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Review paper

Disease control by chemical and biological fungicides in cultivated mushrooms: button mushroom, oyster mushroom and shiitake

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SUMMARY

The most commonly cultivated basidiomycetes worldwide and in Serbia are button mushroom (*Agaricus bisporus*), oyster mushroom (*Pleurotus* sp.) and shiitake (*Lentinus edodes*). Production of their fruiting bodies is severely afflicted by fungal, bacterial, and viral pathogens that are able to cause diseases which affect yield and quality. Major *A. bisporus* fungal pathogens include *Mycogone perniciosa, Lecanicillium fungicola,* and *Cladobotryum* spp., the causal agents of dry bubble, wet bubble, and cobweb disease, respectively. Various *Trichoderma* species, the causal agents of green mould, also affect all three kinds of edible mushrooms. Over the past two decades, green mould caused by *T. aggressivum* has been the most serious disease of button mushroom. Oyster mushroom is susceptible to *T. pleurotum* and shiitake to *T. harzianum*. The bacterial brawn blotch disease, caused by *Pseudomonas tolaasii*, is distributed globally. Disease control on mushroom farms worldwide is commonly based on the use of fungicides. However, evolution of pathogen resistance to fungicides after frequent application, and host sensitivity to fungicides are serious problems. Only a few fungicides are officially recommended in mushroom production: chlorothalonil and thiabendazol in North America and prochloraz in the EU and some other countries. Even though decreased sensitivity levels of *L. fungicola* and *Cladobotryum mycophilum* to prochloraz have been detected, disease control is still mainly provided by that chemical fungicide. Considering such resistance evolution, harmful impact to the environment and human health, special attention should be focused on biofungicides, both microbiological products based on *Bacillus* species and various natural substances of biological origin, together with good programs of hygiene. Introduction of biofungicides has created new possibilities for crop protection with reduced application of chemicals.

Keywords: Fungicides; Biofungicides; Edible mushrooms

INTRODUCTION

Fungal pathogens such as *Lecanicillium (Verticillium) fungicola*, *Mycogone perniciosa, Cladobotryum* (*Dactylium*) spp. and *Trichoderma* spp. afflict the cultivated mushroom *Agaricus bisporus* (Lange) Imbach, causing its most serious fungal diseases:, dry and wet bubble, cobweb disease and green mould, respectively (Potočnik et al., 2008a, 2008b, 2010a, 2010b; Kosanović et al., 2013). Several diseases of cultivated mushrooms are caused by bacteria and viruses (Grogan, 2008; Geels et al., 2008). The most common bacterial disease, distributed worldwide, is the bacterial brown blotch caused by *Pseudomonas tolaasii* (Milijašević-Marčić et al., 2012; Todorović et al., 2012).

Forty years ago, wet and dry bubble diseases caused losses in *A. bisporus* production in the amount of 9 million dollars a year in Pennsylvania, US (Forer et al., 1974), while losses in the UK were up to 5% of total yield (Gaze & Fletcher, 1975). With cobweb disease outbreaks in epidemic proportions in the mid-1990s, yield losses significantly increased up to 40%. The greatest damage was reported in the Republic of Ireland, the UK and the US (Gaze 1996, McKay et al., 1998). Brown blotch disease was found responsible for 8-10% losses worldwide (Olivier et al., 1997). More recently, major *A. bisporus* diseases have been caused by the mushroom virus X complex (Grogan , 2008) and various *Trichoderma* species, fungi that are the causal organisms of green mould (Kosanović et al., 2013). They both account for approximately 25% of the total production value, and are the major pathogens of all three cultivated mushroom species (Soković & van Griensven, 2006).

The spread of pathogens from one country to another through imported goods and machinery increases the risks of unwanted introduction of pathogens. In the mid-1990s, dry and wet bubble diseases largely reduced the quality and yield of *A. bisporus* in Serbia (Potočnik et al., 2008a, 2008b; 2010b). Over the past ten years, cobweb disease has caused significant damage in mushroom cultivation in this region (Potočnik et al., 2009a, 2009b, 2010a). A few years ago, a disease with symptoms resembling green mould became a serious problem in Serbian mushroom farms (Kosanović et al., 2013, 2015).

CHEMICAL CONTROL OF DISEASES OF EDIBLE MUSHROOMS

A common method of pathogen control on farms worldwide is based on the application of various fungicides. Ongoing pesticide reviews in Europe have resulted in many chemicals being no longer approved for use. Thus, a major challenge for mushroom growers in the 21st century is disease control with few or no chemicals at all. Other challenges include fungicide resistance among pathogen populations, managed by the currently available pesticides, and the emergence of new pathogens (Grogan, 2008).

Calcium chloride and chlorinated compounds are the most commonly used chemicals for brown blotch disease control (Solomon et al., 1991).

Fungicide efficiency depends on the frequency of use (Bonnen & Hopkins, 1997), and on fungicide persistence at high concentrations in mushroom casing soil (Grogan & Jukes, 2003). Since studies of fungicide efficacy on cultivated mushrooms by agrochemical companies are very rare, only a few fungicides have beem officially recommended in mushroom production: chlorothalonil and thiabendazol in North America, and prochloraz in the EU and other countries (Beyer & Kremser, 2004; Grogan, 2008). Decreased sensitivity of *L. fungicola* and *Cladobotryum* sp. to prochloraz has been detected (Gea et al., 2005; Grogan, 2006). This combination of circumstances makes prevention of disease outbreaks more challenging (Grogan, 2008).

Excellent control of many fungal diseases was secured when fungicides from the group of methyl benzimidazole carbamates (MBC) were introduced in the late 1960s (Delp, 1987). However, after several years of their intensive use, resistant strains of *L. fungicola* appeared (Bollen & van Zaayen, 1975; Bonnen & Hopkins, 1997; Potočnik et al., 2008b). The resistance of cobweb disease agents to MBC fungicides, especially to benomyl and carbendazim, was reported after their extensive use in the Republic of Ireland in 1992 (McKay et al., 1998). In 1994/95, when cobweb disease took epidemic proportions in British mushroom farms, a majority of *C. mycophillum* isolates were strongly resistant to thiabendazole and weakly resistant to carbendazim (Grogan & Gaze, 2000; Potočnik et al., 2008a). Prochloraz-manganese was still very toxic to Serbian *C. dendroides* isolates when a survey was carried out during 2009 (Potočnik et al., 2009a). Recently, *T. aggressivum* has also developed resistance to benzimidazole fungicides (Romaine et al., 2005). Only *M. perniciosa* has remained sensitive (Potočnik et al., 2010b). In the EU, the benzimidazole fungicide carbendazim has been withdrawn from use on mushrooms, leaving no benzimidazoles approved for control of sensitive pathogens such as *M. perniciosa*. Chlorothalonil was widely used in Europe and worldwide in the past (van Zaayen, 1979), but it is now only approved for use in the US and Canada (Romaine et al., 2005).

Imidazole demethylation inhibitors (DMIs), including prochloraz-manganese, provide effective prevention of all fungal pathogens on mushrooms by inhibition of the demethylation step in ergosterol biosythesis, an essential compound responsible for stability and functioning of lipoprotein membranes in many fungi (van Zaayen & van Adrichem, 1982; Chrysayi-Tokousbalides et al., 2007). Prochloraz-manganese is the most effective fungicide in prevention of *Cladobotryum* spp., but the fungicide has been found no longer able to control the spotting symptoms of cobweb disease (Grogan, 2006). Sensitivity tests of Serbian *C. dendroides* isolates have shown that although they were weakly resistant to thiophanate-methyl, cross resistance was not observed to either benomyl or carbendazim (Potočnik et al., 2009a). Cross resistance to MBC fungicides occured in the mushroom pathogen *L. fungicola*, which was highly resistant to thiabendazole and benomyl (Grogan & Gaze, 2000).

Trifloxystrobin had a satisfactory fungitoxicity to *L. fungicola*, and it was recommended as safe for operators, consumers and the environment (Chrysayi-Tokousbsaliedes et al., 2007). However, Serbian *C. dendroides* isolates were highly resistant to that fungicide. Kresoxim-methyl, another fungicide of the strobilurin group, was even less toxic to the tested *C. dendroides* isolates than trifloxystrobin (Potočnik et al., 2009b). Captan and metalaxyl had satisfactory effectiveness in the control of mushroom diseases in India, the UK and Serbia but less than prochloraz-manganese (Fletcher et al., 1983; Bhatt & Singh, 1992; Potočnik et al., 2009a, 2009b). Spanish isolates of *L. fungicola* were resistant to iprodione, while Serbian isolates were moderately sensitive to that fungicide (Gea et al., 1996; Potočnik et al., 2009b).

The fungicide prochloraz is the only effective chemical for control of *L. fungicola* isolates in European countries, which tend to become more tolerant. A majority of *L. fungicola* isolates, some 70% from Great Britain and Spain have been reported as moderately sensitive to prochloraz-manganese (Grogan et al., 2000; Gea et al., 2005), and some farms reported unsatisfactory levels of control by this fungicide, which was attributed to resistance evolution (Gea et al. 2005). This tolerance has not been associated with any major loss of control probably because of the fact that the sexual state of *Lecanicillium* has not been encountered so far, reducing the risk of increased resistance due to sexual recombination (Grogan, 2008).

The effect of various fungicides on spore germination and mycelial growth of *Trichoderma* isolates aggressive to oyster mushroom (*Pleurotus* spp.) was examined by Hatvani et al (2008). Prochloraz was shown to be the most effective fungicide for inhibition of mycelial growth of green moulds. Captan was toxic to *P. ostreatus*. Prochloraz, benomyl and propineb were found to inhibit spore germination of benomyl-susceptible isolates, while chlorothalonil was effective against benomyl-resistant strains. Prochloraz, thiabendazole and benomyl residues in the fruiting bodies of oyster mushrooms were below the permitted values for fungicide residues in mushrooms. The inhibitory effects of several fungicides commonly used in agriculture (prochloraz, thiabendazole, dichloran, benomyl, propiconazole, thiophanate-methyl) were also tested in the study by Hatvani et al. (2008), and both prochloraz and thiabendazole, the pesticides allowed in edible mushroom production, were found to inhibit the growth of aggressive *Trichoderma* isolates without having a negative effect on *Pleurotus*. Application of calcium hydroxide onto the affected area was suggested in order to prevent the spread of disease (Hatvani et al., 2008).

Rinker and Alm (2008) evaluated *in vitro* and *in vivo* toxicity of fungicides against *Trichoderma* in *A. bisporus*. Thiabendazole was the most effective, followed by benomyl and thiophanate-methyl. Chlorothalonil was ineffective. Adjustment of casing pH is another approach to *Trichoderma* management. High pH in PDA plates reduced spore germination and retarded linear growth. *Trichoderma* was found to prefer acidic substrates as its optimal mycelial growth occurred at 5-7 pH (Kosanović et al., 2015). Romaine et al. (2008) reported benomyl and thiophanate-methyl resistance in *T. aggressivum* f. *aggressivum* (former Ta2) in the US. Imazalil sulfate, an imidazole, was found to be effective as a spawn and supplement treatment for its benzimidazole-resistant aggressive form. Initially, benzimidazole fungicides applied to spawn provided good disease control in the US, but resistance problems began to occur (Romaine et al., 2005). Serbian *Trichoderma* spp. isolates were most susceptible to prochloraz, chlorothalonil and carbendazim, less sensitive to iprodione, weekly resistant to thiophanate-methyl, and resistant to trifloxystrobin. Prochloraz and carbendazim had strong selective toxicity (0.01 and 0.02, respectively), iprodione and chlorothalonil moderate (0.16), and thiophanate-methyl weak, (1.24), while trifloxystrobin toxicity to *A. bisporus* was not tested because of its inefficiency against *Trichoderma* isolates (Kosanović et al., 2015). After reports of benzimidazole mutagenicity, it was recommended to remove that class of fungicides from the market and prochloraz remained the only one registered for use in the EU (Grogan, 2008).

Thiabendazole concentration in casing soil was found to remain high throughout the cropping period, whereas the concentrations of carbendazim and prochloraz manganese dropped considerably by the end of the second flush (Grogan & Jukes, 2003). Despite a very high organic matter content in mushroom casing soil, these trends are similar to data from mineral soils (Yarden et al., 1990). Thiabendazole had better persistence in mushroom casing soil than carbendazim and prochloraz-manganese (Grogan & Jukes, 2003). Carbendazim is known to be susceptible to microbial degradation in soils (Fletcher et al., 1980; Yarden et al., 1990) and it may lead to reduced control of pathogens which are still sensitive to the fungicide. Biodegradation of pesticides is an environmentally desirable trait, so that toxic chemicals do not accumulate in the environment as it had been the case with DDT. But there has to be a balance between the time frame within which a chemical is effective against its target pathogen and its ultimate breakdown to non-toxic components (Grogan, 2008). Recent research has shown that prochloraz can also be degraded by microbes present in prochloraz-treated casing (Papadopoulos, 2006). Under normal growing conditions in a mushroom unit, there was a rapid decline in concentration of the active ingredient with less than 15% remaining by day 45. Further research by Papadopoulos (2006) indicated that ^{14}C-labelled prochloraz degraded much more rapidly in the presence of residual liquid from a fungicide spray tank than in fresh casing, indicating that the source of prochloraz-degrading microbes in casing may be the tank used to apply the chemical.

BIOLOGICAL CONTROL OF DISEASES OF EDIBLE MUSHROOMS

Opposite from the spores of *L. fungicola*, *M. perniciosa* and *Trichoderma* sp. that are sticky and spread by insects, *Cladobotryum* conidia are dry, large and aerodynamic and spread by ventilation to all parts of farms (Adie et al., 2006). Ware (1933) observed that dry bubble disease was associated with the presence of insects. Dispersal by splashing water was found to be very effective, and spreading by staff and equipment has also been reported as important (Wong & Preece, 1987). Infected mushrooms, either in the wild or growing in mushroom farms, could be an important source of inoculum (Zare & Gams, 2008). Anaerobic conditions and low pH are unfavorable for *L. fungicola*. In the UK in 1988, surface peat was mainly used (Visscher, 1988), and *L. fungicola* was able to survive on basidiomycete species that grew in it. However, peat collected from lower peat layers seems to be a less likely habitat for *L. fungicola*. Replacing the casing mixtures of clay, loam and humus with mixtures of sphagnum peat, sand and carbonate led to a considerable reduction in dry bubble incidence in mushroom farms in Denmark (Bech & Riber-Rasmussen, 1967). The pH optimum for *Pleurotus* growth was alkaline (pH 8-9) whereas *Trichoderma* preferred acidic-neutral conditions (pH 5-7). This finding suggests that *Trichoderma* growth could be slowed down by adjusting the substrate pH to 8-9, which would result in a reduced spread of infection (Woo et al., 2004, Kosanović et al., 2015).

However, considering the resistance development, harm caused to the environment and human health, as well as an increase in production costs, special attention should be focused on inventing and developing alternative biological methods of disease control. Introduction of new fungicides of biological origin creates new possibilities for crop protection from fungal pathogens. Environmental awareness has grown, resulting in a more effective enforcement of pollution control laws. This situation stimulates the mushroom industry to develop technologies that ensure production with the least possible harmful effects on the environment (Levanon et al., 1988).

Krupke et al. (2003) suggested that lactonase-producing bacteria should be examined for green mould prevention. Although *T. aggressivum* resisted numerous bacteria isolated from the compost, it was found affected by some strains (Savoie et al., 2001) predominantly belonging to *Bacillus* species. Based on these observations, Serenade® biofungicide (active ingredients: *Bacillus subtilis* and its lipopeptides) was approved for use against *T. aggressivum* in French mushroom farms (Védie & Rousseau, 2008). Rekanović et al. (2007) noted that a significant amount of research work focused on utilization of the mycopathogenic fungus *Pythium oligandrum* for control of *Verticillium* spp. Kosanović et al. (2013) found an antagonistic effect between the commercial biofungicide *B. subtilis* and prochloraz, and recommended their alternating, rather than simultaneous application.

Many compounds have been tested as control agents against edible mushroom diseases. Plant extracts, essential oils and their components have demonstrated strong fungistatic effects (Potočnik et al., 2005; 2010b; Soković et al., 2009; Tanović et al., 2009). Although fungitoxic activity of oils is not strong, they could be used as a supplement to commercial products for disease control, which will minimize the quantity of fungicides used. Essential oils of oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) and their respective major components, carvacrol and thymol, showed very strong activity against *T. aggressivum* f. *europaeum*, *T. harzianum* and *T. atroviride*. Peppermint (*Mentha piperata*) which produces an important essential oil, menthol, also shows strong inhibitory activity against *Trichoderma* (Soković & van Griensven, 2006). Addition of tea tree essential oil (*Melaleuca alternifolia*) to *Pleurotus* substrate resulted in strong to total inhibition of *T. harzianum* (Angelini et al., 2008). Tea tree oil was considerably less toxic than prochloraz-manganese *in vitro* to *C. dendroides* isolates and *A. bisporus* F56 strain. Tea tree oil, the active ingredient of the biofungicide Timorex 66 EC, applied at the standard product application rate caused a significant reduction in cobweb disease levels in an *A. bisporus* experimental growing room (Potočnik et al., 2010a).

Bacillus subtilis QST 713 was found to be toxic to Serbian *Trichoderma* spp. isolates, but tea tree oil did not exhibit a significant antifungal activity (Kosanović et al., 2013).

An efficacy trial *in vivo* showed higher effectiveness of prochloraz-manganese than both tested biofungicides and their respective mixtures with standard fungicide. A biofungicide based on *B. subtilis* demonstrated greater efficacy in preventing disease symptoms than tea tree oil. *B. subtilis* combined with the fungicide revealed less antagonism in effectiveness against the pathogen than tea tree oil (Kosanović et al., 2015). Eco-friendly disinfectants for casing soil based on colloidal silver and hydrogen peroxide showed a significant antimicrobial activity against *P. tolaasii*, the causal agent of bacterial brown blotch (Todorović et al., 2012).

CONCLUSION

Button mushroom, oyster mushroom and shiitake are the most commonly cultivated basidiomycetes worldwide. With the popularization of mushroom farming, mushroom production continues to increase. Commercial strains of cultivated mushrooms produce substantial yield and exhibit attractive morphology and texture, but they are susceptible to a variety of viral, bacterial and fungal diseases. The most serious diseases of cultivated mushrooms are caused by mushroom virus X, pseudomonads and *Lecanicillium*, *Mycogone*, *Cladobotryum* and *Trichoderma* fungi. Disease control is provided by the chemical fungicide prochloraz, biofungicides based on *Bacillus* species and natural substances of biological origin. An important part of disease prevention can be achieved by improvements in farm hygiene, aimed at preventing crop contamination with potentially infected spores and mycelial debris at spawning or casing by preventing the spread of spores by filtration and by cooking out crops at the end cycle.

Application of various fungicides is a common method of pathogen control on farms worldwide. Ongoing pesticide reviews in Europe have resulted in many chemicals being no longer approved for use. A major challenge for mushroom growers in the 21st century is disease control with few or no chemicals at all. Other challenges include fungicide resistance among pathogen populations, and the emergence of new pathogens. Fungicide efficiency depends on the frequency of use, as well as on fungicide persistence at high concentrations in mushroom casing soil. Only a few fungicides are currently officially recommended for use in mushroom production: prochloraz-manganese in the EU countries, chlorothalonil, thiabendazol and tiophanate-methyl in the US and Canada, and prochloraz-manganese, carbendazim and thiabendazole in Australia. The most effective fungicide in mushroom disease control is prochloraz. Decreased sensitivity of the pathogenic fungi *Lecanicillium* and *Cladobotryum* to prochloraz has been already recorded. Special attention is now being focused on a genetic reduction of pathogen virulence by generations of mutants with diminished ability to utilize chitin as a carbon source, and on the possibility of using natural products, such as essential oils of different plants, to inhibit pathogen activity. Tea tree oil, the active ingredient of the biofungicide Timorex, has been found to cause a significant reduction in cobweb disease in *Agaricus* and *Pleurotus*. Brown cultivars of *Agaricus* have been found partially resistant to bacterial blotch, dry bubble disease and green mould. Antibiotics that are produced by *A. bisporus* may play a role in defense against *Lecanicillium* and *Trichoderma* bacteria.

Considering the resistance development, harm to the environment and human health, as well as an increase in production costs, special attention should be focused on inventing and developing alternative biological methods of disease control. Introduction of new fungicides of biological origin creates new possibilities for crop protection from pathogens. Based on these observations, the biofungicide Serenade, based on *Bacillus subtilis*, has been approved for use against *T. aggressivum* in French mushroom farms.

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Suzbijanje bolesti gajenih gljiva: šampinjona, bukovača i šiitaka, sintetičkim i biofungicidima

REZIME

Šampinjon (*Agaricus bisporus*), bukovača (*Pleurotus* sp.) i šiitake (*Lentinus edodes*) su najviše gajene gljive u svetu i kod nas. Patogene gljive, bakterije i virusi nanose velike štete u njihovoj proizvodnji. Najvažnije bolesti *A. bisporus* su *Mycogone perniciosa, Lecanicillium fungicola* i *Cladobotryum* spp., prouzrokovači suve, mokre i paučinaste plesni. Vrste roda *Trichoderma,* prouzrokovači zelene plesni, ugrožavaju sve tri vrste gajenih gljiva. Poslednjih decenija zelena plesan je najvažnija bolest šampinjona, koju izaziva *T. aggressivum*. Parazit bukovače je *T. pleurotum* i šiitake *T. harzianum*. Bakteriozna pegavost šampinjona je veoma rasprostranjena i izaziva je *Pseudomonas tolaasii*. Zaštita šampinjona od bolesti u svetu i kod nas se zasniva na primeni fungicida. Međutim, razvoj rezistentnosti patogena na fungicide nakon česte primene i toksičnosti fungicida na domaćina koji takođe pripada carstvu gljiva sužava izbor adekvatnih preparata. Mali broj fungicida se zvanično preporučuje za primenu: prohloraz u zemljama EU, a hlorotalonil i tiabendazol u Severnoj Americi. Uočena je smanjena osetljivost *L. fungicola* i *Cladobotryum mycophilum* na prohloraz. Zbog razvoja rezistentnosti, potencijalnog rizika za životnu sredinu i ljude, velika pažnja je posvećena održavanju striktne higijene u gajilištima. Uvođenje novih fungicida biološkog porekla donosi nove mogućnosti u zaštiti gajenih gljiva od bolesti.

Ključne reči: Fungicidi; Biofungicidi; Gajene gljive