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In vitro Toxicity of Fungicides of Different Mode of Action to *Agaricus bisporus* (Lange) Imbach

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SUMMARY

Isolates of *Agaricus bisporus* strains F56 and U3 were tested for sensitivity to several selected fungicides *in vitro*. The analysis showed that flusilasole + carbendazim and cyproconazole + carbendazim were the most toxic fungicides to *A. bisporus* strain F56 with respective EC_{50} values of 0.04 and 0.23 mg/l. The least toxic fungicides were carbendazim (EC_{50} = 16.58 mg/l) and trifloxystrobin (EC_{50} = 20.69 mg/l) to *A. bisporus* F56 and benomyl (EC_{50} = 14.99 mg/l) to *A. bisporus* U3.

Keywords: White button mushroom; Fungicides; Toxicity

INTRODUCTION

Production of white button mushroom (*Agaricus bisporus* (Lange) Imbach) in Serbia is severely afflicted by fungal pathogens. Chemical control of fungal diseases requires highly selective fungicides in order to prevent infection without affecting the growth of *A. bisporus*. Diseases of cultivated mushrooms have been controlled so far by dithiocarbamate (Yoder et al., 1950) and methylbenzimidazole carbamate (MBC) fungicides (Fletcher and Yarham, 1976; Gea et al., 1995), except in the case of British and Irish isolates of *Verticillium fungicola* and *Cladobotryum* spp. that have been found resistant to benomyl, carbendazim and thiabendazole (Fletcher and Yarham, 1976; Gaze, 1995; McKay et al., 1998; Grogan and Gaze, 2000). The dicarboximide fungicide iprodione has been used instead, but *V. fungicola* var. *fungicola* isolates resistant to that fungicide have been found in Spain (Gea et al., 1996). Prochloraz, from the group of sterol biosynthesis inhibitors (DMI fungicides), was introduced in the early 1980s owing to its ability to prevent the appearance of mycopathogenic fungi in mushroom units. It is the most commonly used fungicide in mushroom industry in EU countries and Serbia (Gea et al., 1996; Potočnik et al., 2007). However, satisfactory results in control of dry bubble and cobweb diseases were no longer observed after its widespread and continuous usage in the UK and Spain (Grogan et al., 2000; Gea et al., 2005; Grogan, 2006).

The fungicides officially recommended for mushroom cultivation in EU countries are formulations of carbendazim, prochloraz and chlorothalonil (Anonymous, 2005). Fungicide efficacy trials on cultivated mushroooms are very rarely conducted by agrochemical companies because specially designed experimental facilities are required for appropriate evaluation. Such limited trials combined with high registration requirements have led to low availability of commercial fungicides approved in mushroom cultivation (Whitehead, 2002; Stoddart et al., 2004; Anonymous, 2005).

The benzimidazole fungicides benomyl, carbendazim and thiophanate-methyl, dithiocarbamate mancozeb and imidazole prochloraz are widely used in the Serbian mushroom industry (Milenković, 1997; Potočnik et al., 2007, 2008). The aim of this study was to investigate *in vitro* toxicity of the fungicides commonly used in mushroom production to *A. bisporus*. Several fungicides that have never been used for disease control of mushrooms in Serbia were also included in sensitivity tests in order to determine their potential toxicity to the isolates investigated.

MATERIAL AND METHODS

The commercial strains U3 (Sylvan) and F56 (Italspawn) of *A. bisporus* were used in the study. Eleven commercial fungicides: mancozeb, benomyl, carbendazim, thiophanate-methyl, iprodione, prochloraz manganese, carbendazim + cyproconazole, carbendazim + flusilazole, captan, chlorothalonil, and trifloxystrobin were tested against *A. bisporus* (Table 1). The strains were grown on potato dextrose agar (PDA) amended with the tested fungicides.

The selected volumes of fungicide stock solutions were added to molten sterile medium (50°C) in order to make concentration series of active ingredients ranging from 0.01 to 1000.00 mg/l. Preliminary concentrations of all selected fungicides were: 0.01 0.10, 1.00, 10.00, 100.00 and 1000.00 mg/l. Based on previous results, the concentrations selected for further study were: benomyl, carbendazim, thiophanate-methyl and trifloxystrobin 3.17, 6.25, 12.50, 25.00, 50.00 mg/l; cyproconazole + carbendazim 0.19, 0.37, 0.75, 1.50 mg/l; flusilazole + carbendazim 0.01, 0.03, 0.05, 0.10 mg/l; prochloraz manganese 0.019, 0.037, 0.075, 0.150 mg/l; mancozeb and iprodione 1.56, 3.12, 6.25, 12.50, 25.00 mg/l; chlorothalonil and captan 1.00, 5.00, 10.00, 50.00 mg/l. The plates with fungicide-amended and fungicide-free PDA were inoculated with inverted mycelium agar discs (10 mm), taken from the edge of twenty day-old cultures of *A. bisporus*, and incubated at 20°C. Colony diameter was measured after twenty days of cultivation. Mycelial growth on the fungicide-amended PDA was presented as a percentage of growth in the control. Fungicide concentrations inhibiting mycelial growth by 50% (EC_{50}) were determined for each isolate. The data on fungicide concentrations and relative inhibition were analysed using probit analysis, according to Finney (1971). Three replicates per treatment were used.

RESULTS AND DISCUSSION

Toxicity of the selected fungicides to strains U3 and F56 of *A. bisporus* is shown in Tables 2 and 3. The iso-

Table 1. Fungicides data

Chemical class	Fungicide active ingredient	Trade name	Formulation	Supplier
Dithiocarbamates	Mancozeb	Mankogal 80 WP	800 g/kg	Galenika Fitofarmacija
Benzimidazoles	Benomyl	Benfungin WP	500 g/kg	Galenika Fitofarmacija
	Carbendazim	Galofungin WP	500 g/kg	Galenika Fitofarmacija
	Thiophanate-methyl	Tested formulation WP	700 g/kg	Agromarket
Sterol Demethylation inhibitors	Prochloraz managanese	Octave WP	500 g/kg	Bayer Crop Science
Benzimidazoles + Sterol Demethylation inhibitors	Carbendazim + Cyprokonazole	Alto Combi 420 SC	120 + 300 g/l	Syngenta
	Carbendazim + Flusilasole	Alert-S SC	250 + 120 g/l	Syngenta
Dicarboximides	Iprodione	Kidan EC	260 g/l	Bayer Crop Science
Phthalimides	Captan	Captan 50 WP	500 g/kg	Arvesta
Chloronitrils	Chlorothalonil	Bravo 750 SC	720 g/l	Syngenta
Strobilurins	Trifloxystrobin	Zato 50 WP	500 g/kg	Bayer Crop Science

lates of *A. bisporus* were able to grow at mancozeb, iprodione, benomyl, and thiophanate methyl concentrations of 12.50 mg/l, while growth was severely inhibited at concentrations of 25.00 mg/l and higher. The respective EC_{50} values of mancozeb, thiophanate methyl, benomyl, and carbendazim for strain F56 were 6.97, 10.04, and 16.58 mg/l. The EC_{50} value of benomyl for strain U3 was 14.99 mg/l. The values of iprodione EC_{50} for strains U3 and F56 were 1.73 and 13.63, respectively. Growth of the edible mushroom mycelia of F56 was good at trifloxystrobin concentration of 25.00 mg/l and severely inhibited at 50 mg/l. The EC_{50} value of trifloxystrobin for F56 was 20.69 mg/l. Trifloxystrobin was more toxic to strain U3, as its EC_{50} value was 5.20 mg/l. Strain U3 grew well at 12.50 mg/l of trlifloxystrobin, while this fungicide severely inhibited its growth at 25.00 mg/l. Captan, chlorothalonil, and prochloraz manganese applied at the concentration of 1.00 mg/l enabled mycelial growth of *A. bisporus*, inhibiting it severely at 10.00 mg/l. The respective EC_{50} values of captan and chlorothalonil for strain F56 were 2.03 and 2.39 mg/l. The EC_{50} value of prochloraz manganese for strain U3 was 2.97 mg/l. Cyproconazole + carbendazim concentration of 0.19 mg/l failed to affect *A. bisporus* growth, while concentrations of 0.37 mg/l and higher severely inhibited isolate growth. The EC_{50} value of cyproconazole + carbendazim for strain F56 was 0.23 mg/l. *A. bisporus* F56 was capable to grow at flusilazole + carbendazim concentration of 0.05 mg/l, but growth was inhibited at 0.10 mg/l and higher concentrations. The flusilazole + carbendazim EC_{50} for strain F56 was 0.04 mg/l.

It has been reported that treatments with mancozeb, a fungicide from the group of dithiocarbamates, have not produced any evidence of damage to mushroom at any stage of its cultivation (Yoder et al., 1950; Newman and Savidge, 1969). This is consistent with our observations of low growth inhibiting effects of mancozeb. Strain F56 of *A. bisporus* also had low sensitivity to carbendazim. However, in the past, Chalaux et al. (1993) found that carbendazim had toxic effect on *A. bisporus*. Chrysayi-Tokousbalides et al. (2007) reported that strain X22 of *A. bisporus* also had a low sensitivity to carbendazim (EC_{50}=23.20 mg/l). Flusilazole and to a lesser extent cyproconazole were the only fungicides demonstrating toxic affects on *A. bisporus* strain F56. In previous studies, flusilazole had not been reported to limit the growth of *A. bisporus* mycelium significantly (Chalaux et al., 1993). Those previous results also indicated that chlorothalonil was able to induce toxicity problems in mushroom mycelial growth *in vitro* at concentrations between 0.50 and 2.00 mg/l (Challen and Elliott, 1985). However, Chalaux et al. (1993) did not observe any toxicity of that fungicide to *A. bisporus* strains B62, B98, and U3 at concentrations below 2.00 mg/l. It is consistent with our results showing that the tested strain F56 was less sensitive to that fungicide (EC_{50} value exceeded 2 mg/l). Bhatt and Singh (1992) found that captan had a slightly inhibitory effect on the growth of *A. bisporus*. Chalaux et al. (1993) reported that strains B62, B98 and U3 of *A. bisporus* were more sensitive to captan and mancozeb than strain F56 in our study. They assumed that the strains, which were widely cultivated in Europe in the 1990s and later, were apparently more tolerant to fungicides *in vitro* than the older commercial strains used in previous studies. A strain-dependent sensitivity of *A. bisporus* to fungicides has already been reported (Challen and Elliot, 1985). Strain F56 of *A. bisporus* was found to have moderate susceptibility to trifloxystrobin as its EC_{50} exceeded 20.00 mg/l, while strain U3 was more sensitive to this fungicide (EC_{50} = 5.20 mg/l). Diamantopoulou et al. (2006) reported that mycelial growth of an *A. bisporus* strain 2810 (Le Lion) on casing medium in tubes was not affected by trifloxystrobin at 1.00 mg/l. Chrysayi-Tokousbalides et al. (2007) found that strain X22 of

Table 2. *In vitro* toxicity of fungicides to strain U3 of *Agaricus bisporus*

Fungicide active ingredient	EC_{50} (mg/l) CI 95%	EC_{90} (mg/l) CI 95%	b CI 95%	H
Benomyl	14.99 (12.78-17.40)	62.48 (48.09-91.22)	2.07±0.22	1.39
Iprodione	1.73 (0.55-13.09)	4332.18 (231.63-14076.00)	0.38±0.06	0.23
Prohloraz manganese	2.97 (1.01-26.95)	184.45 (93.28-24416.53)	0.72±0.07	2.99
Trifloksystrobin	5.20 (2.91-40.66)	825.31 (73.07-51488000)	0.58±0.19	0.23

A. bisporus was more sensitive to that fungicide than the strains tested in our study, as the EC_{50} of this strain was 1.10 mg/l.

Even if sensitivity is generally higher *in vitro* than *in vivo*, problems with mushroom mycelia growth caused by fungicide residues in casing layer have to be taken seriously. On the other hand, regarding resistance development, damage to the environment and human health risks, as well as increasing production costs, special attention should be focused on developing alternative biological methods for control of mushroom disease.

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Table 3. *In vitro* toxicity of fungicides to strain F56 of *Agaricus bisporus*

Fungicide active ingredient	EC_{50} (mg/l) CI 95%	EC_{90} (mg/l) CI 95%	b CI 95%	H
Mancozeb	6.97 (5.25-8.62)	26.73 (21.36-36.35)	2.19±0.26	0.10
Carbendazim	16.58 (12.34-22.23)	146.68 (92.94-273.06)	1.35±0.11	0.27
Thiophanate-methyl	10.04 (7.66-13.09)	28.60 (21.55-115.47)	1.48±0.12	1.84
Iprodione	13.63 (10.21-20.44)	158.33 (92.99-336.42)	1.34 ± 0.85	0.81
Carbendazim + Cyprokonazole	0.23 (0.18-0.26)	0.69 (0.57-0.96)	2.64±0.36	1.12
Carbendazim + Flusilasole	0.04 (0.03-0.05)	0.34 (0.14-3.49)	1.30±0.31	0.04
Captan	2.03 (0.99-3.83)	361.17 (132.31-1595.49)	0.57±0.07	3.73
Chlorothalonil	2.39 (1.12-4.14)	187.73 (79.05-808.18)	0.68±0.10	1.64
Trifloksystrobin	20.69 (16.11-29.32)	227.88 (120.02-635.86)	0.95±0.12	1.33

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In vitro toksičnost fungicida različitih mehanizama delovanja za *Agaricus bisporus* (Lange) Imbach

REZIME

Ispitana je *in vitro* toksičnost odabranih fungicida za sojeve F56 i U3 *Agaricus bisporus* (Lange) Imbach. Analiza je pokazala da su fungicidi fluzilazol + karbendazim i ciprokonazol + karbendazim pokazali najveću toksičnost za soj F56 *A. bisporus* sa EC_{50} vrednostima 0.04 i 0.23 mg/l. Karbendazim (EC_{50} = 16.58 mg/l) i trifloksistrobin (EC_{50} = 20.69 mg/l) su bili najmanje toksični fungicidi za *A. bisporus* F56 i benomil (EC_{50} = 14.99 mg/l) za *A. bisporus* U3.

Ključne reči: Šampinjon; fungicidi, toksičnost