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THE EFFECT OF SELECTED PREPARATIONS ON *IN VITRO* GROWTH OF *TRICHODERMA HARZIANUM* AND *TRICHODERMA ATROVIRIDE* FOUND IN GARDEN MUSHROOM (*AGARICUS BISPORUS*) CROP

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Abstract

Preparations Biosept 33 SL (0.8%), Grevit 200 SL (0.6%) and a fungicide Bravo 500 SC (0.5%) considerably inhibited *in vitro* growth of garden mushroom pathogens *Trichoderma harzianum* and *T. atroviride*. The mycelium growth inhibition of the fungi was statistically significant and on the fourth day after medium inoculation it was 92.22%, 87.78%, 85.56% (*T. harzianum*) and 89.44%, 87.41%, 88.52% (*T. atroviride*). The other tested preparations exhibited relatively low efficiency.

Key words: garden mushroom, *Trichoderma harzianum*, *Trichoderma atroviride*, mycelial growth, fungicides

Introduction

Within the past 20 years the production of garden mushroom (*Agaricus bisporus*) in Poland increased. However, considerable losses have been observed in edible mushroom growing in the last few years, caused by occurrence of *Trichoderma* spp. These saprotrophic soil fungi are known in plant protection as biological control agents against many diseases (Garibaldi 1983, Pionnat and Tramier 1983, Tramier et al. 1983, Vanachter et al. 1988, Bochow 1989, Coosemans 1989, De Ceuster and Pauwels 1995) and used to produce biopreparations applied against plant pathogenic fungi (Marcinkowska 2003).

Nevertheless, in edible mushroom growing *Trichoderma* spp. are causing agents of green mould, attacking both medium with developing mycelium, and fruiting bodies (Chet and Inbar 1994, Agosin and Aguilera 1998, Hermosa et al. 2000). They constitute a considerable threat to the production of garden mushroom (*A. bisporus*), oyster mushroom (*Pleurotus* spp.) and shiitake (*Lentinula edodes*)

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(Benhamou and Chet 1993, Ziombra 1995, Sharma et al. 1999, Ziombra 2001, Samuels et al. 2002, Szczech 2005).

At present effective methods of edible mushroom protection against green mould are lacking. Available chemical agents are not very effective; moreover, they also create risk of toxic substances accumulation in fruiting bodies. Thus, development of an effective protection program for cultivated mushrooms using natural agents is of great importance.

The aim of the study was laboratory testing of the effect of selected preparations of natural origin and of Bravo 500 SC (fungicide posing limited hazard of active substance accumulation in fruiting bodies) on the growth of *T. harzianum* and *T. atroviride* isolated from garden mushroom (*A. bisporus*) crop.

Material and methods

Plant protection agents of natural origin, i.e. Biochikol 020 PC (chitosan), Bioczos BR (paraffin-coated garlic pulp), Polyversum (oospores of *Pythium oligandrum*), Propolis 10% (alcohol extract of propolis), Biosept 33 SL (extract of grapefruit seeds and flesh), Grevit 200 SL (grapefruit extract) and an organic fungicide Bravo 500 SC (chlorothalonil) constituting a limited threat of accumulation of active substance residue in fruiting bodies of garden mushroom (Ziombra 2001) were taken for the study.

Isolate of *T. harzianum* used in the experiment was obtained on 12 May, 2005 from a mushroom medium based on straw and granulated poultry dung, in the cultivation of garden mushroom at the mushroom-growing cellar in Mikulice near Turek, while the *T. atroviride* isolate was obtained on 20 March, 2005 from a wheat straw substratum at the mushroom-growing cellar in Marcinowo near Koło. The isolates were kept at the Department of Vegetable Crops, Agricultural University of Poznań.

Trichoderma colonies were cultured on potato dextrose agar (PDA medium produced by Merck). Appropriate amounts of individual preparations were added to the medium (Table 1). The control combination was PDA with no fungicide added.

Table 1

Characteristics of preparations used in the study

Preparation	Active substance and its content	Concentration in medium
Biochikol 020 PC	20% chitosan	4.00%
Bioczos BR	10% garlic pulp	1 cube per 100 ml of medium
Biosept 33 SL	33% extract of grapefruit seeds and flesh	0.80%
Bravo 500 SC	50% chlorothalonil	0.50%
Grevit 200 SL	20% grapefruit extract	0.60%
Polyversum	100% oospores of *Pythium oligandrum*	0.15%
Propolis 10%	10% propolis	0.80%

The experiment was performed in three replications. Medium (in 90-mm Petri dishes) was inoculated with 5-mm discs of medium overgrown with mycelium (from edges of seven-day-old cultures). Inoculated discs were incubated at 23°C in the dark. Fungal colonies were measured after two and four days, in two perpendicular directions in order to calculate their diameter. Efficiency of the preparations was determined on the basis of diameters of fungal colonies in relation to control. The results were subjected to statistical analyses based on Duncan's test at the significance level $\alpha = 0.05$.

Results

Preparations Biosept 33 SL, Bravo 500 SC and Grevit 200 SL limited to a considerable degree the *in vitro* growth of *T. harzianum* (Table 2). Inhibition of mycelial growth was statistically significant and on the fourth day after medium inoculation reached 92.22, 87.78 and 85.56%, respectively. A significant inhibition of mycelial growth in relation to control combination was also recorded for the other tested preparations, but they exhibited relatively low efficiency.

Biosept 33 SL, Bravo 500 SC and Grevit 200 SL also significantly inhibited *T. atroviride* growth (Table 2). Inhibition of its mycelial growth on the fourth day after medium inoculation was 89.44, 87.41 and 88.52%, respectively. The other tested preparations exhibited relatively low efficiency.

Discussion

There is scarce information in the literature on control of green mould in garden mushroom. Bodine (1995) reported that the most effective preparations in this respect were benzimidazol fungicides based on benomyl (Benlate 50 WP). Both in Poland and in the other EU countries, these agents are not admissible for application in garden mushroom growing due to the possibility of active substance residue accumulation in fruiting bodies. In Poland only two preparations are recommended to protect garden mushrooms against fungal diseases, i.e. Sporgon 50 WP (prochloraz) and Bravo 500 SC (chlorothalonil). The effectiveness of these agents was not found satisfactory by garden mushroom growers (Maszkiewicz 2004). At the same time even the recommended chemical plant protection agents have a negative effect on proliferation of garden mushroom mycelia; moreover, their application results in reduced yields (Ziombra 2001).

In the performed experiments preparation Biosept 33 SL at a concentration of 0.8%, Bravo 500 SC at a concentration of 0.5% and Grevit 200 SL at a concentration of 0.6% significantly reduced mycelial growth of *T. harzianum* and *T. atroviride*. The effect of Bravo 500 SC on the growth of another *Trichoderma* species, i.e. *T. koningii*, also found in garden mushroom crop, was investigated by Tekiela (2001) who reported that the fungicide exhibited relatively low efficiency – applied at con-

Table 2

Growth of *Trichoderma harzianum* and *T. atroviride* colonies on PDA medium (potato dextrose agar) with the addition of analyzed preparations

Preparation and its concentration in medium	*Trichoderma harzianum*				*Trichoderma atroviride*			
	two days after medium inoculation		four days after medium inoculation		two days after medium inoculation		four days after medium inoculation	
	colony diameter (mm)	mycelium growth inhibition in relation to control (%)	colony diameter (mm)	mycelium growth inhibition in relation to control (%)	colony diameter (mm)	mycelium growth inhibition in relation to control (%)	colony diameter (mm)	mycelium growth inhibition in relation to control (%)
Biosept 33 SL – 0.80%	7.00 a	81.33	7.00 a	92.22	9.50 a	80.94	9.50 a	89.44
Bravo 500 SC – 0.50%	7.00 a	81.33	11.00 b	87.78	9.67 a	80.59	11.33 a	87.41
Grevit 200 SL – 0.60%	7.50 a	80.00	13.00 b	85.56	7.83 a	84.29	10.33 a	88.52
Propolis 10% – 0.80%	10.17 ab	72.88	24.17 c	73.14	10.17 a	79.59	28.00 b	68.89
Biochikol 020 PC – 4.00%	13.33 b	64.45	37.67 d	58.14	10.50 a	78.93	27.00 b	70.00
Bioczos BR – 1 cube per 100 ml of medium	14.50 b	61.33	45.83 e	49.08	14.33 b	71.24	37.33 c	58.52
Polyversum – 0.15%	31.67 c	15.55	53.67 f	40.37	35.33 c	29.10	51.67 d	42.59
Control	37.50 d	–	90.00 g	–	49.83 d	–	90.00 e	–

Means denoted with identical letters do not differ significantly at significance level $\alpha = 0.05$ according to Duncan's test.

centrations of 0.3% and 0.4% it reduced growth rate of the pathogen mycelium only slightly. Apart from Bravo 500 SC, Tekiela (2001) investigated also such preparations as Sporgon 50 WP (prochloraz), Topsin M 70 WP (methylthiophene), Mirage 450 EC (prochloraz), Bioczos BR (garlic pulp) and reported high efficiency of Sporgon 50 WP and Mirage 450 SC, which at a concentration of 0.1% inhibited the growth of *T. koningii* colonies by 95-100%. In contrast, a very low efficiency of Bioczos BR was found when applied at a concentration of 0.1% (efficiency of 2-5%). Also in this study a very poor effect of Bioczos BR on the growth of *T. harzianum* and *T. atroviride* was recorded. A complete inhibition of *T. koningii* growth was reported by Tekiela (2001) after the application of sodium chloride (NaCl) at a concentration of 50%. However, Sakson (2004) pointed out that in the case of noxious *Diptera* presence in the cultivation chamber sodium chloride may be transported by these insects, which would lead to the damage of fruiting bodies.

Results of this study, obtained under laboratory conditions, indicate that it may be possible to select preparations of natural origin, strongly reducing the growth of *Trichoderma* genus fungi, green mould agents of garden mushroom. In future studies it will be essential to determine the efficiency of selected preparations *in vivo*, additionally taking into consideration the effect of substratum and mushroom species grown on it. It would be a major breakthrough to find an effective agent of natural origin for control of green mould. The application of these agents would undoubtedly contribute to the development of an effective protection of cultivated mushroom, at the same time being completely safe for both producers and consumers.

Conclusion

Preparations Biosept 33 SL, Grevit 200 SL and Bravo 500 SC, strongly reducing under laboratory conditions mycelial growth of *Trichoderma harzianum* and *T. atroviride*, are promising for control of these species in garden mushroom (*Agaricus bisporus*) crop.

Streszczenie

WPŁYW WYBRANYCH PREPARATÓW NA WZROST *IN VITRO* GRZYBÓW *TRICHODERMA HARZIANUM* I *TRICHODERMA ATROVIRIDE* WYSTĘPUJĄCYCH W UPRAWIE PIECZARKI DWUZARODNIKOWEJ (*AGARICUS BISPORUS*)

W latach 2005–2006 badano wpływ wybranych preparatów na wzrost *in vitro* grzybów *Trichoderma harzianum* i *T. atroviride*, występujących w uprawie pieczarki dwuzarodnikowej i będących sprawcami zielonej pleśni. Podłożem dla wzrostu grzybni *Trichoderma* był agar ziemniaczano-glukozowy (pożywka PDA), do którego

dodawano odpowiednie ilości testowanych preparatów. Kombinację kontrolną stanowiła pożywka PDA bez dodatku preparatów. Mierzono średnice kolonii grzybów w poszczególnych kombinacjach, po dwóch i czterech dobach od inokulacji pożywki.

Preparaty Biosept 33 SL w stężeniu 0,8%, Grevit 200 SL w stężeniu 0,6% oraz fungicyd Bravo 500 SC w stężeniu 0,5% w znacznym stopniu ograniczały wzrost grzybów *T. harzianum* i *T. atroviride*. Zahamowanie wzrostu grzybni badanych gatunków, rosnących na pożywkach z dodatkiem wymienionych preparatów, było statystycznie istotne. Po czterech dniach od inokulacji pożywki wynosiło ono dla *T. harzianum* odpowiednio 92,22%, 87,78% i 85,56%, natomiast dla *T. atroviride*: 89,44%, 87,41% i 88,52%. Pozostałe testowane preparaty wykazywały stosunkowo małą efektywność działania.

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