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Sensitivity of *Cladobotryum* spp., a Pathogen of the Button Mushroom (*Agaricus bisporus*), to Some Fungicides

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SUMMARY

Thirteen isolates of *Cladobotryum* spp., isolated from diseased fruiting bodies of *Agaricus bisporus*, sampled from mushroom farms in Serbia during 2003-2006, were studied. Sensitivity to several selected fungicides was tested and all isolates were found to be highly sensitive to iprodione (EC_{50} = 0.35-2.30 mg L^{-1}), benomyl (EC_{50} = 0.14-0.97 mg L^{-1}) and especially to prochloraz-Mn (EC_{50} = 0.02-0.09 mg L^{-1}). The minimum inhibitory concentration (MIC) of benomyl was 4 mg L^{-1}, while those of prochloraz-Mn and iprodione exceeded 1000 mg L^{-1}. The minimum fungicide concentration (MFC) of benomyl was 4 mg L^{-1}, and none of the tested concentrations of prochloraz-Mn and iprodione were lethal to any of the investigated *Cladobotryum* spp. isolates.

Keywords: *Cladobotryum* spp.; Antifungal activity; Benomyl; Iprodione; Prochloraz-Mn

INTRODUCTION

White button mushroom (*Agaricus bisporus* (Lange) Imbach) is the most common cultivated mushroom species in Serbia (Bugarski and Gvozdenović, 1998). Its production is severely afflicted by fungal, bacterial and viral pathogens that may cause diseases resulting in significant yield losses. *Verticillium fungicola* var. *fungicola*, *Mycogone perniciosa* and *Cladobotryum* spp. are the most important fungal pathogens of *A. bisporus*. Untill the mid-1990s *Cladobotryum* spp., the causal agents of cobweb disease, had predominantly affected later flushes in mushroom production, but cobweb mould is now considered to be the most problematic disease affecting mushroom cultivation (Gaze, 1995).

Cladobotryum spp. is a soil-inhabiting fungus that may be introduced into mushroom casing in the form of spores or mycelium (McKay et al., 1999). It produces verticillately branched conidiophores bearing two-, three- and four-celled conidia. Colonies on mushroom casing are usually cottony fluffy, white and circular in appearance, and may turn yellow or pink with age. Patches of mycelium rapidly colonize the casing surface, eventually causing the affected mushrooms to turn brown and rot (McKay et al., 1998). Massive sporulation usually occurs once mushrooms are encountered and dry spores, which are easily dislodged when watering the mushroom crop, are responsible for the brown spotting symptoms on mushroom fruit bodies (Grogan and Gaze, 2000).

Control of *A. bisporus* diseases is based on the use of chemicals. Development of pathogen resistance to fungicides has become a serious problem. In 1970s resistance to benzimidazole fungicides had been recorded in *Verticillium fungicola* (Fletcher and Yarham, 1976). The fungicides iprodione and prochloraz-Mn were introduced because of their ability to prevent the appearance of *V. fungicola*, *M. perniciosa* and *Cladobotryum* spp. (Gea et al., 1996). *V. fungicola* var. *fungicola* isolates resistant to iprodione have been found in Spain (Gea et al., 1996), and isolates moderately resistant to prochloraz-Mn in Great Britain and Spain (Grogan et al., 2000; Gea et al., 2003). By the mid-1980s, the first signs of *Cladobotryum* spp. resistance to benzimidazole fungicides occurred in Great Britain (Gaze, 1995). In 1992, following an extensive use of carbendazim in Ireland, resistance of cobweb disease organisms to benzimidazole fungicides was reported (Doyle and Morris, 1993). A study of cobweb isolates sampled during 1995 in Great Britain indicated that 75% of isolates were benzimidazole resistant. This fungicide resistance is believed to have contributed to the severity of outbreaks experienced in 1994-1995 (Adie and Grogan, 2000).

The most commonly used fungicides in mushroom industry in Serbia are benomyl, mancozeb and prochloraz-Mn. Althought iprodione is not used to control *A. bisporus* diseases in Serbia, it is a common fungicide in Europe (Gea et al., 1996). A survey of *V. fungicola* var. *fungicola* isolates in Serbia during 2002-2003 indicated that all investigated isolates were highly resistant to benomyl, having the EC_{50} values exceeding 200.00 mg L^{-1}, moderately sensitive to iprodione, whose EC_{50} values ranged between 11.93 and 22.80 mg L^{-1}, and highly sensitive to prochloraz-Mn with EC_{50} values below 3.00 mg L^{-1}. Prochloraz-Mn was highly toxic to *M. perniciosa* isolates, collected in the same period, with EC_{50} values being below 0.008 mg L^{-1}. Benomyl and iprodione were also very toxic to *M. perniciosa* isolates and had EC_{50} values below 0.46 and 4.08 mg L^{-1}, respectively (Potočnik, 2006). In the mid-1990s the cobweb disease was still rare before a third or fourth flush in mushroom farms in Serbia and it rarely caused a serious problem (Potočnik et al., 2004).

This study aimed to investigate the sensitivity of 13 *Cladobotryum* spp. isolates to the fungicides benomyl, iprodione and prochloraz-Mn. The isolates were collected from mushroom farms in Serbia during 2003-2006. Iprodione, although never used for disease control in mushrooms in Serbia, was included in order to determine its potential toxicity to the isolates investigated.

MATERIAL AND METHODS

Isolates

Sensitivity of 13 *Cladobotryum* spp. isolates to the selected fungicides was tested. All isolates were obtained from diseased fruiting bodies of *A. bisporus* collected from 13 farms in Serbia during 2003-2006 (Table 1). Isolation was done by sampling small pieces (2 x 2 x 5 mm) of fruiting bodies with cobweb disease symptoms and immersing them in a 1% sodium hypochlorite solution (for 1 min). All isolates were grown on potato dextrose agar medium (PDA) in 90 mm Petri plates at 18°C. The isolates have been kept on PDA, at 5°C, in the culture collection of the Institute of Pesticides and Environmental Protection.

Fungicides

Commercial formulations of the two most commonly used fungicides in the Serbian mushroom industry, benomyl (Benfungin WP, 50%, Galenika-Fitofarmacija), and prochloraz-Mn (Octave WP, 50%, Bayer CropScience), were tested in this study. Iprodione (Kidan EC, 25.5%, Bayer CropScience), a

Table 1. *Cladobotryum* spp. isolates
Tabela 1. Izolati *Cladobotryum* spp.

Code of isolate Naziv izolata	Origin Poreklo izolata	Year of collection Vreme izolacije
SPC4	Smederevska Palanka	2003
P1C1	Požarevac	2003
BaC1	Beograd – Banjica	2004
BC1	Beograd – Savski venac	2004
KuC1	Kurjače	2004
NSlC1	Novi Slankamen	2004
OBC2	Ovčar Banja	2004
P7C1	Požarevac	2004
Res1C1	Resnik	2004
VG3C2	Vračev Gaj	2004
BečC1	Bečej	2006
JakC1	Jakovo	2006
KalC1	Kaluđerica	2006

common fungicide in Europe, was included in sensitivity tests.

Benomyl is a benzimidazole fungicide, which binds to fungal beta-tubulins and inhibits the microtubule function (Smith, 1994). Prochloraz-Mn is a sterol C-14 demethylation inhibitor, which impairs biosynthesis of ergosterol, an essential compound for the stability and functioning of lipoprotein membranes (Scheinpflug, 1994; Kuck et al., 1995). Iprodione inhibits germination of spores and growth of fungal mycelium (Sisler, 1994).

Freshly-made stock solutions were prepared to give specific concentrations of active ingredient in mg L^{-1}. Volumes of stock solution were added to molten (50°C) sterile culture media prior to pouring, producing active ingredient concentrations ranging from 0.01 to 1000.00 mg L^{-1} (Grogan and Gaze, 2000).

Antifungal activity

Cladobotryum spp. isolates grown on PDA medium amended with the fungicides: benomyl, iprodione or prochloraz-Mn, were used for sensitivity tests. Preliminary concentrations of all selected fungicides were: 0.01, 0.10, 1.00, 10.00, 100.00 and 1000.00 mg L^{-1}. Based on the results obtained, the selected concentrations of benomyl for further study were: 1.50, 0.75, 0.37 and 0.19 mg L^{-1}; iprodione: 5.00, 2.50, 1.00 and 0.50 mg L^{-1}; prochloraz-Mn: 0.150, 0.075, 0.037 and 0.019 mg L^{-1}. Each plate was inoculated with an inverted mycelium agar disc (10 mm), taken from the edge of a four day-old culture of *Cladobotryum* spp. isolates, placed centrally onto the fungicide-amended and fungicide-free medium (control) and incubated at 18°C. Three replicates per treatment were used. Colony diameter was measured after three days of cultivation. Mycelium growth on the fungicide-amended medium was measured as a percentage against control. The EC_{50} (fungicide concentration which inhibits mycelial growth by 50%) was determined for each isolate and data of fungicide concentration and relative inhibition were analysed using probit analysis, according to Finney (1971). The resistance factor (RF) was expressed as the ratio of the highest EC_{50} and the lowest EC_{50} of the isolates tested (Gouot, 1994). The means were separated by Duncan's test.

The fungicide concentration inhibiting completely mycelium growth is defined as the minimum inhibitory concentration (MIC), and concentration at which mycelium growth has not been observed after 7 days of reisolation on PDA without fungicides as the minimum fungicidal concentration (MFC).

The minimum inhibitory concentration and minimum fungicidal concentrations of the fungicides were tested by macrodilution method (Ishii, 1995) in PDA medium amended with benomyl, iprodione or prochloraz-Mn at concentrations of: 100, 200, 300, 400, 500, 600, 700, 800, 900, and 1000.00 mg L^{-1}. Benomyl test was similarly repeated using concentrations of: 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mg L^{-1}. The minimum inhibitory concentrations were examined by measuring mycelium growth on the 7^{th}, 14^{th}, and 21^{st} day of incubation. The minimum fungicidal concentration was determined after 7 days of reisolation on fungicide-free PDA. Three replicates per treatment and isolate were used.

RESULTS

Antifungal activity of benomyl, iprodione and prochloraz-Mn

All *Cladobotryum* spp. isolates demonstrated an ability to tolerate benomyl at low concentrations. They were capable of good growth at 1 mg L^{-1}, but most isolates were severely inhibited at concentrations of 2 mg L^{-1} or higher. The EC_{50} values were between 0.14 and 0.97 mg L^{-1}. The highest resistance factor was found for the isolate OBC2 (RF=6.93). All isolates were sensitive to benomyl. Statistically significant differences were found between the EC_{50} values of different isolates (Table 2).

The investigated *Cladobotryum* spp. isolates were strongly susceptibile to prochloraz-Mn. They were capable to grow well at 0.15 mg L^{-1} concentration, but were severely inhibited at 0.20 mg L^{-1} and higher (Table 3). The EC_{50} values were between 0.02 and 0.09 mg L^{-1}. The isolate BečC1 had the highest value of resistance factor (RF = 4.50). Statistically significant differences were found between the EC_{50} values of different isolates.

The isolates were capable of good growth at 5 mg L^{-1} iprodione concentration, but were severely inhibited at 6 mg L^{-1} and above. The EC_{50} values were between 0.35 and 2.30 mg L^{-1} (Table 4). The isolates OBC2 and KalC1 had the highest values of resistance factor (RF = 6.57). All investigated isolates were found to be sensitive to iprodione. Statistically significant differences were found between the EC_{50} values of different isolates.

Table 2. *In vitro* sensitivity of *Cladobotryum* spp. isolates to benomyl
Tabela 2. *In vitro* osetljivost izolata *Cladobotryum* spp. na benomil

Code of isolate Naziv izolata	Benomyl – Benomil					
	EC_{50} (mg L^{-1})		b		H	RF
	Mean[a] Vrednost	Range Opseg	Mean Vrednost	Range Opseg		
BC1	0.23 efg	0.18-0.27	2.45	2.22-2.68	0.32	1.64
BaC1	0.33 def	0.26-0.39	2.15	1.90-2.38	1.96	5.36
BečC1	0.14 g	0.09-0.19	2.08	1.77-2.39	0.27	1.00
JakC1	0.39 de	0.31-0.48	1.45	1.24-1.66	0.25	2.78
KalC1	0.43 cd	0.38-0.47	3.3	3.01-3.56	0.61	3.07
KuC1	0.32 def	0.28-0.37	2.71	2.44-2.98	1.83	2.28
NSlC1	0.68 b	0.57-0.86	1.71	1.47-1.95	1.87	4.85
OBC2	0.97 a	0.81-1.23	1.83	1.60-2.06	1.33	6.93
SPC4	0.32 def	0.28-0.36	2.70	2.43-2.97	1.57	2.28
P1C1	0.19 fg	0.13-0.23	1.99	1.76-2.22	1.89	1.35
P7C1	0.37 de	0.28-0.45	1.53	1.33-1.73	0.66	2.85
Res1C1	0.40 de	0.31-0.49	1.59	1.39-1.79	1.82	2.85
VG3C2	0.57 bc	0.52-0.62	4.35	3.99-4.71	0.02	4.07

EC_{50} = Fungicide concentration which inhibits mycelial growth by 50%/Koncentracija fungicida koja inhibira rast micelije 50%
[a] The means followed by different letters are significantly different (Duncan's test, P<0.05)/Vrednosti označene različitim slovima se statistički značajno razlikuju (Dankanov test, P<0.05)
RF = The resistance factor is expressed as the ratio of the higest EC_{50} to the lowest EC_{50} of the isolates tested/Faktor rezistentnosti predstavlja odnos najveće i najmanje EC_{50} za testirane izolate
b = Regression coefficient at the 95% confidence level/Koeficijent regresije na nivou poverenja 95%
H = Heterogenity/Heterogenost

Table 3. *In vitro* sensitivity of *Cladobotryum* spp. isolates to prochloraz-Mn
Tabela 3. *In vitro* osetljivost izolata *Cladobotryum* spp. na prochloraz-Mn

Code of isolate Naziv izolata	Prochloraz-Mn					
	EC50 (mg L^{-1})		b		H	RF
	Mean[a] Vrednost	Range Opseg	Mean Vrednost	Range Opseg		
BC1	0.08 ab	0.06-0.11	1.24	1.04-1.44	1.15	4.00
BaC1	0.04 c	0.02-0.07	1.25	1.03-1.47	2.46	2.00
BečC1	0.09 a	0.08-0.11	2.31	2.55-2.07	1.93	4.50
JakC1	0.04 cd	0.01-0.05	0.64	0.39-0.83	0.18	2.00
KalC1	0.04 cd	0.03-0.05	2.32	2.09-2.55	0.44	2.00
KuC1	0.05 cd	0.04-0.05	1.53	1.33-1.73	0.78	2.50
NSlC1	0.06 bc	0.04-0.08	1.02	0.83-1.21	0.28	3.00
OBC2	0.04 cd	0.02-0.05	1.55	1.29-1.81	0.83	2.00
SPC4	0.08 ab	0.06-0.12	1.17	0.97-1.37	0.22	4.00
P1C1	0.04 cd	0.03-0.05	1.09	0.90-1.28	1.98	2.00
P7C1	0.02 d	0.01-0.03	0.78	0.57-0.97	1.07	1.00
Res1C1	0.05 dc	0.04-0.06	1.50	1.30-1.70	1.08	2.50
VG3C2	0.06 bc	0.05-0.07	2.60	2.34-2.84	2.40	3.00

EC_{50} = Fungicide concentration which inhibits mycelial growth by 50%/Koncentracija fungicida koja inhibira rast micelije 50%
[a] The means followed by different letters are significantly different (Duncan's test, P<0.05)/Vrednosti označene različitim slovima se statistički značajno razlikuju (Dankanov test, P<0.05)
RF = The resistance factor is expressed as the ratio of the higest EC_{50} to the lowest EC_{50} of the isolates tested/Faktor rezistentnosti predstavlja odnos najveće i najmanje EC_{50} za testirane izolate
b = Regression coefficient at the 95% confidence level/Koeficijent regresije na nivou poverenja 95%
H = Heterogenity/Heterogenost

Table 4. *In vitro* sensitivity of *Cladobotryum* spp. isolates to iprodione
Tabela 4. *In vitro* osetljivost izolata *Cladobotryum* spp. na iprodion

Code of isolate Naziv izolata	Iprodione – Iprodion					
	EC_{50} (mg L^{-1})		b		H	RF
	Mean[a] Vrednost	Range Opseg	Mean Vrednost	Range Opseg		
BC1	1.69 b	1.49-1.94	2.56	2.34-2.78	0.52	4.83
BaC1	1.04 c	0.85-1.25	2.28	2.07-2.49	3.78	2.97
BečC1	0.44 d	0.23-0.65	1.07	0.89-1.25	1.12	1.26
JakC1	1.57 b	1.39-1.78	2.91	2.68-3.14	0.95	4.48
KalC1	2.30 a	1.93-2.79	1.82	1.61-2.01	0.30	6.57
KuC1	0.35 d	0.30-0.43	2.39	2.06-2.72	0.99	1.00
NSlC1	1.26 bc	1.08-1.46	3.21	2.94-3.48	0.04	3.60
OBC2	2.30 a	1.93-2.79	1.82	1.59-2.01	0.30	6.57
SPC4	1.73 b	1.40-2.18	1.74	1.54-1.94	1.24	4.94
P1C1	1.73 b	1.55-1.92	3.31	3.07-3.55	0.72	4.94
P7C1	0.42 d	0.38-0.55	2.08	1.75-2.42	0.60	1.20
Res1C1	2.30 a	1.93-2.79	1.82	1.61-2.01	0.30	6.57
VG3C2	1.55 b	1.25-1.86	2.40	2.16-2.64	0.19	4.43

EC_{50} = Fungicide concentration which inhibits mycelial growth by 50%/Koncentracija fungicida koja inhibira rast micelije 50%
[a] The means followed by different letters are significantly different (Duncan's test, P<0.05)/Vrednosti označene različitim slovima se statistički značajno razlikuju (Dankanov test, P<0.05)
RF = The resistance factor is expressed as the ratio of the higest EC_{50} to the lowest EC_{50} of the isolates tested/Faktor rezistentnosti predstavlja odnos najveće i najmanje EC_{50} za testirane izolate
b = Regression coefficient at the 95% confidence level/Koeficijent regresije na nivou poverenja 95%
H = Heterogenity/Heterogenost

The minimum inhibitory concentration (MIC) of benomyl for all *Cladobotryum* spp. isolates studied was 4 mg L^{-1}, and over 1000 mg $L^{-1}$of prochloraz-Mn and iprodione. The minimum fungicide concentration (MFC) of benomyl was 4 mg L^{-1}, while prochloraz-Mn and iprodione were not lethal for the studied *Cladobotryum* spp. isolates at any of the selected concentrations. Prochloraz-Mn and iprodione had only fungistatic activity.

DISCUSSION

The study showed high sensitivity of *Cladobotryum* spp. isolates to iprodione (EC_{50} 0.35-2.30 mg L^{-1}), benomyl (EC_{50} 0.14-0.97 mg L^{-1}), as well as to prochloraz-Mn (EC_{50} 0.02-0.09 mg L^{-1}). The highest resistance factors to benomyl, prochloraz-Mn and iprodione exceeded 3 and, according to criteria established by Gouot (1994), the isolates were weakly resistant. It does not necessarily imply that resistance will become a problem, because the obtained EC_{50} values were very low. A wide range of EC_{50} values and low mean EC_{50} of all fungicides showed a wider range in sensitivity among the studied isolates (Tables 2, 3, and 4).

In the 1970s, a survey of *Cladobotryum* spp. isolates in Great Britain showed that none of them grew at 5 mg L^{-1} benomyl and, so that the isolates were sensitive (Fletcher and Yarham, 1976). In the British mushroom industry benomyl has been largely replaced by carbendazim, which is a major primary breakdown product of benomyl (Hassall, 1990). In the 1980s, benomyl, thiabendazole and prochloraz-Mn were still effective against *Cladobotryum* spp. in cropping experiments (Fletcher et al., 1983). In a later survey of British farms in 1993, some of *Cladobotryum* spp. isolates were capable of growing at 20 mg L^{-1} thiabendazole (Fletcher and Jaffe, 1993). In 1994/95, outbreaks of cobweb were on the increase and a survey in Great Britain reported that *Cladobotryum* spp. isolates were inhibited at 10 mg L^{-1} concentration of carbendazim (Grogan and Gaze, 2000). Resistance of *Cladobotryum* spp. to benzimidazole fungicides was also reported in Ireland in 1992. Further investigation confirmed that these isolates were resistant to the benzimidazole fugnicides benomyl and carbendazim at up to 10 mg L^{-1} (McKay et al., 1998). Grogan and Gaze

(2000) recorded that a majority of *Cladobotryum* spp. isolates in Great Britain were strongly resistant to thiabendazole (EC_{50} > 200 mg L^{-1}), and weakly resistant to carbendazim (EC_{50} 3.5-4.4 mg L^{-1}). Our findings indicate that isolates obtained from mushroom crops in Serbia are still sensitive to benzimidazole fungicides as EC_{50} values of benomyl were between 0.14 and 0.97 mg L^{-1}. The investigated isolates grew at 1 mg L^{-1}, but most of them were inhibited at 2 mg L^{-1}. The obtained values were much lower than those recorded for benomyl by McKay et al. (1998) in Ireland. It is important, therefore, to test the pathogen's responce to a wide range of fungicide concentrations in order to determine more precisely the degree of isolate susceptibility (Grogan, 2006).

Studies in India (Bhatt and Singh, 1992) showed that prochloraz-Mn inhibited the growth of *Cladobotryum* spp. isolates at lower concentrations. Contrary to these results, prochloraz-Mn EC_{50} values were higher for 25% of the isolates examined in Great Britain and ranged from 0.14 to 7.8 mg L^{-1} (Grogan and Gaze, 2000). Grogan and Gaze (2000) reported that 75% of *Cladobotryum* spp. isolates were weakly resistant to prochloraz-Mn (EC_{50} 1.1-3.7 mg L^{-1}). The isolates collected in Serbian mushroom units were inhibited at 0.20 mg L^{-1} with EC_{50} values between 0.02 and 0.09 mg L^{-1}. They were much more sensitive to prochloraz-Mn than the isolates in Great Britain, as shown by Grogan and Gaze (2000).

The studied *Cladobotryum* spp. isolates from mushroom crops in Serbia were inhibited at 6 mg L^{-1} iprodione, with EC_{50} values between 0.35 and 2.30 mg L^{-1}. No data are available on the activity of iprodione against *Cladobotryum* spp. isolates in other regions. Iprodione could be recommended for use against cobweb disease in Serbia after *in vivo* biological efficacy testing.

According to criteria established by Gea et al. (1996, 2003), resistance to benomyl, iprodione or prochloraz-Mn was not detected in any of the studied isolates of *Cladobotryum* spp. in Serbia. Prochloraz-Mn showed the highest toxicity of all fungicides tested against *Cladobotryum* spp. isolates in Serbia. A wide range of EC_{50} values was observed in *Cladobotryum* spp. isolates. With an expanding range of susceptibility, there is always a growing risk of individuals becoming less susceptible to fungicides than the rest of their population. Gradual selection pressure exerted on the pathogen by the fungicide, and the fact that a few applications are required each year to control *A. bisporus* diseases, contribute to increasing the pathogen's resistance to fungicides (Gea et al., 2005). Although the risk of resistance developing in *Cladobotryum* spp. in Serbia seems low, farm hygiene measures should be used to reduce the incidence of the disease and minimize that risk.

Grogan (2006) reported a *Cladobotryum* spp. isolate resistant *in vitro* to thiabendazole to be also cross-resistant to carbendazim, another benzimidazole fungicide. The emergence of benzimidazole resistance reduces the value of benzimidazoles in mushroom pathogens control. However, a lack of effective alternatives makes fungicides useful in cases where pathogens are still sensitive but this requires regular resistance monitoring (Grogan, 2006). Also, many studies in Belgium, Great Britain, and Spain show a gradually diminishing sensitivity of *V. fungicola* to prochloraz-Mn (Desrumeaux et al.,1998; Grogan et al., 2000; Gea et al., 2005). Mushroom growers can no longer rely exclusively rely on prochloraz-Mn as a primary management tool (Gea et al., 2005). Management strategies for *A. bisporus* diseases should be focused on integrated disease control.

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Osetljivost *Cladobotryum* spp., patogena šampinjona (*Agaricus bisporus*), na neke fungicide

REZIME

Trinaest izolata *Cladobotryum* spp., dobijeno je iz obolelih plodonosnih tela *Agaricus bisporus* iz gajilišta šampinjona u Srbiji tokom 2003-2006. godine. Ispitivanje osetljivosti izolata na fungicide je pokazalo da su svi izolati visoko osetljivi na benomil (EC_{50} = 0.14-0.97 mg L^{-1}), iprodion (EC_{50} = 0.35-2.30 mg L^{-1}) i naročito na prohloraz (EC_{50} = 0.02-0.09 mg L^{-1}). Minimalna inhibitorna koncentracija (MIC) benomila je bila 4 mg L^{-1}, a prohloraza i iprodiona veća od 1000 mg L^{-1}. Minimalna fungicidna koncentracija (MFC) benomila je takođe bila 4 mg L^{-1}, a nijedna od testiranih koncentracija prohloraza i iprodiona nije bila letalna za *Cladobotryum* spp. izolate.

Ključne reči: *Cladobotryum* spp.; antifungalna aktivnost; benomil; iprodion; prohloraz-Mn