OF *AGARICUS BISPORUS* AND CONTROL OF COBWEB DISEASE

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ABSTRACT

Cladobotryum dendroides is the most frequently found soil-borne mushroom pathogen in Serbia [1]. Although many investigations have been carried out to find an adequate disinfectant of mushroom casing, so far, none has been found to be completely effective thus non-toxic to humans. The aim of this study was to explore the potential of Ecocute, based on colloidal silver [2-3] and Peral-S (active oxygen) [4-5], as environmentally friendly casing disinfectants. Efficacies of the disinfectants and incidence of cobweb disease were evaluated in bags of compost cased with a black peat/lime casing soil, previously disinfected with Ecocute at 30 mg L^{-1} per m^2 and Peral-S at 1.7 ml L^{-1} per m^2. Plots were inoculated with suspension set on 1000 conidia per m^2 of *C. dendroides*. Both disinfectants were applied alone and in combination with prochloraz-manganese. Biological efficiency of colloidal silver applied alone was higher than in treatments with active oxygen in all trials. The colloidal silver did not control cobweb disease satisfactorily, and resulted in diseased mushrooms in all three trials (1.1%, 5.7% and 5.9%). Application of colloidal silver did not decrease the total productivity of *Agaricus bisporus*, and resulted in comparatively higher cumulative biological efficiency than that of active oxygen. On the other hand, active oxygen provided better disease control compared to colloidal silver. Treatment of active oxygen with prochloraz-manganese had the highest effectivenes in disease control and also satisfactorily mushroom productivity.

Keywords: Ecocute; Peral-S; *Cladobotryum dendroides*.

INTRODUCTION

Soil-borne pathogens, such as *Lecanicillium fungicola*, *Mycogone perniciosa*, *Cladobotryum* spp. and *Pseudomonas tolaasii*, cause the most serious growing mushroom diseases, respectively, dry and wet bubble, cobweb disease and bacterial brown blotch [6-7]. *Cladobotryum dendroides* is the most frequently found soil-inhabiting mushroom pathogen in Serbia [1]. The disease symptoms were: cottony fluffy white or yellowish to pink colonies on mushroom casing, rapid colonization of casing surface, covering of host basidiomata by mycelia, and their decay. Infection intensity depended on the development stage of fruiting bodies. The use of both peat casing and indoor disinfectants, such as formalin, sodium hypochlorite, potassium permanganate, sulphur, calcium chloride and chlorinated compounds, is a general practice in mushroom cultivation process. The selective fungicide prochloraz-manganese is officially recommended in mushroom production facilities in EU countries [6]. However, the use

of chemicals induces a problem with residues in mushroom fruiting bodies. So far, many investigations have been carried out to find an adequate disease control in edible mushroom industry, but none has been found to be completely effective thus non-toxic to humans. Many alternative compounds, e.g. biopesticides, disinfectants, plant extracts, essential oils and their components, have been tested as control agents against mushroom pathogens in general [1, 8-10]. Environmental awareness has grown, with the result that the enforcement of pollution control laws has become more and more effective. This situation forced the mushroom industry to develop technologies that ensure production with the least possible harmful effects on the environment [11]. Evaluations of the commercially available disinfectants effects on mushroom pathogenic bacterium *Pseudomonas tolaasii* and fungi *Trichoderma harzianum* and *C. dendroides*, have already been reported [12-13]. Reducing environmental pollutants and microorganisms by colloidal silver has been developed in hospitals, diaries, food processing and even drinking water disinfection [14-15]. Environmentally friendly solution based on silver combined with hydrogen peroxide showed synergism having strong bactericidal and antiviral effects [2-3]. Other ecological disinfectant is active oxygen, arising as a result of break-down of peracetic acid with its high oxidizing potential. It is broadly effective against microorganisms for indoor use on hard surfaces. Its use sites include agricultural premises, food establishments, medical facilities etc. [4-5]. The aim of this study was to explore the potential of colloidal silver and active oxygen, as ecological casing disinfectants, against Serbian *C. dendroides* isolate.

MATERIALS AND METHODS

Commercial fungicide formulation prochloraz-manganse (Octave WP, Bayer Crop Science, Germany, prochloraz-manganese complex 50%, kaolin 35%, other ingredients 15%) was used in this study. The ecologically friendly disinfectants Peral-S (Vetprom, Belgrade, Serbia, active oxigen 0.9%) and Ecocute (IHIS Techno Experts, Belgrade, Serbia, colloidal silver 30 mg, hydrogen peroxide 1 L) were tested as potential antifungal agents against Serbian *C. dendroides* isolate in a mushroom growing room.

An isolate of *C. dendroides*, strain Vegr2C7, was grown on potato dextrose agar (PDA) at 20^{o}C for four days. Conidia were harvested by flooding the plates with 10 ml of sterile distilled water and Tween 20 (v/v 0.01 %) (REANAL Finomvegyszergyar Rt., Hungary, No.: 805383) followed by filtration through double layers of cheesecloth. Each plot of the infested casing was treated with a total volume of 10 ml of conidial suspension at a rate of 1000 conidia per m^2.

Plastic bags, 0.60 x 0.40 x 0.25 m (*l* x *w* x *h*), filled with 18 kg of compost spawned with *A. bisporus* strain 737, (Sylvan, Hungária zRt), were incubated (spawn-run) for 18 days at 24^{o}C. Compost surface was divided by wooden barriers into two sections so that each experimental compartment, measuring 0.30 x 0.40 x 0.25 m, had a total area of 0.12 m^2, and contained 9 kg of spawned substrate. Each plot was cased with a 40-50 mm layer of black peat/lime casing soil (Ramski rit – Treset, Veliko Gradište, Serbia) and incubated at 21^{o}C for 8 days (case-run), and then air temperature was reduced to 16^{o}C. Drench applications of prochloraz-manganese were applied on day 5 and 20, and relevant plots inoculated with conidial suspension of *C. dendroides* Vegr2C7 isolate 7 days after casing. The treatments were as follows: *(1)* uninoculated and untreated control; *(2)* inoculated and untreated control; *(3)* inoculated, treated with prochloraz-manganese at standard product application rate (0.6 g a.i. in 1.8 l H_2O m^{-2} of mushroom bed area); *(4)* inoculated, treated with prochloraz-manganese at standard product application rate and Peral-S ((active oxygen 0.9%) 1.7 ml in 1 L H_2O m^{-2} of mushroom bed area); *(5)* inoculated, treated with prochloraz-manganese at standard product application rate and Ecocute ((colloidal silver 30 mg in 1 L hydrogene peroxide) 250 ml in 1 L H_2O m^{-2} of mushroom bed area); *(6)* inoculated, treated with Peral-S (1.7 ml in 1 L H_2O m^{-2} of mushroom bed area); *(7)* inoculated, treated with Ecocute (250 ml in 1 L H_2O m^{-2} of mushroom bed area). The plots were arranged in

a randomized block design with three replicates per treatment. All experiments were repeated three times.

The mushrooms were hand-picked in three successive production flushes. The harvested mushrooms were weighed, counted, and divided in two groups based on visual observation: fruiting bodies without symptoms and those covered with *C. dendroides* mycelium. Disease incidence was recorded as a percentage value, based on the number of diseased sporophores visually recorded. The effect of fungicides on mushroom productivity was evaluated by the biological efficiency (BE) calculated as the ratio of the fresh weight of total yield of harvested mushrooms (healthy and diseased) to the weight of dry substrate at spawning and expressing the fraction as kg/100 kg compost [16]. The incidence of cobweb disease in inoculated plots was calculated as percentages of diseased mushrooms of all mushrooms harvested.

Fungicide effectiveness was calculated by using Abbott's formula [17]:

% effectivness= [(Ic -It)/Ic] x 100

(where Ic = disease incidence of the control; It = disease incidence of the treatment)[18].

Analysis of variance was performed in order to determine treatment effects. Data were analysed using ANOVA and the means separated by Duncan's multiple range test.

RESULTS AND DISCUSSION

The incidence of cobweb disease in untreated inoculated plots in three trials was 4.6%, 7.6% and 4.5%, calculated as percentages of all mushrooms harvested. Prochloraz-manganese prevented disease development in one trial, while disease symptoms were recorded in the two other trials (3.1% and 2.3%, respectively). The disinfection with colloidal silver did not control cobweb disease satisfactorily, and resulted in diseased mushrooms in all three trials (1.1%, 5.7% and 5.9%). Active oxygen gave better control than colloidal silver, and disease incidence was 0%, 5.9% and 3.0%.

Table 1. Biological efficiency (BE %) of the different treatments on *Agaricus bisporus* artificially inoculated with *Cladobotryum dendroides*

Treatments ($g\ m^{-2}$)	BE (%)		
	Trial I	Trial II	Trial III
Uninoculated and untreated	74.28 c[1]	90.14 b	79.63 e
Inoculated Untreated	64.70 f	85.47 d	108.94 c
Inoculated Untreated Peral S 1.7 ml L^{-1}	57.08 g	66.09 e	72.78 f
Inoculated Untreated Ecocute 250 ml L^{-1}	67.46 e	88.38 c	101.79 d
Inoculated Octave WP 1.2 mg L^{-1} Peral S 1.7 ml L^{-1}	97.48 a	91.62 b	110.42 b
Inoculated Octave WP 1.2 mg L^{-1} Ecocute 250 ml L^{-1}	70.98 d	94.78 a	127.47 a
Inoculated Octave WP 1.2 mg L^{-1}	91.16 b	84.89 d	50.08 g

[1]Means within the same column followed by the same letter are not significantly different (P=0.005).

Regarding the effect of treatments on mushroom productivity evaluated by biological efficiency, the greatest productivity was obtained by the combination of prochloraz-manganese and active oxygen in the first trial, and with both disinfectants in the second and the third trial (Table 1). The fungicide also showed high productivity when applied alone in the first trial. The poorest results were obtained with active oxygen. Biological efficiency of colloidal silver applied alone was higher than in treatments with active oxygen in all trials. Additionally, yields in the treatment with colloidal silver, both applied alone and in combination with prochloraz-manganese, exceeded the yields in uninoculated control in the third trial. When both

disinfectants were combined with fungicide, efficiency of colloidal silver was higher than of active oxygen in two trials.

In the first trial, a 100% effectiveness was shown in treatment when active oxygen was involved, alone or with prochloraz-manganese (Fig. 1). Prochloraz-manganese applied alone exhibited the least effectiveness in the first trial (32.6%). Colloidal silver was effective both applied alone and with fungicide (76.1 and 45.7%, respectively). In the second trial, the 100% effectiveness was attained in all treatments with prochloraz-manganese. Active oxygen and colloidal silver had much lower effectiveness, 22.4 and 25% respectively. In the third trial, fully effective was a combination of fungicide and active oxygen. Active oxygen applied alone also was highly effective (93.3%). Less effective was fungicide applied alone and with colloidal silver (48.9 and 48.7%) and the least effective was colloidal silver applied alone.

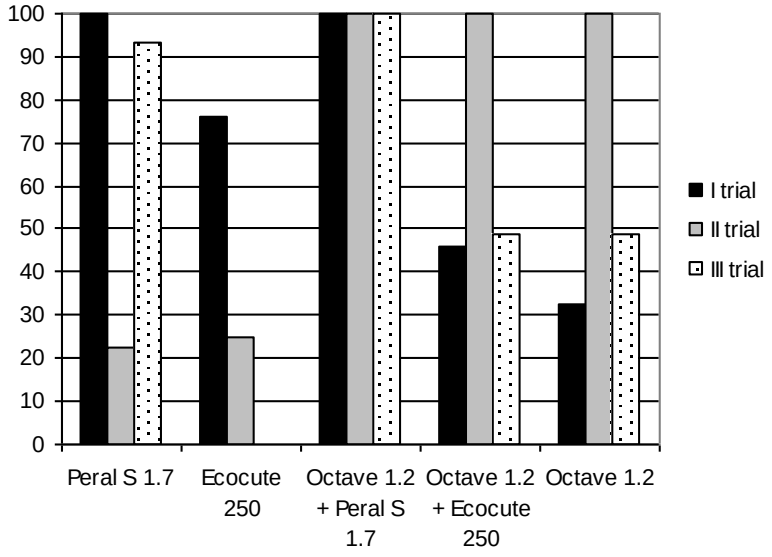

Figure 1. Effectiveness (%) of different treatments on control of *Cladobotryum dendroides* in artificially infected *Agaricus bisporus*; LSD_{005} =68.52.

Application of colloidal silver did not decrease the total productivity of *A. bisporus*, and it resulted in comparatively higher cumulative biological efficiency than that of active oxygen.

CONCLUSION

Colloidal silver caused a significant reduction in cobweb disease levels, but less than active oxygen. Results indicated that colloidal silver had no negative interference with *A. bisporus* physiology. Active oxygen provided better disease control compared to colloidal silver, but this disinfectant had negative influence on *A. bisporus* yield. On the other hand, treatment of active oxygen with prochloraz-manganese had the highest effectiveness in disease control and also satisfactorily mushroom productivity. Based on this finding colloidal silver and active oxygen should be tested further to examine effectiveness against other *A. bisporus* pathogens.

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