

Possibility of environmentally-safe casing soil disinfection for control of cobweb disease of button mushroom

Ivana Potočnik^{1*}, Emil Rekanović¹, Miloš Stepnović¹, Svetlana Milijašević-Marčić¹, Biljana Todorović¹, Ljiljana Nikolić-Bujanović² and Milan Čekerevac²

¹*Institute of Pesticides and Environmental Protection, Laboratory for Applied Phytopathology, Banatska 31b, 11080 Belgrade, Serbia*

²*IHIS Techno-Experts, Batajnički put 23, 11080, Belgrade, Serbia*
(*ivana.potocnik@pestring.org.rs)

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SUMMARY

The soil-borne pathogen *Cladobotryum dendroides* causes cobweb disease of button mushroom (*Agaricus bisporus*) and its significant yield losses. Casing soil disinfection by toxic formaldehyde is a widespread practice. The aim of this study was to investigate the potential of two environmentally friendly substances, colloidal silver and peracetic acid, against *C. dendroides*. Their biological efficacy (impact on mushroom yield), effectiveness (disease control) and type of interactions between them and the fungicide prochloraz-manganese were evaluated.

Black peat/lime casing soil was applied to a colonized substrate with the white button mushroom strain 737, then inoculated with *C. dendroides* and treated with the fungicide prochloraz-manganese and two environmentally friendly disinfectants based on peracetic acid and colloidal silver. The effects of fungicides on mushroom productivity were evaluated as biological efficacy and calculated as a ratio of fresh weight of total mushroom yield to the weight of dry substrate. Fungicide effectiveness and synergy factor were calculated by Abbott's (1925) formula. Tests for synergism between prochloraz-manganese and both other substances were performed using Limpel's formula.

The highest biological efficacy, exceeding 92.00, was achieved in treatments with prochloraz-manganese, applied alone or in combination with both other disinfectants. The highest effectiveness of 93.33% was attained in treatments with peracetic acid combined with prochloraz-manganese. Trials against cobweb disease revealed a synergistic reaction between the fungicide and peracetic acid and antagonistic between the fungicide and colloidal silver.

Peracetic acid provided better disease control, compared to colloidal silver applied alone or in combination with the fungicide. Based on these findings, peracetic acid should be recommended as an environmentally friendly casing soil disinfectant against cobweb disease of *A. bisporus*.

Keywords: Soil disinfection; Colloidal silver; Peracetic acid; Prochloraz; *Cladobotryum dendroides*; *Agaricus bisporus*.

INTRODUCTION

Soil-borne pathogens, such as *Lecanicillium fungicola*, *Mycogone perniciosus*, *Cladobotryum* spp., *Trichoderma* spp. and *Pseudomonas tolaasii*, cause the most serious diseases of edible mushroom *Agaricus bisporus* (Lange) Imbach in Serbia, dry and wet bubble, cobweb disease and bacterial brown blotch, respectively (Potočnik et al., 2008; Milijašević-Marčić et al., 2012). Besides *Trichoderma*, the micromycete *C. dendroides* is the most frequent soil-inhabiting mushroom pathogen in Serbia (Potočnik et al., 2010). Still, Fletcher and Ganney (1968) noted that mushroom diseases were mostly transmitted by casing soil and suggested formaldehyde as a control method. Casing soil disinfection by indoor disinfectants, such as formaldehyde, sodium hypochlorite, potassium permanganate, sulphur, calcium chloride and chlorinated compounds, is currently a widespread practice in mushroom cultivation (Sharma & Guleria, 1999). The selective fungicide prochloraz-manganese has been officially recommended for mushroom production in all EU countries (Potočnik et al., 2010). So far, many studies have been carried out to find the most adequate method of disease control in edible mushroom industry, but none has been found to be completely effective while being at the same time 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 (Tanović et al., 2009; Savić et al., 2012). The effects of commercially available disinfectants on the mushroom pathogens *P. tolaasii*, *Trichoderma harzianum* and *C. dendroides* (Wong & Preece, 1985; Abosriwil & Clancy, 2002; Todorović et al., 2012) and on plant pathogenic organisms (Lewandowski & Hayes, 2009) have already been evaluated. Environmental awareness has grown with enforcement of pollution control laws becoming more and more effective. In 1954, silver was registered in the US as a disinfectant and fungicide in hospitals, food processing facilities and for drinking water disinfection (Varner et al. 2010). In 1991, the US EPA determined an oral reference dose of 0.005 mg/kg/day for silver, while exposure to ambient silver should be less than 0.010 mg/m³ (Varner et al., 2010). Silver nano-particles are considered to be a slow-release stream of silver ions which react with the thiol groups of proteins and interfere with DNA replication (Morones et al., 2005). A solution of colloidal silver in hydrogen peroxide, known as Ecocute, had been previously tested by Nikolić-Bujanović et al. (2006), Čekerevac et al. (2006) and Todorović et al. (2012), and was found to have a strong bactericidal and antiviral effect.

Since the early 1950s, peracetic acid has been used for bacteria and fungi removal from fruits and vegetables. It is now broadly effective against microorganisms in products for indoor use on hard surfaces in agricultural premises, medical facilities, etc. It is easily biodegradable and leaves no residues. In water, it disintegrates to hydrogen peroxide and acetic acid, which further breaks down to water, active oxygen and carbon dioxide. The oxidation potential of peracetic acid, producing active oxygen, outranges that of chlorine and chlorine dioxide. The activity period of active oxygen is very short, destroying microorganisms within 5-15 minutes. There are many reports showing that peracetic acid is active as a bactericide and fungicide (Greenspan & MacKellar, 1951; Gershenfeld & Davis, 1952). Little is known about the mechanism of action of this disinfectant, but it is believed to work similarly to other oxidizing agents, denaturing proteins, disrupting cell wall permeability, etc. (Rutala & Weber, 2008). The disinfectant Peral-S, based on active oxygen resulting from a break-down of 15% peracetic acid, has proved to have a powerful bactericidal, antiviral and fungicidal effect (Durisić et al. 1990; Ašanin & Mišić 2006). The aim of this study was to explore the potential of colloidal silver and peracetic acid as environmentally-safe disinfectants of mushroom casing material, to be used as agents against *C. dendroides*, the causal agent of cobweb disease of *A. bisporus*.

MATERIAL AND METHODS

Chemicals: The commercial fungicide formulation prochloraz-manganese (Octave WP, Bayer Crop Science, Germany, prochloraz-manganese complex 50%, kaolin 35%, other ingredients 15%) was used in this study. The environmentally friendly disinfectants Oksanil (Jokasan, Belgrade, Serbia, peracetic acid 0.9%) and Ecocute (IHIS Techno Experts, Belgrade, Serbia, colloidal silver 30 mg, hydrogen peroxide 1 L) were tested as potential antifungal agents against an isolate of *C. dendroides* in a mushroom growing room.

Isolate: The isolate of *C. dendroides*, strain Vegr2C7, was grown on potato dextrose agar (PDA) at 20°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 Finomvegyszergyár Rt., Hungary, No.: 805383), followed by filtration through double layers of cheesecloth. Each plot of infested casing was treated with a total volume of 10 ml of conidial suspension at a rate of 1000 conidia per m².

Evaluation of biological efficiency, effectiveness and synergy factor: 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°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, with a total area of 0.12 m², contained 9 kg of spawned substrate. Each plot was cased with a 40–50 mm layer of black peat/ lime casing soil (Terahum, Treset d.o.o., Veliko Gradište, Serbia) and incubated at 21°C for 8 days (case-run). After incubation, air temperature was reduced to 16°C. Drench amounts of prochloraz-manganese were applied on days 5 and 20, and relevant plots were inoculated with the conidial suspension of *C. dendroides*, Vegr2C7 isolate, 7 days after casing. Treatment data are shown in Table 1.

Table 1. Treatments of casing soil plots in mushroom growing room

No.	Treatments
1	Uninoculated and untreated control
2	Inoculated and untreated control
3	Inoculated and treated with prochloraz-manganese at standard product application rate (0.6 g a.i. in 1.8 l H ₂ O m ⁻² of mushroom bed area)
4	Inoculated and treated with prochloraz-manganese at standard product application rate and peracetic acid 0.9%, (1.7 ml in 1 L H ₂ O m ⁻² of mushroom bed area)
5	Inoculated and treated with prochloraz-manganese at standard product application rate and colloidal silver
6	Inoculated and treated with 0.9 % of peracetic acid (1.7 ml in 1 L H ₂ O m ⁻² of mushroom bed area)
7	Inoculated and treated with colloidal silver (30 mg in 1 L hydrogen peroxide) 250 ml in 1 L H ₂ O m ⁻² of mushroom bed area.

The plots were arranged in a randomized block design with three replicates per treatment. An analysis of variance was performed in order to determine treatment effects. Data were analysed using ANOVA and the means were separated by Duncan's multiple range test. Means were separated by the least significant difference (LSD) test at the 5% level of probability.

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. The incidence of cobweb disease in inoculated plots was calculated as percentages of diseased mushrooms of all mushrooms harvested. The effects of the fungicide and disinfectants on mushroom productivity was evaluated as biological efficiency (BE) and calculated as the ratio of fresh weight of total mushroom yield (healthy and

diseased) to the weight of dry substrate at spawning, expressing the fraction as kg/100 kg compost (Chrysai-Tokousbalides et al., 2007). Fungicide effectiveness was calculated by Abbott's (1925) formula: % effectiveness = $[(I_c - I_t)/I_c] \times 100$ (where I_c = disease incidence in the control; I_t = disease incidence of treatment) (Gea et al., 2010). Tests for synergism between prochloraz-manganese and disinfectants were performed using Limpel's formula (Richer, 1987): $E_e = (X + Y) - (XY)/100$ [E_e stands for the expected effect from additive responses of two inhibitory agents (disinfectants and fungicide); X and Y represent the percentage of inhibition caused by both disinfectants, i.e. by colloidal silver and control fungicide, or peracetic acid and fungicide, respectively]. Abbott's formula (Abbott, 1925) was used to determine the synergy factor (SF). SF stands for Observed Inhibition/ Expected Inhibition, where $SF > 1$ is synergistic reaction, $SF < 1$ is antagonistic reaction, and $SF = 1$ is additive reaction.

RESULTS AND DISCUSSION

Incidence of disease symptoms: The highest incidence of cobweb disease was found in untreated inoculated plots in all three trials, 7.69, 6.56 and 6.02%, calculated as percentages of all mushrooms harvested. Low disease symptoms were recorded in all three trials of prochloraz-manganese treatment (1.08, 2.35, and 1%). Disinfection with colloidal silver did not control cobweb disease satisfactorily, and it resulted in diseased mushrooms in all three trials in which it was applied alone (4.41, 3.37 and 2.56%), and in combination with prochloraz-manganese (2.82, 2.11 and 0.87%). Peracetic acid provided better control than colloidal silver, and disease incidence was 1.09, 1.27 and 2.74% when the former was applied alone, while no symptoms were found in the first two trials and the incidence was 0.9% in the third trial with prochloraz-manganese added. The highest incidence of disease in the first two trials, besides the untreated inoculated control, was found in the exclusive treatment with colloidal silver. In the third trial, the highest incidence was observed in treatment with peracetic acid alone. The lowest disease incidence in the first two trials with no symptoms at all was found in the treatment combining peracetic acid and prochloraz-manganese, and in the third trial (0.87%) in treatment with combined colloidal silver and the fungicide. Apart from the inoculated control, treatment with colloidal silver without the fungicide resulted in the highest disease incidence, while the lowest was the result of treatment with the disinfectant peracetic acid combined with prochloraz-manganese.

Impact of chemicals on *Agaricus bisporus* yield: Regarding the effect of disinfectants on mushroom productivity, the highest biological efficiency (BE) of 97.48 in the first trial was obtained by the combination of prochloraz-manganese and active oxygen (peracetic acid) (Table 2). High *A. bisporus* productivity in the first trial and BE exceeding 90.00 was also found in individual treatments of prochloraz-manganese and peracetic acid. The lowest yield in the first trial was found in the inoculated control, BE=64.70. In the second trial, the highest yield was achieved in plots treated with the combination of prochloraz-manganese and colloidal silver, 94.78. Also, BE exceeded 90.00 in uninoculated control and in plots treated with the combination of fungicide and peracetic acid. The lowest value of BE in the second trial

was found in inoculated control, 60.94. In the third trial, the greatest mushroom productivity with BE exceeding 100.00 was achieved under individual treatment with prochloraz-manganese and its combinations with both disinfectants. The lowest yield in the third trial was found in treatment with peracetic acid, BE=72.78. The highest BE exceeding 90.00 was attained in both uninoculated control and treatments with prochloraz-manganese alone or in combination with both disinfectants. The lowest mean value of BE of all three trials was found in inoculated control, 69.36. Peracetic acid as a disinfectant showed higher BE than colloidal silver. Prochloraz-manganese treatment had no significant influence on total biological efficiency which is in accordance with data reported by Chrysayi-Tokousbalides et al. (2007).

Table 2. Biological efficiency (BE) % of different treatments of *Agaricus bisporus* artificially inoculated with *Cladobotryum dendroides* Vegr2C7; BE% = ratio of fresh weight of total mushroom yield and weight of dry spawned substrate

Treatments (mg/ml L ⁻¹ m ⁻²)	BE (%)					
	Trial I		Trial II		Trial III	
	Mean	SD ¹	Mean	SD	Mean	SD
Uninoculated control	74.28 c ²	8.00	90.14 c	5.05	113.62 b	12.50
Inoculated control	64.70 f	10.16	60.94 g	9.86	82.42 e	6.44
Peracetic acid 1.7	91.08 b	8.87	78.25 f	8.58	72.78 g	6.42
Colloidal silver 30	67.46 e	4.04	88.38 d	9.68	77.31 f	8.09
Prochloraz-Mn 1.2	97.48 a	6.77	91.62 b	7.08	110.42 c	3.88
+ Peracetic acid 1.7						
Prochloraz-Mn 1.2	70.98 d	7.08	94.78 a	6.94	115.11 a	9.47
+ Colloidal silver 30						
Prochloraz-Mn 1.2	91.16 b	4.96	84.89 e	7.95	100.16 d	4.41

¹SD-standard deviation of means (degrees of freedom = 2).

²Means in the same column marked by the same letter are not significantly different ($P=0.005$).

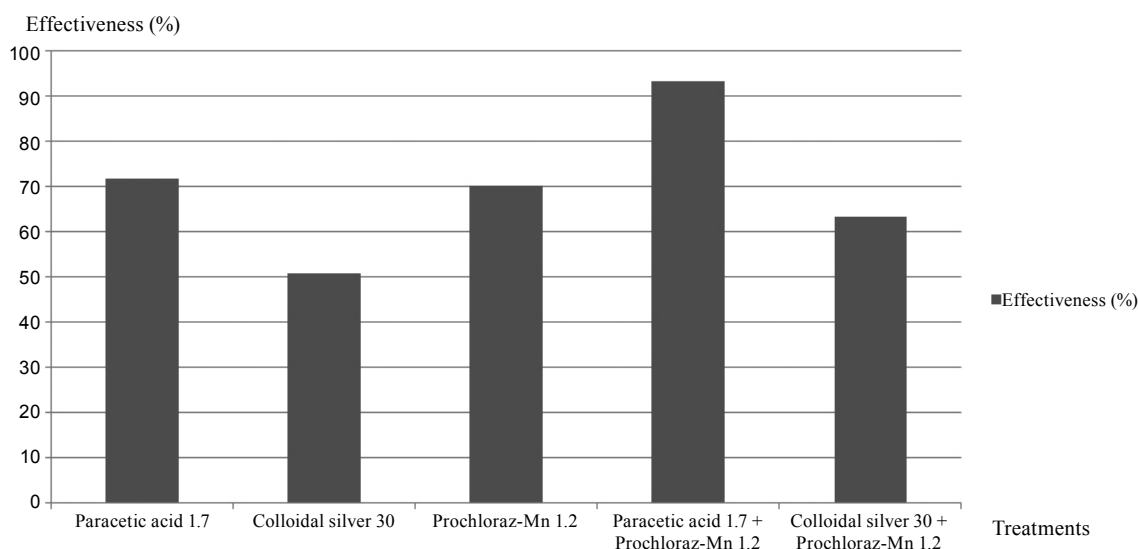


Figure 1. Effectiveness (%) of different treatments against *Cladobotryum dendroides* Vegr2C7 on artificially inoculated *Agaricus bisporus*; fungicide effectiveness % = $[(Ic - It)/Ic] \times 100$, Ic - disease incidence in inoculated control, It - disease incidence in treated samples; standard deviation of means = 15.56 (degrees of freedom = 10).

Effectiveness in inhibition of disease symptoms: Effectiveness was calculated based on disease incidence recorded in both the first and second flushes. Diseased fruiting bodies of *A. bisporus* were found in control treatments in the third flush as cross-contamination occurred and data from the third flush concerning effectiveness were not considered. The highest effectiveness of 93.33% was attained in treatments involving prochloraz-manganese combined with peracetic acid (Figure 1). Highly effective were prochloraz-manganese and peracetic acid, their individual application exhibited similar values, 70.00 and 71.67%, respectively. Less effective was colloidal silver, both when it was used alone and combined with prochloraz-manganese (50.57 and 63.33%, respectively). Peracetic acid provided better disease control than colloidal silver, both alone and combined with the fungicide.

Intereaction between chemicals: Calculation for synergism based on Limpel's formula (Richer, 1987) and Abbott's formula (Abbott, 1925) showed a synergistic reaction ($SF > 1$) of 1.02 in the effectiveness of peracetic acid and prochloraz-manganese (Table 3). Antagonistic reaction ($SF < 1$) of 0.74 was found in the other combination of the fungicide prochloraz-manganese with colloidal silver disinfectant. The trials against cobweb disease revealed a slightly synergistic reaction between the fungicide and peracetic acid, as their synergy factor was somewhat higher than 1, and antagonistic between the fungicide and colloidal silver.

There are not many reference data relating to casing soil treatments and only several of them have reported about disinfectants based on chlorine in bacterial blotch control (Wong & Preece, 1985; Abosriwil & Clancy, 2002). The authors reported that sodium hypochlorite inhibited the growth of pathogenic bacteria in water, casing and mushroom tissue. Sodium hypochlorite is effective only against bacteria and some viruses, while on the other hand it is a very dangerous and corrosive substance. Formaldehyde is widely used as a casing soil disinfectant in Serbia and worldwide (Fletcher & Ganney, 1968; Sharma & Guleria, 1999). In view of toxicity and volatility, exposure to formaldehyde is a significant consideration for human health (Rutala & Weber, 2008). At the Pennsylvania State University, Chikthimmah (2006) found significantly

reduced bacterial populations in fresh mushrooms after using irrigation water amended with acidic electrolyzed oxidizing water (EOA) and/or 0.3% calcium chloride (CaCl_2). Irrigation treatments containing 0.3% CaCl_2 decreased overall yield by 3.9 to 6.6%. A combination of 0.75% hydrogen peroxide and 0.3% CaCl_2 reduced bacterial populations in fresh mushrooms by 87% without affecting crop yield. The author reported that there was no significant difference in bacterial abundance in mushrooms grown using either unpasteurized casing or one pasteurized by steam temperature of 60°C for 2 hours. Crop yield decreased by 11.0% on pasteurized casing soil. It is presumed that steam treatment may negatively affect beneficial soil microflora while inhibiting soilborne pathogens.

CONCLUSIONS

In conclusion, colloidal silver with hydrogen peroxide caused a significant reduction in cobweb disease levels but less than peracetic acid. The results indicate that peracetic acid and colloidal silver had positive effect on *A. bisporus* physiology. Peracetic acid provided better disease control than colloidal silver. Treatment with peracetic acid and prochloraz-manganese revealed a synergistic reaction, and showed the highest effectiveness in disease control and satisfactory mushroom productivity. In available literature, there are no studies on effects of a disinfectant based on peracetic acid (active oxygen) against *C. dendroides* to compare our results to. Based on these findings, peracetic acid should be recommended as a successful casing soil disinfectant against cobweb disease of *A. bisporus*. It does not contain chlorine, aldehydes or phenols, which makes it applicable in food industry. It is easily biodegradable to water, active oxygen and carbon dioxide. It may be considered as an environmentally friendly alternative to other highly toxic disinfectants that are in daily use in mushroom production facilities. Further *in vivo* experiments aimed to determine the recommended application rates of different products based on active oxygen, are in progress. The disinfectant based on colloidal silver should be tested further, analysing especially its residues in mushrooms.

Table 3. Interaction between prochloraz-manganese and two disinfectants in effectiveness against *Cladobotryum dendroides* Vegr2C7

Treatment	Observed effectiveness (%)	Expected effectiveness (%)	Synergy factor
	Mean \pm SD ¹		
Peracetic acid 1.7 + Prochloraz-Mn 1.2	93.33 \pm 11.55	91.50	1.02
Colloidal silver 30 + Prochloraz-Mn 1.2	63.33 \pm 15.27	85.17	0.74

¹SD - standard deviation of means (degrees of freedom = 2).

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Mogućnost zaštite šampinjona od paučinaste plesni sterilisanjem pokrivke ekološkim sredstvima

REZIME

Zemljišna mikromiceta *Cladobotryum dendroides* izaziva paučinastu plesan šampinjona (*Agaricus bisporus*) i nanosi značajne štete u proizvodnji. Uobičajen način dezinfekcije pokrivke za gajenje šampinjona je tretiranje formaldehidom koji ima toksična svojstva. Cilj ovog rada je ispitivanje mogućnosti primene dva ekološka dezinfekciona sredstva na bazi koloidnog srebra i persirćetne kiseline u suzbijanju *C. dendroides*. Određena je njihova efikasnost, uticaj na prinos šampinjona i međusobna reakcija sa fungicidom prohloraz-Mn.

Pokrivka od crnog treseta i kreča je stavljena na supstrat zasejan micelijom šampinjona soj 737, inokulisana patogenom *C. dendroides* i tretirana fungicidom prohloraz-Mn i ekološkim dezinficijensima na bazi persirćetne kiseline i koloidnog srebra. Uticaj antifungalnih supstanci na prinos (biološka efikasnost) je određen kroz srazmeru mase ukupnog prinosa šampinjona i mase suvog supstrata. Efikasnost fungicida u suzbijanju patogena i sinergistički faktor su izračunati primenom Abotove formule. Sinergizam između prohloraz-Mn i oba dezinficijensa je određen pomoću Limpelove formule.

Najveća biološka efikasnost je utvrđena u tretmanima sa prohloraz-Mn, preko 92,00, primenjenim pojedinačno ili u kombinaciji sa oba dezinficijensa. Najveća efikasnost u suzbijanju patogena, 93.33%, postignuta je u kombinovanoj primeni fungicida sa persirćetnom kiselinom. Ustanovljen je sinergizam između fungicida i persirćetne kiseline i antagonizam u kombinovanoj primeni fungicida i koloidnog srebra.

Primenom persirćetne kiseline omogućena je veća efikasnost u poređenju sa koloidnim srebrom u suzbijanju prouzrokovala paučinaste plesni, bilo da je primenjena pojedinačno ili u kombinaciji sa fungicidom. Na osnovu ovih ispitivanja, može se preporučiti primena persirćetne kiseline kao ekološkog dezinficijensa pokrivke za gajenje šampinjona i zaštitu od paučinaste plesni.

Ključne reči: Dezinfekcija zemljišta; koloidno srebro; persirćetna kiselina; prohloraz; *Cladobotryum dendroides*; *Agaricus bisporus*