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Review paper

Impact of Fungicides Used for Wheat Treatment on Button Mushroom Cultivation

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SUMMARY

Little information is currently available on the potential environmental risks that fungicides applied during wheat cultivation and remaining in straw may have for mushroom production. The substrate for many cultivated mushrooms is mostly based on cereal straw. This review aimed to answer the question whether residues of the fungicides commonly used in wheat production and remaining in straw could be directly or indirectly responsible for changes in yields of *Agaricus bisporus*. Potential chemical risks of eight fungicides (for wheat treatments) for *A. bisporus*: mancozeb, carbendazim, thiophanate-methyl, carbendazim+cyproconazole, carbedazim+flusilasole, captan, chlorothalonil and trifloxystrobin are disscused. Only the value of maximum residue level of flusilasole and its formulation was evaluated as higher than medium effective concentration of the fungicide for *A. bisporus*. As a conclusion, flusilazole treatment could be a limiting factor for using straw for composting and mushroom cultivation.

Keywords: Mushroom; *Agaricus bisporus*; Cultivation; Fungicides; Resiclues; Straw; Wheats

INTRODUCTION

White button mushroom, *Agaricus bisporus* (Lange) Imbach, is the most commonly cultivated mushroom species in Serbia. Most of the substrates used for mushroom production are organic wastes or by-products, involving agricultural, municipal or industrial wastes. The substrate for its cultivation is normally fermented compost, mostly based on animal manure and cereal straw. The process of composting has two distinct phases. During phase I, various microorganisms break down the straw. In phase II, pests are eliminated and the substrate decomposes under the activity of thermophilic microorganisms (Chang and Miles, 2004). Mushroom industry is part of complex agricultural system: animal husbandry – wheat – edible mushroom – spent mushroom substrate (Manjunath and Korikanthimath, 2009). New EU regulations considered that organic mushrooms must be grown on organic straw and manure. At a present, transition period is being introduced to allow producers to adopt the new requirements as straw and manure cannot currently be obtained in sufficient quantities for organic production (Anonymous, 1998, 2010).

LITERATURE REVIEW

Fungicides are normally applied during intensive wheat production. Wheat diseases can be drastically reduced by using fungicides from the group of ergosterol biosynthesis inhibitors and strobilurins (Savčić-Petrić and Sekulić, 2007; Balaž et al., 2008). In Serbia, fusarium head blight has been a growing problem over the past three decades. The highest frequency and intensity of up to 67.2% was detected in 2005 for *Fusarium graminearum*, followed by *F. poae* (up to 20.4%) (Lević et al., 2008a). *F. langsethiae* was isolated for the first time in Serbia in 2005 (Lević et al., 2008b). The fungicides widely used in Serbia for control of fusarium head blight, leaf rust, septoria leaf blotch, and powdery mildew are: mancozeb, carbendazim, carbendazim + cyproconazole, captan, chlorothalonil, epoxyconazole + thiophanate-methyl, carbendazim + flusilazole, trifloxystrobin + cyproconazole, tebuconazole + triadimenol + spiroxamine, prochloraz + tebuconazole, prochloraz + propiconazole (Savčić-Petrić and Sekulić, 2007; Balaž et al., 2008). However, there is little information on the potential effects of these fungicides on mushroom production (Chalaux et al., 1993). In France, Savoie et al. (1992) detected prochloraz and carbendazim residues in wheat straw at concentrations below 1.00 mg kg^{-1}, while the amount of flusilazole residues ranged from 0.2 to 0.3 mg kg^{-1}.

Potential chemical risks of several fungicides commonly used in wheat production in Serbia were evaluated for *A. bisporus*, regarding fungicide medium effective concentration (EC_{50}) (Potočnik 2006, 2009; Potočnik et al., 2009a, 2009b), in order to determine whether fungicide residues in straw could be directly or indirectly responsible for changes in mushroom yields. In addition, fungicides with different mode of action are currently used commercially in mushroom industry to provide control of many serious fungal diseases (Potočnik, 2006, 2009; Potočnik et al., 2005, 2008, 2010a, 2010b; Tanović et al., 2009). The maximum tolerated levels of fungicide residues in grains, wheat straw and cultivated mushrooms are shown in table 1. The EC_{50} values of eight fungicides: mancozeb, carbendazim, thiophanate-methyl, carbendazim + cyproconazole, carbendazim + flusilazole, captan, chlorothalonil, and trifloxystrobin, for *A. bisporus* are presented in table 2.

Table 1. Maximum residue levels (MRL) of fungicides in cereal grain, straw, and cultivated mushrooms.

Fungicide class	Fungicide active ingredient (a.i.)	MRL (mg kg^{-1})		
		Cereal grain	Straw	Cultivated mushrooms
Dithiocarbamates	Mancozeb	1[1]	1[1]	0.05[1]
Benzimidazoles	Carbendazim	0.1[1]	0.1[2]	0.1[2]
	Thiophanate-methyl	0.05[1]	0.05[2]	0.1[2]
Ergosterol Biosynthesis Inhibitors	Cyprokonazole	0.1[1]	0.1[1]	0.05[1]
	Flusilasole	0.1[1]	0.1[1]	0.02[1]
Phthalimides	Captan	0.02[1]	0.02[3]	0.02[1]
Chloronitrils	Chlorothalonil	0.1[1]	0.1[1]	2[1]
Strobilurins	Trifloxystrobin	0.05[1]	0.05[2]	0.02[2]

1 Anonymous (2005)
2 Anonymous (2006)
3 Anonymous (2007)

Table 2. Selected fungicides and their EC_{50} values for Agaricus bisporus F56.

Fungicide active ingredient (a.i.)	Trade name	Formulation	Supplier	EC_{50} (mg l^{-1})
Mancozeb	Mankogal 80 WP	800 g kg^{-1}	Galenika Fitofarmacija, Serbia	6.97[2]
Carbendazim	Galofungin WP	500 g kg^{-1}	Galenika Fitofarmacija, Serbia	16.58[1]
Thiophanate-methyl	Tested formulation WP	700 g kg^{-1}	Agromarket, Serbia	10.04[1]
Carbendazim + Cyprokonazole	Alto Combi 420 SC	120 + 300 g l^{-1}	Syngenta, Serbia	0.23[1]
Carbendazim + Flusilasole	Alert-S SC	250 + 120 g l^{-1}	Syngenta, Serbia	0.04[1]
Captan	Captan 50 WP	500 g kg^{-1}	Vins 2000, Serbia	2.03[2]
Chlorothalonil	Bravo 750 SC	720 g l^{-1}	Syngenta, Serbia	2.39[2]
Trifloxystrobin	Zato 50 WP	500 g kg^{-1}	Bayer Crop Science, Serbia	20.69[2]

1 Potočnik et al. (2009a)
2 Potočnik et al. (2009b)

Potočnik et al. (2009a) reported that strain F56 of *A. bisporus* was able to grow at mancozeb, thiophanate methyl, and carbendazim concentrations of 12.50 mg l^{-1}, while growth was severely inhibited at concentrations of 25.00 mg l^{-1} and higher. The respective EC_{50} values of mancozeb, thiophanate methyl, and carbendazim were 6.97, 10.04, and 16.58 mg l^{-1}. Growth of the edible mushroom mycelia was good at trifloxystrobin concentration of 25.00 mg l^{-1} and severely inhibited at 50 mg l^{-1} of this fungicide. EC_{50} value of trifloxystrobin was 20.69 mg l^{-1}. Both captan and chlorothalonil at the concentration of 1.00 mg l^{-1} enabled mycelial growth of *A. bisporus*, inhibiting it severely at 10.00 mg l^{-1}. The respective EC_{50} values of captan and chlorothalonil were 2.03 and 2.39 mg l^{-1} (Potočnik et al., 2009b). The cyproconazole + carbendazim concentration of 0.19 mg l^{-1} failed to affect *A. bisporus* growth, while concentrations of 0.37 mg l^{-1} and higher severely inhibited isolate growth. The EC_{50} value of cyproconazole + carbendazim was 0.23 mg l^{-1}. Isolate of *A. bisporus* was capable to grow at flusilazole+carbendazim concentration of 0.05 mg l^{-1}, but growth was inhibited at 0.10 mg l^{-1} and higher concentrations. The flusilazole + carbendazim EC_{50} for *A. bisporus* was 0.04 mg l^{-1} (Potočnik et al., 2009a).

Strain F56 of *A. bisporus* demonstrated the lowest sensitivity to trifloxystrobin and slightly higher to carbendazim (EC_{50} values were 20.69 mg l^{-1} and 16.58 mg l^{-1}, respectively) (Potočnik et al., 2009a, 2009b). Diamantopoulou et al. (2006) reported that, in their study, mycelial growth of *A. bisporus* 2810 (Le Lion) was not affected by trifloxystrobin at concentration of 1.00 mg l^{-1}, while *A. bisporus* X22 was sensitive to the fungicide, having an EC_{50} value of 1.10 mg l^{-1} (Chrysayi-Tokousbalides et al., 2007). Likewise, according to Chalaux et al. (1993), carbendazim was the only fungicide with a toxic effect on *A. bisporus* (Chalaux et al., 1993), while Chrysayi-Tokousbalides et al. (2007) reported its very low toxicity to *A. bisporus* X22 (EC_{50} value of 23.20 mg l^{-1}). However, carbendazim in a mixture with flusilazole or cyproconazole showed the highest *in vitro* toxicity to *A. bisporus* F56, with EC_{50} values of 0.04 and 0.23 mg l^{-1}, respectively (Potočnik et al., 2009a). In the previous studies flusiazole was not found to significantly limit the growth of *A. bisporus* (Chalaux et al., 1993).

Fungicides chlorothalonil and captan showed higher toxicity than thiophanate-methyl, mancozeb, carbendazim and trifloxystrobin to *A. bisporus* F56, reported by Potočnik et al. (2009a, 2009b). In other studies, the use of mancozeb, a fungicide from the group of dithiocarbamates, was not found to cause any damage to *A. bisporus* at any stage of cultivation (Yoder et al., 1950; Newman and Savidge, 1969). Previous results had 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^{-1} (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^{-1}. Bhatt and Singh (1992) also noted a slight inhibitory effect of captan on growth of *A. bisporus*, while

Chalaux et al. (1993) reported that strains B62, B98 and U3 were more sensitive to captan and mancozeb than strain F56. They assumed that the strains widely cultivated in Europe in 1990s and later were apparently more tolerant to fungicides *in vitro* than the older commercial strains. Likewise, a strain-dependent sensitivity to fungicides had already been reported previously by Challen and Elliot (1985).

CONCLUSION

Since the residues of a majority of fungicides used in wheat cultivation remain in straw in low quantities, it is less likely for them to have significant effects on composting and mushroom cultivation and yields. However, flusilazole and its formulation were shown to induce some toxicity to mycelia *in vitro*. Flusilazole and, to a lower extent, cyproconazole were reported as the only fungicides demonstrating toxic effects on *A. bisporus*. *In vitro* tolerance of *A. bisporus* to flusilazole was lower, than maximum residue level in straw of 0.10 mg kg^{-1}.

Although there are no specific data on the fate of fungicides during composting, Miller (1991) reported that composting could result in decomposition of hazardous materials. Chalaux et al. (1993) also postulated that carbendazim may be partly degraded during composting. Similarly, it can be postulated that flusilazole may be partly degraded during composting, but it should be further investigated in composting experiments.

Even if the sensitivity of *A. bisporus* to fungicides is generally higher *in vitro* than *in vivo*, the problems in mushroom compost production caused by fungicide residues from wheat straw have to be taken into account. *In vitro* tolerance of flusilazole and its formulation was lower than the maximum residue level allowed in straw. Therefore, flusilazole treatment could be a limiting factor for using straw for mushroom cultivation. However, with regard to resistance development, damage to the environment and human health, as well as the increasing production costs, special attention should be focused on developing alternative biological methods to protect crops from diseases.

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Uticaj primene fungicida u zaštiti strnih žita na proizvodnju šampinjona

REZIME

U literaturi postoji veoma malo podataka o mogućim uticajima primene fungicida u zaštiti strnih žita na proizvodnju gajenih gljiva. Supstrat za rast mnogih gajenih gljiva se uglavnom sastoji od slame žitarica. Ovaj rad ima za cilj da odgovori na pitanje da li ostaci fungicida koji se uobičajeno primenjuju u zaštiti strnih žita mogu neposredno ili posredno da utiču na proizvodnju *Agaricus bisporus*. Procenjen je potencijalni rizik primene osam fungicida (za zaštitu strnih žita) za *A. bisporus*: mankozeb, karbendazim, tiofanat-metil, karbendazim+ciprokonazol, karbendazim+fluzilazol, kaptan, hlorotalonil i trifloksistrobin. Jedino je vrednost dozvoljenog maksimalnog nivoa ostataka fluzilazola u strnim žitima bila veća od dobijene srednje efektivne koncentracije ovog fungicida i njegovih formulacija za *A. bisporus*. Prema tome, tretiranje žita fluzilazolom može biti ograničavajući faktor za korišćenje slame u pripremi komposta za gajenje šampinjona.

Ključne reči: Gljiva; *Agaricus bisporus*; gajenje; fungicidi; ostaci pesticida; slama; pšenica