

# Impact of Fungicides Used for Wheat Treatment on Button Mushroom Cultivation

Ivana Potočnik<sup>1</sup>, Jelena Vukojević<sup>2</sup>, Mirjana Stajić<sup>2</sup>, Dejana Kosanović<sup>1,2</sup>,  
Emil Rekanović<sup>1</sup>, Miloš Stepanović<sup>1</sup> and Svetlana Milijašević-Marčić<sup>1</sup>

<sup>1</sup>*Institute of Pesticides and Environmental Protection, Banatska 31B, 11080 Belgrade, Serbia (ivana.potocnik@pesting.org.rs)*

<sup>2</sup>*University of Belgrade, Faculty of Biology, Institute of Botany, Takovska 43, 11000 Belgrade, Serbia*

*Received: December 12, 2011*

*Accepted: March 3, 2012*

## 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, carbendazim+flusilazole, captan, chlorothalonil and trifloxystrobin are discussed. Only the value of maximum residue level of flusilazole 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; Residues; 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 (Chaloux et al., 1993). In France, Savoie et al. (1992) detected prochloraz and carbendazim residues in wheat straw at concentrations below 1.00 mg kg<sup>-1</sup>, while the amount of flusilazole residues ranged from 0.2 to 0.3 mg kg<sup>-1</sup>.

Potential chemical risks of several fungicides commonly used in wheat production in Serbia were evaluated for *A. bisporus*, regarding fungicide medium effective concentration (EC<sub>50</sub>) (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<sub>50</sub> 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 <sup>-1</sup> )		
		Cereal grain	Straw	Cultivated mushrooms
Dithiocarbamates	Mancozeb	1 <sup>1</sup>	1 <sup>1</sup>	0.05 <sup>1</sup>
	Carbendazim	0.1 <sup>1</sup>	0.1 <sup>2</sup>	0.1 <sup>2</sup>
Benzimidazoles	Thiophanate-methyl	0.05 <sup>1</sup>	0.05 <sup>2</sup>	0.1 <sup>2</sup>
	Cyprokonazole	0.1 <sup>1</sup>	0.1 <sup>1</sup>	0.05 <sup>1</sup>
Ergosterol Biosynthesis Inhibitors	Flusilasole	0.1 <sup>1</sup>	0.1 <sup>1</sup>	0.02 <sup>1</sup>
	Captan	0.02 <sup>1</sup>	0.02 <sup>3</sup>	0.02 <sup>1</sup>
Chloronitrils	Chlorothalonil	0.1 <sup>1</sup>	0.1 <sup>1</sup>	2 <sup>1</sup>
Strobilurins	Trifloxystrobin	0.05 <sup>1</sup>	0.05 <sup>2</sup>	0.02 <sup>2</sup>

1 Anonymous (2005)

2 Anonymous (2006)

3 Anonymous (2007)

**Table 2.** Selected fungicides and their EC<sub>50</sub> values for *Agaricus bisporus* F56.

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

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

Strain F56 of *A. bisporus* demonstrated the lowest sensitivity to trifloxystrobin and slightly higher to carbendazim (EC<sub>50</sub> values were 20.69 mg l<sup>-1</sup> and 16.58 mg l<sup>-1</sup>, 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<sup>-1</sup>, while *A. bisporus* X22 was sensitive to the fungicide, having an EC<sub>50</sub> value of 1.10 mg l<sup>-1</sup> (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<sub>50</sub> value of 23.20 mg l<sup>-1</sup>). However, carbendazim in a mixture with flusilazole or cyproconazole showed the highest *in vitro* toxicity to *A. bisporus* F56, with EC<sub>50</sub> values of 0.04 and 0.23 mg l<sup>-1</sup>, respectively (Potočnik et al., 2009a). In the previous studies flusilazole 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<sup>-1</sup> (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<sup>-1</sup>. Bhatt and Singh (1992) also noted a slight inhibitory effect of captan on growth of *A. bisporus*, while

Chaloux 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<sup>-1</sup>.

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. Chaloux 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.

## ACKNOWLEDGEMENT

This study was carried out as part of the projects TR 31043 and No. 173032 financially supported by the Ministry of Education and Science of the Republic of Serbia.

## REFERENCES

- Anonymous:** UKROFS Standards for Organic Food Production. Chapter IA Principles of Organic Production at Farm Level. B Mushroom Production. Department for Environment Food and Rural Affairs. Article 2 of Commission Regulation 1900/98 of 4 September 1998 – OJ L247/6 of 5/9/98. DEFRA, London, UK. 1998. <http://adlib.everysite.co.uk/adlib/defra/content.aspx?id=000IL3890W.16NTC1KYYGY2YL> Accession date: March 8, 2012.
- Anonymous:** Pesticide EU – MRLs Database, Annexes of Regulation (EC) No. 396/2005. Official Journal of the European Union (Plant Protection – Pesticide Residues – Community Legislation), 2005.
- Anonymous:** The Pesticides, Maximum Residue Levels in Crops, Food and Feeding Stuff, Statutory Instrument No. 2922 (Amendment No. 3). Regulations 2006, England and Wales., 2006.
- Anonymous:** Annual Report of the Pesticide Residues Committee 2007. Pesticides Safety Directorate, York, UK, 2007.
- Anonymous:** Organic mushroom production. Department of Agriculture and Rural Development. Belfast, UK. 2010. [www.dardni.gov.uk/ruralni/index/bussys/organic\\_production/organic\\_horticulture/mushroom.htm](http://www.dardni.gov.uk/ruralni/index/bussys/organic_production/organic_horticulture/mushroom.htm). Accession date: February 29, 2012.
- Balaž, F., Bagi, F., Stojšin, V. and Mastilović, J.:** Efficacy of chemical control against wheat head blight and impact on yield and technological quality. 3rd International FHB Symposium, Szeged, Hungary – Cereal Research Communications, Supplement B, 36: 701-702, 2008.
- Bhatt, N. and Singh, R.P.:** Cobweb disease of *Agaricus bisporus*: incidence, losses and effective management. Indian Journal of Mycology and Plant Pathology, 22: 178-181, 1992.
- Chaloux, N., Savoie, J.M. and Olivier, J.M.:** Growth inhibition of *Agaricus bisporus* and associated thermophilic species by fungicides used in wheat cultivation. Agronomie, 13: 407-412, 1993.
- Challen, M.P. and Elliott, T.J.:** The *in vitro* responses to a range of fungicides of two strains of the mushroom *Agaricus bisporus* and the pathogen *Verticillium fungicola*. Mycopathology, 90: 161-164, 1985.
- Chang, S.-T. and Miles, P.G.:** Mushrooms. Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact. 2nd ed., 451. Boca Raton, USA: CRC Press, 2004.
- Chrysayi-Tokousbalides, M., Kastanias, M.A., Philippoussis, A. and Diamantopoulou, P.:** Selective fungitoxicity of famaxadone, tebuconazole and trifoxystrobin between *Verticillium fungicola* and *Agaricus bisporus*. Crop Protection, 26: 469-475, 2007.

- Diamantopoulou, P., Philippoussis, A., Kastanias, M., Flouri, F. and Chrysayi-Tokousbalides, M.A.:** Effect of famaxadone, tebuconazole and trioxystrobin on *Agaricus bisporus* productivity and quality. *Scientia Horticulturae*, 109: 190-195, 2006.
- Finney, D.J.:** Probit analysis. 3<sup>rd</sup> ed., University Press, Cambridge, UK, 1971, pp. 1-383.
- Lević, J., Stanković, S., Ivanović, D., Krnjaja, V., Kovačević, T., Tančić, S. and Bočarov-Stančić, A.:** Fusarium head blight and grain yield losses of wheat in Serbia. 3<sup>rd</sup> International FHB Symposium, Szeged, Hungary – Cereal Research Communications, Supplement B, 36: 513-514, 2008a.
- Lević, J., Stanković, S., Krnjaja, V., Kovačević, T., Tančić, S. and Bočarov-Stančić, A.:** Pathogenicity and phytotoxicity of *Fusarium langsethiae* on wheat seedlings. 3<sup>rd</sup> International FHB Symposium, Szeged, Hungary – Cereal Research Communications, Supplement B, 36: 515-516, 2008b.
- Manjunath, B.L. and Korikantimath, V.S.:** Sustainable rice production through farming systems approach. *Journal of Sustainable Agriculture*, 33(3): 272-284, 2008.
- Miller, F.C.:** Biodegradation of solid wastes by composting. In: *Biological Degradation of Wastes. 1 Waste Material Disposal* (Martin A.M., ed.), Elsevier Science Publication Ltd., Essex, UK, 1991, pp. 1-30.
- Newman, R.H. and Savidge, M.:** Mancozeb dust – breakthrough in mushroom disease control. *MGA Bulletin*, 232: 161-162, 1969.
- Potočnik, I.:** Prouzrokovaci truleži šampinjona (*Agaricus bisporus* (Lange) Imbach) i njihova osetljivost na fungicide. Magistarska teza. Univerzitet u Beogradu, Biološki fakultet, 2006, str. 1-63.
- Potočnik, I.:** *Cladobotryum dendroides* (Bull.) W. Gams & Hooz., uzročnik paučinaste plesni šampinjona (*Agaricus bisporus* (Lange) Imbach) i njegova osetljivost na fungicide i bio-fungicide. Doktorska disertacija. Univerzitet u Beogradu, Biološki fakultet, 2009, str. 1-131.
- Potočnik, I., Milijašević, S., Rekanović, E., Todorović, B. and Stepanović, M.:** Sensitivity of *Verticillium fungicola* var. *fungicola*, *Mycogone perniciosa* and *Cladobotryum* spp. to fungicides in Serbia. In: *Science and Cultivation of Edible and Medicinal Fungi: Mushroom Science XVII* (van Greuning M., ed.), International Society for Mushroom Science, Cape Town, South Africa, 2008a, pp. 615-627.
- Potočnik, I., Vukojević, J., Stajić, M., Rekanović, E., Milijašević, S., Todorović, B. and Stepanović, M.:** *In vitro* toxicity of selected fungicides from the groups of benzimidazoles and demethylation inhibitors to *Cladobotryum dendroides* and *Agaricus bisporus*. *Journal of Environmental Science and Health, Part B*, 44(4): 365-370, 2009a.
- Potočnik, I., Vukojević, J., Stajić, M., Rekanović, E., Milijašević, S., Stepanović, M. and Todorović, B.:** Toxicity of fungicides with different modes of action to *Cladobotryum dendroides* and *Agaricus bisporus*. *Journal of Environmental Science and Health, Part B*, 44(8): 823-827, 2009b.
- Potočnik, I., Vukojević, J., Stajić, M., Rekanović, E., Stepanović, M., Milijašević, S. and Todorović, B.:** Toxicity of biofungicide Timorex 66 EC to *Cladobotryum dendroides* and *Agaricus bisporus*. *Crop Protection*, 29(3): 290-294, 2010a.
- Potočnik, I., Vukojević, J., Stajić, M., Tanović, B. and Rekanović, E.:** Sensitivity of *Mycogone perniciosa*, pathogen of culinary-medicinal button mushroom *Agaricus bisporus* (J. Lge) Imbach (Agaricomycetidae), to selected fungicides and essential oils. *International Journal of Medicinal Mushrooms*, 12(1): 91-98, 2010b.
- Potočnik, I., Vukojević, J., Stajić, M., Tanović, B. and Todorović, B.:** Fungicide sensitivity of selected *Verticillium fungicola* isolates from *Agaricus bisporus* farms. *Archives of Biological Sciences*, 60(1): 151-158, 2008b.
- Potočnik, I., Tanović, B., Milijašević, S., Rekanović, E. and Todorović, B.:** Response of the mushroom pathogen *Verticillium fungicola* (Preuss) Hasebrauk (dry bubble) to some essential oils. *ESNA Meeting, Amiens, France – Revue de Cytologie et de Biologie Végétale – Le Botaniste*, 28 (s.i.): 388-392, 2005.
- Savić-Petrić, S. and Sekulić, J.S.:** Pesticides on the Serbian Market. *Plant Doctor (Biljni lekar)* 35: 2-3, 2007 (In Serbian)
- Savoie, J.M., Chalaux, N. and Olivier, J.M.:** Variability in straw quality and mushroom production: importance of fungicide schedules on chemical composition and potential degradability of wheat straw. *Bioresource Technology*, 41: 161-166, 1992.
- Tanović, B., Potočnik, I., Delibašić, G., Ristić, M., Kostić, M. and Marković, M.:** *In vitro* effect of essential oils from aromatic and medicinal plants on mushroom pathogens: *Verticillium fungicola* var. *fungicola*, *Mycogone perniciosa*, and *Cladobotryum* sp. *Archives of Biological Sciences*, 61(2): 231-238, 2009.
- Yoder, J.B., Sinden, J.W. and Hauser, E.:** Experience with zink ethylene bisdithiocarbamate fungicide in mushroom cultivation. *Mushroom Science*, 1: 100-108, 1950.

# 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