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In vitro Sensitivity of the Mushroom Pathogen *Cladobotryum* spp. to Thiophanate-Methyl and Different Carbendazim Formulations

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SUMMARY

A survey of *in vitro* sensitivity of 13 isolates of the mushroom pathogen *Cladobotryum* spp. to the fungicides thiophanate-methyl, carbendazim, cyproconazole+carbendazim and flusilazole+carbendazim was undertaken. The isolates were collected from diseased fruiting bodies of *Agaricus bisporus* sampled from mushroom farms in Serbia over the period 2003-2006. Sensitivity to the selected fungicides was tested and all isolates were found to be highly sensitive to carbendazim (EC_{50} = 0.24 - 2.92 mg l^{-1}), cyproconazole+carbendazim (EC_{50} = 0.33 - 1.82 mg l^{-1}) and especially to flusilazole+carbendazim (EC_{50} = 0.02 - 0.11 mg l^{-1}). All the isolates tested were weakly resistant to thiophanate-methyl and had EC_{50} values in the region of 6.53 to 12.09 mg l^{-1}.

Keywords: *Cladobotryum* spp.; Antifungal activity; Carbendazim; Cyproconazole + carbendazim; Flusilazole + carbendazim; Thiophanate-methyl

INTRODUCTION

Cladobotryum spp., the causal agent of cobweb disease, is one of the major fungal pathogen of button mushroom (*Agaricus bisporus* (Lange) Imbach) (Van Zaayen and Van Andrichem, 1982). The pathogen is a soil-inhabiting cosmopolitan fungus (McKay et al., 1999). *Cladobotryum* spp. produces verticillately or irregularly branched conidiophores bearing two-, three- and four-celled conidia. Colonies on mushroom casing are cottony fluffy, white, yellowish to pink. Its mycelia rapidly colonizes the casing surface, covers hymenophore, pileus and stipe of *A. bisporus*, causing decay of host basidiomata (McKay et al., 1998). Outbreaks of cobweb disease in Serbian mushroom farms have been sporadic for many years and the disease controlled by available fungicides, but cobweb mould is now considered to be the most problematic disease affecting mushroom cultivation (Potočnik et al., 2007).

After being first introduced in the late 1960s, the benzimidazole fungicides, i.e. benomyl, carbendazim and thiophanate-methyl, provided excellent control of

many fungal diseases (Delp, 1987). After several years of intensive use it was found that a selection of resistant strains of target fungi reduced the usefulness of these compounds (Smith, 1988). The use of benzimidazole fungicides to control fungal mushroom diseases was first reported in 1970 (Wuest and Cole, 1970), resulting in a sharp decline in the incidence of fungal disease in mushroom crops (Fletcher, 1973). Resistance of *Verticillium fungicola* to benomyl was reported soon after its release (Fletcher and Yarham, 1976). Grogan and Gaze (2000), working in Great Britain, reported that benomyl was being largely replaced by the active ingredient carbendazim, which is a major primary breakdown product of benomyl (Hassall, 1990) and would be expected to have similar activity as benomyl against individual pathogens. By the mid-1980s, the first sign of *Cladobotryum* spp. isolates developing resistance to benzimidazole fungicides occurred in Great Britain (Gaze, 1995). A majority of *Cladobotryum* spp. isolates were found to be strongly resistant to thiabendazole (EC_{50} > 200 mg l^{-1}). Thiabendazole-resistant isolates were weakly resistant to carbendazim (EC_{50} 1-10 mg l^{-1}). In 1992, resistance of cobweb disease organisms to benzimidazole fungicides was reported in the Republic of Ireland after extensive use of carbendazim (Doyle and Morris, 1993). This disease outbreak differed from earlier problems as its spreading in growing units was very rapid having the potential to spread throughout a crop over a brief period of 24-48 h. Colony morphology on casing was distinctly different; a dense, granular mat was produced over the casing layer, with little or no characteristic "pink" colouration. Further investigations confirmed that these isolates were resistant to the benzimidazole fungicides benomyl and carbendazim at rates of up to 10 mg l^{-1} active ingredient (McKay et al., 1998).

The imidazole fungicide prochloraz-Mn was introduced becasue of its ability to prevent the appearance of *V. fungicola*, *Mycogone perniciosa* and *Cladobotryum* spp. (Gea et al., 1996). It is the most commonly used fungicide in mushroom industry in EU countries and Serbia (Gea et al., 1996; Potočnik et al., 2007). However, *V. fugnicola* var. *fungicola* isolates weakly resistant to prochloraz-Mn have been found in Great Britain and Spain (Grogan et al., 2000; Gea et al., 2003). Very few efficacy trials of other sterol biosynthesis inhibitors (DMI fungicides) have been conducted on cultivated mushrooms. Chrysayi-Tokousbalides et al. (2006) reported that tebuconazole, a triazole DMI fungicide, was not as toxic as prochloraz-Mn to *V. fungicola*.

The benzimidazole fungicides benomyl, carbendazim and thiophanate-methyl and imidazole prochloraz-Mn are frequently applied in the Serbian mushroom industry (Milenković, 1997). A survey of Serbian isolates conducted over the period 2003-2006 showed that *V. fungicola* var. *fungicola* has developed a resistance to benzimidazole fungicides, having the EC_{50} values of benomyl that exceed 200.00 mg l^{-1} (Potočnik, 2006). Potočnik et al. (2007) reported that benomyl and prochloraz-Mn tolerance was not detected in *Cladobotryum* spp.

The withdrawal of benomyl from the market due to registration restrictions (Anonymous, 2002a; Anonymous, 2002b) and development of resistance to benzimidazole fungicides (Bonnen and Hopkins, 1997) have additionally reduced the number of available fungicides that would be effective against fungal diseases. The fungicides still officially recommended for mushroom cultivation in EU countries are formulations of carbendazim, prochloraz-Mn complex and chlorothalonil. Fungicide efficacy trials on cultivated mushrooms are very rarely conducted by agrochemical companies because specially designed experimental facilities are required for appropriate evaluation (Chrysayi-Tokousbalides et al., 2006). As a result, very few commercial fungicides have been approved for mushrooms (Whitehead, 2002; Stoddart et al., 2004; Anonymous, 2005). This study aimed to investigate the sensitivity of 13 *Cladobotryum* spp. isolates, collected in Serbia over the period 2003-2006, to the fungicides carbendazim, cyproconazole+carbendazim, flusilazole+carbendazim and thiophanate-methyl. Cyproconazole+carbendazim and flusilazole+carbendazim formulations, although never used in mushroom units in Serbia, were included in sensitivity tests in order to determine their potentials as alternative fungicides against mycopathogenic fungi.

MATERIAL AND METHODS

Isolates

The sensitivity of 13 *Cladobotryum* spp. isolates to several selected fungicides was tested. All isolates were obtained from diseased fruiting bodies of *A. bisporus* collected from 13 farms in Serbia from 2003 to 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 hypo-

chlorite solution (for 1 min). All isolates were grown on potato dextrose agar medium (PDA) in 90 mm Petri plates at 18°C. The isolates are 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, namely carbendazim (Galofungin WP, 500 g kg^{-1}, Galenika-Fitofarmacija), and thiophanate-methyl (tested formulation WP, 700 g kg^{-1}, Agromarket), were tested in this study. Cyproconazole+carbendazim (Alto Combi SC, 160 g l^{-1} + 300 g l^{-1}, Syngenta) and flusilazole+carbendazim (Alert-S SC, 125 g l^{-1} + 250 g l^{-1}, Syngenta) were included in sensitivity tests.

Carbendazim, a benzimidazole fungicide, is a major primary breakdown product of benomyl (Hassall, 1990). Thiophanate-methyl is also considered a benzimidazole fungicide for being transformed to carbendazim. The mode of action of benzimidazole fungicides is interference with the division of cell nuclei by disrupting the assembly of tubulin into microtubules (Smith, 1988; Hassall, 1990). The Alto Combi formulation is a mixture of two active ingredients: cyproconazole and carbendazim, while Alert-S is a combination of flusilazole and carbendazim. Cyproconazole and flusilazole, which are triazole fungicides, are DMI fungicides, which impair biosynthesis of ergosterol, an essential compound for the stability and functioning of lipoprotein membranes (Buchenauer, 1987; Nene and Thapliyal,1993; Gisi and Hermann, 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: carbendazim, cyproconazole+carbendazim, flusilazole+carbendazim or thiophanate-methyl, were used for sensitivity tests. Preliminary concentrations of all selected fungicides were: 1000.00, 100.00, 10.00, 1.00, 0.10, and 0.01 mg l^{-1}. Based on the results obtained, the selected concentrations of carbendazim and cyproconazole+carbendazim for further study were: 1.50, 0.75, 0.37 and 0.19 mg l^{-1}; flusilazole+carbendazim: 0.10, 0.05, 0.03 and 0.013 mg l^{-1}; thiophanate-methyl: 50.00, 25.00, 12.50 and 6.25 mg l^{-1}. Each plate was inoculated with an inverted mycelium agar disc (10 mm), taken from the edge of four day-old cultures of *Cladobotryum* spp. isolates, placed centrally onto the fungicide-amended and fungicide-free media and incubated at 18°C. Three replicates per treatment were used. Colony diameter was measured after three days of cultivation. Mycelial growth on the fungicide-amended media 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 on fungicide concentration and relative inhibition were analysed using probit analysis, according to Finney (1971). The resistance factor (RF)

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

Code of isolate / Naziv izolata	Origin / Poreklo izolata	Year of collection / Vreme izolacije
SPC_4	Smederevska Palanka	2003
P_1C_1	Požarevac	2003
BaC_1	Beograd – Banjica	2004
BC_1	Beograd – Savski venac	2004
KuC_1	Kurjače	2004
$NSlC_1$	Novi Slankamen	2004
OBC_2	Ovčar Banja	2004
P_7C_1	Požarevac	2004
Res_1C_1	Resnik	2004
VG_3C_2	Vračev Gaj	2004
$BečC_1$	Bečej	2006
$JakC_1$	Jakovo	2006
$KalC_1$	Kaluđerica	2006

was expressed as the ratio of EC_{50} and the lowest EC_{50} of the isolates tested (Gouot, 1994).

RESULTS

Antifungal activity of carbendazim, cyproconazole + carbendazim, flusilazole + carbendazim and thiophanate-methyl

All *Cladobotryum* spp. isolates demonstrated an ability to tolerate carbendazim at low concentrations. They were able to grow well at 1.50 mg l^{-1}, but were severely inhibited at 2.00 mg l^{-1} or above. The EC_{50} values were between 0.24 and 2.92 mg l^{-1}. The highest resistance factor was found for the isolate $Beč_1C_1$ (RF=12.17). All isolates were sensitive to carbendazim. Only isolate SP_1C_4 had different EC_{50} range (Table 2).

The tested *Cladobotryum* spp. isolates were capable of good growth at 1.50 mg l^{-1} cyproconazole+carbendazim concentration, but were severely inhibited at 2.00 mg l^{-1} and above. The EC_{50} values were between 0.33 and 1.82 mg l^{-1}. The isolate OB_1C_2 had the highest values of resistance factor (RF=5.52). All investigated isolates were found to be sensitive to cyproconazole+carbendazim. Only isolate SP_1C_4 had different EC_{50} range (Table 3).

The investigated *Cladobotryum* spp. isolates were strongly susceptible to flusilazole+carbendazim. They were able to grow well at 1.00 mg l^{-1} concentration, but were severely inhibited at 2.00 mg l^{-1} and above (Table 3). The EC_{50} values were between 0.02 and 0.11 mg l^{-1}. The isolate $Beč_1C_1$ had the highest resistance factor (RF=5.50). Statistically significant differences were found between the EC_{50} values of different isolates (Table 4).

All *Cladobotryum* spp. isolates demonstrated some ability to tolerate thiophanate-methyl. They were capable of growth at 25.00 mg l^{-1}, but were severely inhibited at 55.00 mg l^{-1} or above. The EC_{50} values were between 6.53 and 12.09 mg l^{-1}. The highest resistance factor was found for the isolate OB_1C_2 (RF=1.91). All of these isolates were considered to be weakly resistant to thiophanate-methyl. Statistically significant differences were very slight between the EC_{50} values of different isolates (Table 5).

DISCUSSION

Cladobotryum spp. were isolated from mushroom farms in Serbia over the period 2003-2006. Of the 13 Serbian *Cladobotryum* spp. isolates collected, all we-

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

Code of isolate Naziv izolata	Carbendazim – Karbendazim					
	EC_{50} (mg l^{-1}))		b		H	RF
	Value Vrednost	Range Opseg	Value Vrednost	Range Opseg		
SP_1C_4	0.24	0.16-0.31	1.52	1.30-1.74	0.59	1.00
P_3C_1	1.38	0.93-3.09	0.89	0.70-1.08	0.65	5.75
Ba_1C_1	0.93	0.59-2.35	0.66	0.47-0.85	0.30	3.88
B_1C_1	0.92	0.68-1.46	1.02	0.82-1.22	2.21	3.83
Ku_1C_1	0.82	0.53-1.88	0.66	0.47-0.85	0.12	3.42
NSl_1C_1	0.47	0.35-0.61	1.13	0.96-1.32	0.53	1.96
OB_1C_2	1.27	0.87-2.71	0.89	0.70-1.08	0.45	5.29
P_7C_1	1.14	0.85-1.84	1.14	0.96-1.34	2.55	4.75
Res_2C_1	0.71	0.42-1.78	0.57	0.38-0.76	0.16	2.96
VG_3C_1	1.89	1.33-3.53	1.29	1.05-1.53	2.47	7.88
$Beč_1C_1$	2.92	1.47-25.19	0.69	0.40-0.88	0.18	12.17
Jak_1C_1	1.37	0.87-4.03	0.75	0.56-0.94	0.60	5.71
Kal_1C_1	0.55	0.43-0.71	1.23	1.03-1.43	0.70	2.29

EC_{50} – Fungicide concentration which inhibits mycelial growth by 50%; Koncentracija fungicida koja inhibira rast micelije 50%
RF –The resistance factor was expressed as the ratio of the EC_{50} and the lowest EC_{50} for the isolates tested; Faktor rezistentnosti predstavlja odnos EC_{50} i najmanje EC_{50} vrednosti za testirane izolate
b – Regression coefficient at 95% confidence level; Koeficijent regresije na nivou poverenja 95%
H – Heterogenity; Heterogenost

Tabela 3. *In vitro* sensitivity of *Cladobotryum* spp. isolates to cyproconazole+carbendazim
Table 3. *In vitro* osetljivost izolata *Cladobotryum* spp. na ciprokonazol+karbendazim

Code of isolate Naziv izolata	Cyproconazole+carbendazim – Ciprokonazol+karbendazim					
	EC_{50} (mg l^{-1})		b		H	RF
	Value Vrednost	Range Opseg	Value Vrednost	Range Opseg		
SP_1C_4	0.33	0.28-0.39	2.20	1.97-2.43	1.78	1.00
P_3C_1	0.67	0.54-0.84	1.43	1.23-1.63	0.67	2.03
Ba_1C_1	1.27	0.84-3.11	0.80	0.61-0.99	0.23	3.85
B_1C_1	0.46	0.40-0.53	2.79	2.56-3.02	2.91	1.39
Ku_1C_1	1.42	0.81-13.40	1.00	0.68-1.32	0.28	4.30
NSl_1C_1	1.01	0.73-1.74	0.96	0.77-1.15	1.52	3.06
OB_1C_2	1.82	1.21-4.07	1.04	0.83-1.25	0.24	5.52
P_7C_1	0.95	0.75-1.33	1.33	1.13-1.53	0.93	2.88
Res_2C_1	1.13	0.76-2.99	1.38	1.06-1.71	0.04	3.42
VG_3C_1	1.03	0.78-1.55	1.19	0.99-1.39	0.38	3.12
$Beč_1C_1$	0.56	0.47-0.68	1.65	1.44-1.86	0.33	1.70
Jak_1C_1	0.78	0.62-1.04	1.33	1.13-1.53	0.05	2.36
Kal_1C_1	1.45	0.98-3.19	0.93	0.73-1.13	0.04	4.39

EC_{50} – Fungicide concentration which inhibits mycelial growth by 50%; Koncentracija fungicida koja inhibira rast micelije 50%
RF –The resistance factor was expressed as the ratio of the EC_{50} and the lowest EC_{50} for the isolates tested; Faktor rezistentnosti predstavlja odnos EC_{50} i najmanje EC_{50} vrednosti za testirane izolate
b – Regression coefficient at 95% confidence level; Koeficijent regresije na nivou poverenja 95%
H – Heterogenity; Heterogenost

Tabela 4. *In vitro* sensitivity of *Cladobotryum* spp. isolates to flusilaozole+carbendazim
Table 4. *In vitro* osetljivost izolata *Cladobotryum* spp. na flusilazol+karbendazim

Code of isolate Naziv izolata	Flusilazole+carbendazim – Flusilazol+karbendazim					
	EC_{50} (mg l^{-1})		b		H	RF
	Value Vrednost	Range Opseg	Value Vrednost	Range Opseg		
SP_1C_4	0.02	0.01-0.03	1.48	1.27-1.69	2.24	1.00
P_3C_1	0.08	0.06-0.12	1.35	1.14-1.56	0.48	4.00
Ba_1C_1	0.10	0.07-0.18	1.08	0.87-1.29	1.30	5.00
B_1C_1	0.03	0.02-0.04	1.08	0.88-1.28	0.28	1.50
Ku_1C_1	0.06	0.05-0.09	1.41	1.19-1.63	2.05	3.00
NSl_1C_1	0.07	0.05-0.10	1.30	1.08-1.52	2.20	3.50
OB_1C_2	0.04	0.03-0.06	0.96	0.77-1.15	1.06	2.00
P_7C_1	0.05	0.04-0.06	1.54	1.33-1.75	0.75	2.50
Res_2C_1	0.06	0.05-0.08	1.39	1.08-1.60	1.05	3.00
VG_3C_1	0.05	0.04-0.07	1.39	1.17-0.22	1.98	2.50
$Beč_1C_1$	0.11	0.07-0.32	0.82	0.62-1.02	0.12	5.50
Jak_1C_1	0.03	0.02-0.04	1.60	1.30-1.90	1.04	1.50
Kal_1C_1	0.07	0.06-0.09	1.75	1.55-1.95	1.38	3.50

EC_{50} – Fungicide concentration which inhibits mycelial growth by 50%; Koncentracija fungicida koja inhibira rast micelije 50%
RF –The resistance factor was expressed as the ratio of the EC_{50} and the lowest EC_{50} for the isolates tested; Faktor rezistentnosti predstavlja odnos EC_{50} i najmanje EC_{50} vrednosti za testirane izolate
b – Regression coefficient at 95% confidence level; Koeficijent regresije na nivou poverenja 95%
H – Heterogenity; Heterogenost

Tabela 5. *In vitro* sensitivity of *Cladobotryum* spp. isolates to thiophanate-methyl
Table 5. *In vitro* osetljivost izolata *Cladobotryum* spp. na na tiofanat-metil

Code of isolate Naziv izolata	Thiophanate-methyl – Tiofanat-metil					
	EC_{50} (mg l^{-1})		b		H	RF
	Value Vrednost	Range Opseg	Value Vrednost	Range Opseg		
SP_1C_4	6.87	5.18-9.07	1.52	1.38-1.66	1.03	1.05
P_3C_1	9.43	5.82-13.07	1.05	0.85-1.25	1.38	1.44
Ba_1C_1	6.53	3.92-11.50	0.77	0.70-0.84	0.25	1.00
B_1C_1	7.27	5.58-9.47	1.69	1.53-1.85	0.06	1.11
Ku_1C_1	8.87	6.16-12.96	1.12	0.99-1.25	1.56	1.36
NSl_1C_1	10.42	7.85-13.84	1.49	1.09-1.89	0.10	1.60
OB_1C_2	7.78	6.38-9.01	2.74	2.37-3.11	0.09	1.91
P_7C_1	6.76	4.31-10.99	0.97	0.89-1.05	0.17	1.04
Res_2C_1	12.09	10.28-14.00	2.92	2.45-3.19	0.62	1.85
VG_3C_1	7.22	4.80-10.91	0.99	0.75-1.11	2.39	1.06
$Beč_1C_1$	7.96	5.82-9.91	1.68	1.46-1.90	2.68	1.22
Jak_1C_1	10.26	6.17-18.15	0.81	0.74-0.88	0.78	1.57
Kal_1C_1	7.90	5.75-10.79	1.27	1.15-1.39	1.82	1.21

EC_{50} – Fungicide concentration which inhibits mycelial growth by 50%; Koncentracija fungicida koja inhibira rast micelije 50%
RF –The resistance factor was expressed as the ratio of the EC_{50} and the lowest EC_{50} for the isolates tested; Faktor rezistentnosti predstavlja odnos EC_{50} i najmanje EC_{50} vrednosti za testirane izolate
b – Regression coefficient at 95% confidence level; Koeficijent regresije na nivou poverenja 95%
H – Heterogenity; Heterogenost

re weakly resistant to thiophanate-methyl (EC_{50} 6.53 - 12.09 mg l^{-1}) and sensitive to carbendazim (EC_{50} 0.24 - 1.89 mg l^{-1}), cyproconazole+carbendazim (EC_{50} 0.33 - 1.82 mg l^{-1}) and flusilazole+carbendazim (EC_{50} 0.02 - 0.11 mg l^{-1}). The highest resistance factors to carbendazim, cyproconazole+carbendazim, as well as flusilazole+carbendazim exceeded 3 mg l^{-1} 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 EC_{50} values obtained were very low. The wide range of EC_{50} values and low mean EC_{50} for carbendazim formulations showed a wider range in sensitivity among the studied isolates (Tables 2, 3, 4 and 5).

Grogan and Gaze (2000) recorded that during the height of the cobweb epidemic in 1995, 25% of the British *Cladobotryum* spp. isolates were weakly resistant to thiabendazole (EC_{50} 1 – 10 mg l^{-1}) and sensistive to carbendazim (EC_{50} < 1 mg l^{-1}). The remaining 75% of British isolates were all strongly resistant to thiabendazole (EC_{50} > 200 mg l^{-1}) and weakly resistant to carbendazim (EC_{50} 2.00 - 10.00 mg l^{-1}). These isolates were inhibited at 10 mg l^{-1} concentration of carbendazim (Grogan and Gaze, 2000). Resistance of *Cladobotryum* spp. to the benzimidazole fungicides benomyl and carbendazim was also reported in Ireland in 1992. Further investigation confirmed that the isolates were tolerant of these fungicides at up to 10 mg l^{-1} (McKay et al., 1998). Studies in India (Bhatt and Singh, 1992) showed that carbendazim inhibited the growth of *Cladobotryum* spp. isolates at lower concentrations. Our findings indicate that isolates obtained from mushroom crops in Serbia are still sensitive to carbendazim as EC_{50} values were below 3 mg l^{-1}. Serbian *Cladobotryum* spp. isolates were severely inhibited by 2 mg l^{-1} or higher rates of carbendazim, cyproconazole+carbendazim and flusilazole+carbendazim. The obtained values were much lower than those recorded for carbendazim by McKay et al. (1998) in Ireland and Grogan and Gaze (2000) in Great Britain. It has been reported that Serbian isolates are still sensitive to benomyl, another benzimidazole fungicide, as most of them were inhibited at 2 mg l^{-1}, and EC_{50} values were below 1.00 mg l^{-1} (Potočnik et al. 2007). Serbian *Cladobotryum* spp. isolates were only weakly resistant to thiophanate-methyl as they were severely inhibited at 55.00 mg l^{-1}. All *Cladobotryum* spp. isolates have EC_{50} > 6.00 mg l^{-1} for thiophanate-methyl, so it is unlikely that this fungicide would control outbreaks of cobweb disease.

The molecular structure of thiabendazole is, however, sufficiently different from that of benomyl and car-

bendazim to imply that their abilities to bind to tubulin may differ (Hassall, 1990). Differences in benzimidazole sensitivity have been shown to reflect differences in the beta-tubulin gene mutations, which confer resistance (Davidse, 1986), so that a mutation which leads to poor binding of, and therefore resistance to, thiabendazole may not necessarily affect the binding of carbendazim to the same extent. Bonnen and Hopkins (1997) also reported that 12% of *V. fungicola* isolates tested were not cross resistant to either thiabendazole or benomyl, indicating that at least two different mutation sites may have been involved. Grogan and Gaze (2000) supposed that perhaps the structure of tubulin of thiabendazole-resistant *Cladobotryum* spp. (Type II) is different from that of thiabendazole-sensitive *Cladobotryum* spp. (Type I). This recently developed benzimidazole resistance may have occurred as a result of regular use of benzimidazole fungicides to control other mushroom pathogens and this phenomenon is widely recognized following bezimidazole use (Hassall, 1990; Bonnen and Hopkins, 1997).

Cyproconazole and flusilazole are components of two tested mixtures with carbendazim. Cyproconazole and flusilazole are DMI fungicides along with prochloraz-Mn. Applying the criteria established by Gea et al. (1996; 2003), resistance to cyproconazole+carbendazim, as well as flusilazole+carbendazim was not detected in any of the studied isolates of *Cladobotryum* spp. in Serbia. Their respective EC_{50} values were lower than 2.00 and 0.20 mg l^{-1}. The flusilazole+carbendazim mixture showed the highest toxicity of all fungicides tested in this study. Its toxicity (EC_{50} = 0.02 - 0.11 mg l^{-1}) corresponded to prochloraz-Mn in a previous study (EC_{50} = 0.02 – 0.09 mg l^{-1}) reported by Potočnik et al. (2007). It is clear therefore that DMI supplements amended the effect of carbendazim, especially flusilazole. Grogan et al. (2000) reported that 25% British *V. fungicola* isolates were weakly resistant to prochloraz-Mn. The development of strains resistant to prochloraz-Mn may additionally reduce the number of available effective fungicides against bubble disease of *A. bisporus* caused by *V. fungicola*. It has been suggested that growers can no longer rely on prochloraz-Mn, as a primary management tool (Gea et al., 2003). Carbendazim and its mixture with cyproconazole and especially flusilazole might be expected to be very effective against *Cladopotryum* spp. after *in vivo* biological efficacy testing. Chalaux et al. (1993) confirmed that flusilazole did not significantly limit the radial growth of *A. bisporus* mycelium.

The fungicide resistance profile of individual mushroom pathogens gives valuable information on whether or not a given fungicide will be effective in controlling a disease outbreak. With the evolution of strongly, or even weakly, resistant isolates, the efficacy of fungicides may be significantly compromised (Grogan and Gaze, 2000). The emergance of benzimidazole resistance reduces the value of benzimidazoles in the control of mushroom pathogens. However, a lack of effective alternatives, makes fungicides useful in cases where pathogens are still sensitive but this requires regular resistance monitoring (Grogan, 2006).

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In vitro osetljivost patogena šampinjona *Cladobotryum* spp. na tiofanat-metil i različite formulacije karbendazima

REZIME

Iz obolelih plodonosnih tela *Agaricus bisporus* prikupljenih u gajilištima šampinjona u Srbiji tokom 2003-2006. dobijeno je trinaest izolata *Cladobotryum* spp. Ispitana je njihova osetljivost na fungicide tiofanat-metil, karbendazim, ciprokonazol+karbendazim i flusilazol+karbendazim *in vitro*. Ispitivanje osetljivosti izolata na fungicide je pokazalo da su svi izolati visoko osetljivi na karbendazim (EC_{50}=0,24-2,92 mg l^{-1}), ciprokonazol+karbendazim (EC_{50}=0,33-1,82 mg l^{-1}) i naročito na flusilazol+karbendazim (EC_{50}=0,02-0,11 mg l^{-1}). Svi testirani *Cladobotryum* spp. izolati su bili umereno rezistentni na tiofanat-metil sa EC_{50} vrednostima u rasponu od 6,53 do 12,09 mg l^{-1}.

Ključne reči: *Cladobotryum* spp.; antifungalna aktivnost; tiofanat-metil; karbendazim; ciprokonazol+karbendazim; flusilazol+karbendazim