The effect of *Trichoderma* isolates, from family mushroom growing farms, on the yield of four *Agaricus bisporus* (Lange) Imbach strains

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Abstract: The effect of different *Trichoderma* species on the yield of *Agaricus bisporus* strains was investigated in this study. For the first time, the effect of different *Trichoderma* species on the yield of *Agaricus bisporus* strains was determined under fully controlled conditions. Four button mushroom strains were used: Somycel 53, Somycel 11, Amycel 2200, and Polmycel 31. The cultivation substrate was inoculated with the following *Trichoderma* species: *T. aggressivum* f. *europaeum, T. atroviride, T. hamatum, T. harzianum, T. inhamatum, T. koningii,* and *T. longibrachiatum.* Except for *T. atroviride*, all the *Trichoderma* isolates reduced the yield of the button mushroom strains.

Key words: button mushroom, green moulds, inoculation, substrate, yield

Introduction

The green moulds of the *Trichoderma* genus are one of the most dynamic fungal groups in the world. They are perfectly adapted to decompose organic materials, and are especially adept at decomposing cellulose. They also have a set of enzymes which are capable of decomposing very complicated compounds. The conditions in the cultivation chamber, *i.e*. abundance of organic matter, high temperature, and humidity, provide the perfect conditions for their growth and development (Savoie *et al*. 2001).

Infestation with green moulds causes considerable losses on mushroom farms. So far no effective methods of eradicating *Trichoderma* fungi from these farms have been developed. The following *Trichoderma* species found on mushroom growing farms have been described in detail: *T. harzianum, T. viride, T. aureoviride, T. koningi, T. hamatum, T. piluriferum, T. psedokoningii, T. longibrachiatum, T. inhamatum, T. croceum, T. stricipile, T. atroviride, T.* cf. *virens,* and *T. parceramosum.* Among the above mentioned species, the greatest losses in button mushroom production are caused by *T. harzianum*, found in four strains or biotypes (Sharma *et al*. 1999; Fletcher and Gaze 2008).

The aggressive strains of the *T. harzianum* species were identified as Th2 and Th4. The unaggressive strains of the species were identified as Th1 and Th3. At present in reference publications, the aggressive Th2 strain is identified as the *T. aggressivum* f. *europaeum* (Th2) species, whereas the Th4 strain is identified as *T. aggressivum* (Th4). It is found in North America. The aggressive strains of the *T. harzianum* species, *i.e.* Th2 and Th4, are distinguished from the morphologically similar strains of *T. harzianum* and *T. atroviride* by their growth rate (Seaby 1996).

Many scientific centres all over the world are conducting research on the potential fungi eradication of the *Trichoderma* genus, from button mushroom and oyster mushroom farms. Issues related with the development, identification, classification, and physiology of the *Trichoderma* fungi in mushroom growing, are being investigated at the biological, phytopathological, and molecular levels, usually applying the latest analytical and computer techniques (Seaby 1996; Chen *et al*. 1999; Samuels *et al*. 2002; Savoie and Mata 2003).

In recent years, *Trichoderma* strains have been characterised on the basis of: the growth rate of the pathogen's mycelium at different temperatures and on different culture media, their ability to stain the media, and colony odour. Apart from that, spore and shape were analysed by optical and electron microscopy. The size, shape, and distribution of spore clusters were also investigated (Seaby 1996; Williams *et al*. 2003).

Materials and Methods

Four strains of button mushroom (*Agaricus bisporus*) were used in the experiment: Somycel 53 and Somycel 11, grown in Poland in the 1960s and 1970s, as well as Amycel

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Table 1. Origin of *Trichoderma* sp. isolates derived from Polish mushroom farms

No.	*Trichoderma* species	Isolate symbol	Year of isolate collection	Place	Source of isolate
1	*T. aggresivum* f. *europaeum*	T. agg. 7/19	2009	Kłoda near Leszno	substrate
2	*T. atroviride*	T. at. 11/2	2007	Skierniewice	casing soil
3	*T. hamatum*	T. ham. 20/3	2009	Poznań	substrate
4	*T. harzianum*	T. har. 4/11	2008	Poznań	casing soil
5	*T. inhamatum*	T. inh 23/2	2009	Skierniewice	casing soil
6	*T. koningii*	T. kon. 16/4	2008	Gniezno	substrate
7	*T. longibrachiatum*	T. lon 7/2	2009	Poznań	substrate

2200 and Polmycel 31, grown in Poland in the 2000s. The strains came from the collection of cultivated and medicinal mushrooms of the Department of Vegetable Crops, the Poznań University of Life Sciences, Poland. Isolates of different fungal species from the *Trichoderma* genus used in the experiments came from the same collection (Table 1).

The cultivation experiment was conducted on the phase II substrate from a composting facility. The cultivation was located in an air-conditioned chamber in containers of 38 × × 30 × 18 cm. Incubation was conducted at a temperature of 25°C for 12 days; the relative humidity was 85–90%. Next, when the substrate was overgrown with mycelium, it was inoculated with the granular mycelium of the investigated *Trichoderma* isolates. Each time, 20 g of the mycelium was introduced to the cultivation container and mixed with the substrate. Incubation was continued at a temperature of 25°C; the relative humidity was 85–90%. The substrate overgrown with mycelium was covered with a 5-cm layer of high-moor peat neutralised with calcium carbonate to a pH of 7.5. Fruiting bodies were collected for seven weeks and the yield was calculated per 1 m^2 of the cultivated area. Each time, the substrate which was not inoculated with a *Trichoderma* isolate, was used as the control.

The experiment was performed in four replications in two cultivation cycles. The experimental method is described in detail in Sobieralski *et al*. (2012).

Results

In most cases infestation of the substrate with different fungal isolates of the *Trichoderma* genus caused a considerable yield reduction. The greatest decrease in yield was caused by substrate infestation with the isolate of *T. aggresivum* f. *europaeum* (Fig. 1). In all of the cases, a very high yield reduction could be observed. In the Amycel 2200 strain, the yield from the non-infested control was 15.6 kg/m^2, whereas it was as low as 1.5 kg/m^2 when the strain was infested with the aforementioned isolate. Substrate infestation with the T. at. 11/2 isolate of *T. atroviride* did not result in a statistically significant yield reduction of any of the strains (Fig. 2). Substrate infestation with the T. ham. 20/3 isolate caused a yield reduction in three strains, except for Amycel 2200, where the yield was similar both on the infested and non-infested substrate (Fig. 3). Substrate infestation with the other isolates, *i.e.* T. har. 4/11 of *T. harzianum*, T. inh. 23/2 of *T. inhamatum*, T. kon. 16/4 of *T. koningii* and T. lon. 7/2 of *T. longibrachiatum*, caused a statistically significant yield reduction (Fig. 4–7).

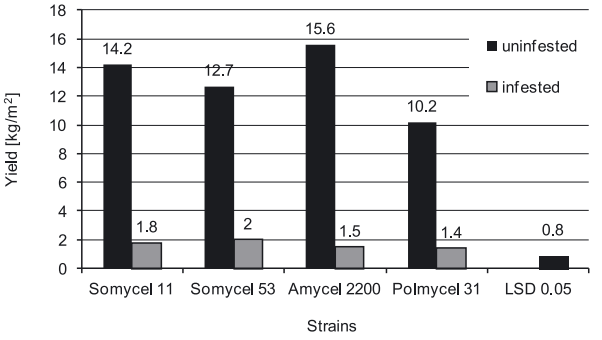

Fig. 1. Yield of a cultivated strain *A. bisporus* infested with the T. agg. 7/19 isolate of *T. aggressivum*

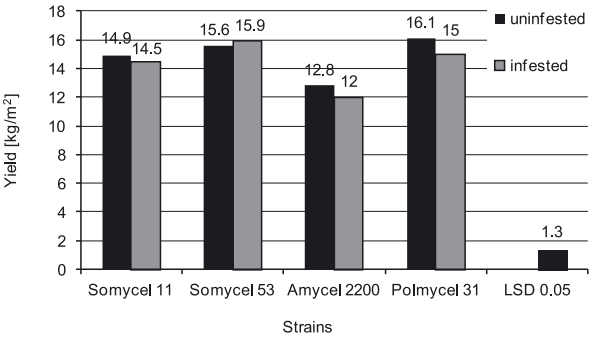

Fig. 2. Yield of a cultivated strain *A. bisporus* infested with the T. at. 11/2 isolate of *T. atroviride*

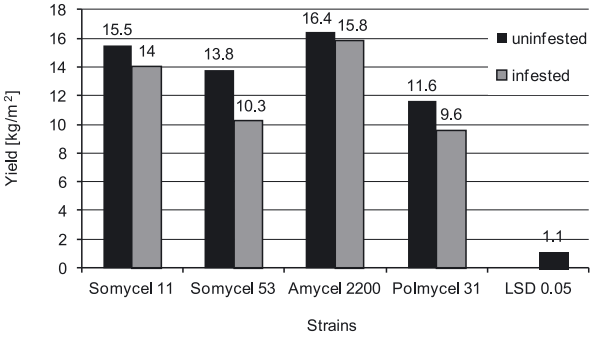

Fig. 3. Yield of a cultivated strain *A. bisporus* infested with the T. ham. 20/3 isolate of *T. hamatum*

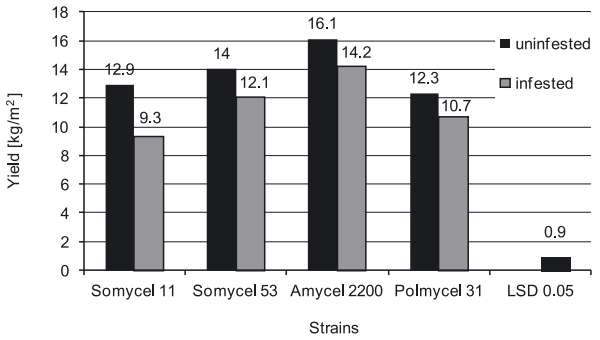

Fig. 4. Yield of a cultivated strain *A. bisporus* infested with the T. har. 4/11 isolate of *T. harzianum*

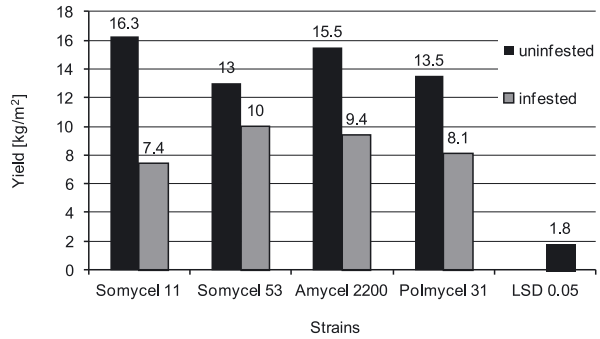

Fig. 5. Yield of a cultivated strain *A. bisporus* infested with the T. inh. 23/2 isolate of *T. inhamatum*

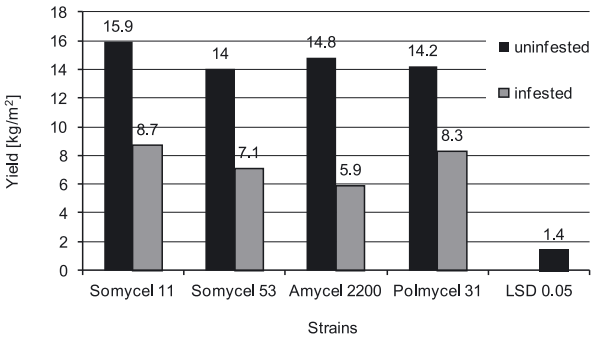

Fig. 6. Yield of a cultivated strain *A. bisporus* infested with the T. kon 16/4 isolate of *T. koningii*

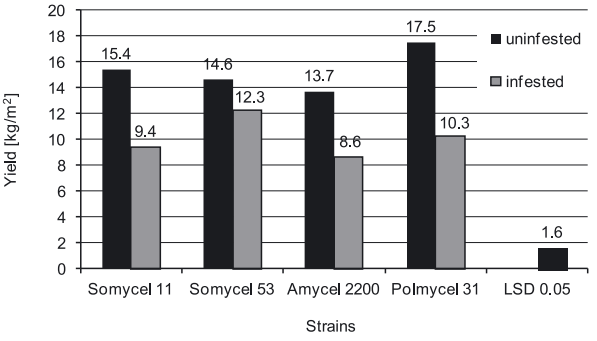

Fig. 7. Yield of a cultivated strain *A. bisporus* infested with the T. lon. 7/2 isolate of *T. longibrachiatum*

Discussion

Our study results partly confirmed the results reported by other authors. According to many researchers, a wide range of fungal species of the *Trichoderma* genus may be found in the substrate for button mushroom cultivation. These fungi do not have any significant effect on the button mushroom yield. Pathogenicity of: *T. viride, T. aureoviride, T. pseudokoningii,* and *T. hamatum* is relatively low (Fletcher and Gaze 2008). In contrast, strain Th2, which is now identified as *Trichoderma aggressivum* f. *europaeum*, is very aggressive (Seaby 1996; Williams *et al*. 2003).

Our study showed that the yield of the button mushroom strains was very strongly limited by the *T. aggressivum* f. *europaeum* isolate T. agg. 7/19. These findings are in agreement with the findings by Seaby (1996) and Williams *et al*. (2003). A study conducted by those authors showed that a wide range of *Trichoderma* isolates which are considered to be less aggressive, may cause a statistically significant yield reduction in different button mushroom strains.

Of the isolates of different investigated *Trichoderma* species, only the *T. atroviride* isolate T. at 11/2 caused no statistically significant yield reduction in the four button mushroom strains under analysis. The T. ham. 20/3 isolate of *T. hamatum* reduced yields in three of the analysed strains, but it did not have a significant effect on the yield of Amycel 2200. Our findings largely confirm the earlier findings of other authors who showed diversification in the yield of button mushroom strains grown on a substrate infested with different *T. aggressivum* f. *europaeum* isolates. Brown button mushroom strains responded with a considerably lower yield than white strains (Sobieralski *et al*. 2009). Other studies showed that *T. aggressivum* f. *europaeum* isolates always caused a reduction in the number of primordia, and a considerable yield decrease (Sobieralski *et al*. 2010).

There was considerable diversification in the strains under investigation, as they were grown in different decades in Poland. Our study indicated that in spite of considerable diversification, the responses of the strains were very similar. All of the strains under investigation responded with a very high yield reduction when infested with the T. agg. 7/19 isolate of *T. aggressivum* f. *europaeum* species. The response to all the other fungal isolates of the *Trichoderma* genus was very similar. Earlier studies showed diversified yields of the *Agaricus bisporus* strains grown in natural sites on a substrate infested with different *T. aggressivum* f. *europaeum* strains. The yield decrease ranged from 22.7% to 75% (Sobieralski *et al*. 2010).

To sum up, the findings of the study show that most fungal isolates of the *Trichoderma* genus reduced yields of the button mushroom strains to varying degrees. Only the T. at. 11/2 isolate of the *T. atroviride* species did not result in a statistically significant yield reduction in any of the four button mushroom strains.

Acknowledgements

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