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THE EFFECT OF INFESTATION WITH ISOLATES OF *Trichoderma* sp. ON MYCELIUM GROWTH AND YIELDING IN SINGLE-SPORE HETEROKARYOTIC CULTURES OF *Agaricus bisporus* (Lange) Imbach

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Abstract. The occurrence of fungi from the genus *Trichoderma* constitutes a serious problem in the culture of *Agaricus bisporus* leading to considerable losses. The aim of the investigations was to compare mutual interaction between some isolates of *Trichoderma* sp. and single-spore heterokaryotic cultures of *A. bisporus* on agar media and in cultivation conditions. The first stage of the study comprised assessment of growth for isolates of *T. harzianum*, *T. agrressivum*, *T. atroviride* and *T. hamatum* on PDA medium and growth of four heterokaryotic single-spore and strain Amycel 2600 *A. bisporus* cultures on peat-manure substrate. Moreover, the individual biotic effect (IEB) between isolates of *Trichoderma* and the tested *A. bisporus* cultures was also analysed. The second stage consisted in the evaluation of the volume of *A. bisporus* yield on the substrate, to which mycelia isolates of *Trichoderma* sp. were introduced. It was found that the analysed cultures and the cultivar strain of *A. bisporus* responded differently to the presence of *Trichoderma* sp. isolates both in terms of mycelium growth and the yield volume.

Key words: green mould, button mushroom, biotic effect, yield

INTRODUCTION

A major threat to common mushroom growing is connected with the occurrence of green mould caused by pathogenic fungi from the genus *Trichoderma* [Błażej and Tekiela 2002, Samuels et al. 2002, Kredics et al. 2010]. These fungi attack both mycelium and fruiting bodies of *A. bisporus* [Hermosa et al. 2000, Samuels et al. 2002],

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resulting in a deteriorated quality and a considerable loss of yields [Mumpuni et al. 1998, Sharma et al. 1999, Mamoun et al. 2000b, Savoie et al. 2001, Sobieralski et al. 2009a; 2010]. The sources of infection may include the substrate, casing soil or spawn [Błażej and Tekiela 2002, Tekiela 2005, Sobieralski et al. 2009b].

The occurrence of numerous *Trichoderma* species in different substrates (soil, compost, garden soil, forest soil, forest wood, mushroom compost, triticale kernel) at 49 sites in Poland has been reported by Błaszczyk et al. [2011]. As it was indicated by Hatvani et al. [2007], in recent years in Central Europe considerable losses in mushroom growing have been caused by *T. aggressivum* f. *europaeum*. The occurrence of this fungus species also in Polish mushroom plantations has been confirmed by studies by Szczech et al. [2008] and Sobieralski et al. [2009b]. Pudełko and Pyżalski [2010] identified – using molecular techniques – *T. aggressivum* f. *aggressivum*, a biotype widespread in North America [Ospina-Giraldo et al. 1999, Samuels et al. 2002].

As it was shown by numerous studies, an interaction occurs between *A. bisporus* and *Trichoderma* fungi [Mumpuni et al. 1998, Mamoun et al. 2000a, Savoie et al. 2001, Krupke et al. 2003, Savoie and Mata 2003, Williams et al. 2003, Largeteau and Savoie 2010]. However, the mechanism of interaction between these fungi has not been fully clarified [Savoie et al. 2001, Largeteau and Savoie 2010].

Single-spore cultures are characterised by greater variability that multi-spore cultures, which is used in production of new strains of *A. bisporus*. As a result of breeding work Fritsche [1972] and Kneebone et al. [1976] obtained single-spore cultures producing better yields than the original strain. Sobieralski et al. [2000] showed that single-spore cultures typically produce a lower number of primordia in comparison to the original strain, but they may exhibit faster mycelium growth in the casing soil and substrate. At present in order to produce new strains of *A. bisporus*, exhibiting low susceptibility to pathogens, biotechnological methods based on genetic modification are being applied with increasing frequency.

The aim of this study was to assess the interaction between isolates of *Trichoderma* sp. and single-spore heterokaryotic cultures of *A. bisporus* and to evaluate the effect of inoculation of the culture medium with tested pathogens on yielding in these cultures.

MATERIAL AND METHODS

The experiment was conducted at the laboratory of the Department of Vegetable Crops at the Poznań University of Life Sciences. During the first stage of the investigations, growth of four isolates of *Trichoderma* : *T. harzianum* T.7/23, *T. aggressivum* f. *europaeum* T.11/5, *T. atroviride* T.10/4, *T. hamatum* T.3/36 on the above-mentioned PDA medium (Oxoid Ltd., England) was determined. Their characteristics are presented in Table 1. Prior to use in analyses the above mentioned isolates were identified using molecular techniques, conducted at the Institute of Plant Genetics PAS in Poznań. The experiments were conducted using also four single-spore heterokaryotic cultures of *A. bisporus* (A.J./16/3, A.J./24/1, A.J./27/9, A.J./31/7) produced from the strain Amycel 2600 as well as the above mentioned original strain. Tested cultures came from the collection of cultivated mushrooms of the Department of Vegetable Crops, PULS. The

above mentioned cultures and the cultivated strain were grown on peat-manure agar medium. This medium was also used in the comparisons of their mycelium growth rates. The composition of the peat-manure medium and the method of their preparation are provided below.

Table 1. Charactericties of *Trichoderma* sp. isolates derived from Polish mushroom farms
Tabela 1. Charakterystyka izolatów *Trichoderma* sp. pochodzących z polskich pieczarkarni

Species Gatunek	Isolate designation Symbol izolatu	Year of isolate collecting Rok uzyskania izolatu	Place Miejscowość	Site of isolate obtaining Miejsce uzyskania izolatu
T. harzianum	T.7/23	2007	Kłoda (k. Leszna)	casing soil okrywa
T. aggressivum f. *europaeum*	T.11/5	2010	Jarocin	substrate podłoże
T. atroviride	T.10/4	2006	Wolsztyn	casing soil okrywa
T. hamatum	T.3/36	2008	Łobez	substrate podłoże

The substrate was produced by weighing 50 g manure and 5 g peat, which were flooded with 1dm^3 distilled water and boiled for 30 minutes. Next the entire volume was strained through a sieve in order to obtain the extract. The extract was supplemented with 1.5 g glucose and 22 g agar and made up to the volume 1dm^3 with distilled water. The substrate was sterilised in an autoclave, similarly as the PDA medium, for 20 minutes at a temperature of 121°C.

Inoculations were performed in a laminar-airflow cabinet by putting mycelial discs (5 mm diameter) of the examined *Trichoderma* isolate in the center of the medium in a Petri dish (9 cm in diameter). Discs were cut out from the PDA medium overgrown with the mycelium of the examined strains. Incubation was carried out in an incubator with no light access, at a temperature of 25°C and relative air humidity ranging from 80 to 85%. The diameter of the fungus colony was measured after 5 days of incubation. In order to compare mycelium growth of *A. bisporus* the above mentioned procedure was also followed; however, in this case the peat-manure medium was used and measurements were taken after 14-day incubation period.

In the course of the second stage of the experiment, the individual biotic effect (IBE) was estimated with the index of biotic relations developed by Mańka [1974]. For this purpose, mycelial discs of four *A. bisporus* cultures and the Amycel 2600 strain and competitive *Trichoderma* isolates were placed on a Petri dish with peat-manure medium at a distance of 4 cm from each other. Using a plastic pipe of 5 mm in diameter, the discs were cut out from PDA or peat-manure media overgrown with the mycelium of the examined fungi. The mycelial discs of examined *Trichoderma* isolates were inoculated for 7 days after the inoculation of the tested *Agaricus* cultures. Incubation was carried out under the conditions described above. Mycelium growth measurements of the investigated fungi were taken every 24 hours. Assessments of interactions between

the developing mycelia were determined. The observations recorded the degree of one colony surrounding the other, width of the inhibition zone, and growth limitation or infestation of one colony by the other. A precise description of the experimentation method was given by Frużyńska-Jóźwiak et al. [2011].

The experiment was established in six replications in a random design. Two series of the experiments were conducted.

In the growing experiment the culture medium was stage II substrate purchased at a company producing substrates for *A. bisporus* growing. The experiment was run in a climate chamber. Culture was grown in plastic boxes of 38 × 30 × 18 cm. Boxes were filled with 6 kg substrate and next spawned with grain mycelium of *A. bisporus*. The grain mycelium was prepared according to the formulation given by Lemke [1971]. Incubation was run for 12 days at a temperature of 25°C and relative humidity of 85–90%. Next substrates were inoculated with *Trichoderma* isolates. Substrates were infested with mycelia of isolates prepared on wheat grain at 100 cm^3 per culture box. Next the substrate was thoroughly mixed with mycelia of the tested isolates and subjected to further incubation at approx. 25ºC and relative humidity of 85–90%. Casing soil was prepared from highmoor peat, which was limed in order to obtain pH 7.5. The amount of added chalk was determined on the basis of the neutralisation curve. Casing soil was steam-disinfected at 70°C for 8 h. The layer of the casing soil, placed after the substrate had been completely overgrown with the mycelium, was 5 cm thick. Four independent experiments were conducted in the same design for the four analysed *Trichoderma* isolates. Fruiting bodies of *A. bisporus* were harvested for a period of 6 weeks. Yield of fruiting bodies comprised the weight of harvested fruiting bodies per 1 m^2 culture area. The control comprised growing of a culture or strain of *A. bisporus* run in a substrate free from *Trichoderma* isolates.

The experiments were established in a complete random design in four replications. Two culture cycles were run.

Results of studies on agar media were evaluated on the basis of mean values from two series, while the results of cultivation tests – from two culture cycles, respectively. Data were analysed using the analysis of variance for factorial experiments at $\alpha = 0.05$.

RESULTS AND DISCUSSION

On the basis of mycelium growth measurements for four isolates of *Trichoderma* cultured on PDA medium it was found that the fastest growth rate was observed for the isolate of *T. aggressivum* f. *europaeum* T.11/5, while slower growth was found in *T. harzianum* T.7/23. The slowest mycelium growth was recorded for isolates *T. hamatum* T.3/36 and *T.atroviride* T.10/4, between which no significant difference was observed (fig. 1).

Among the four compared heterokaryotic single-spore cultures of *A. bisporus* and the strain Amycel 2600 the fastest mycelium growth on the peat-manure medium was recorded for the strain Amycel 2600 and culture of A.J./24/1. A slower growth was recorded in cultures of A.J.27/9 and A.J./16/3. In turn, the slowest mycelium growth was found in case of culture of A.J./31/7 (fig. 2).

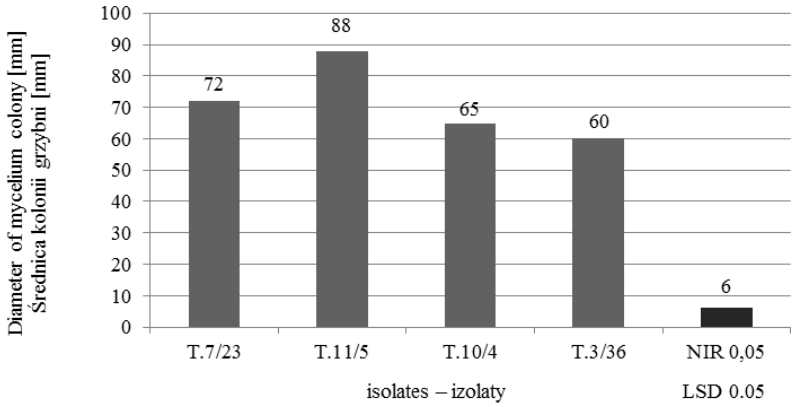

Fig. 1. Mycelium growth of four *Trichoderma* isolates on PDA medium, mm
Rys. 1. Wzrost grzybni czterech izolatów *Trichoderma* na pożywce PDA, mm

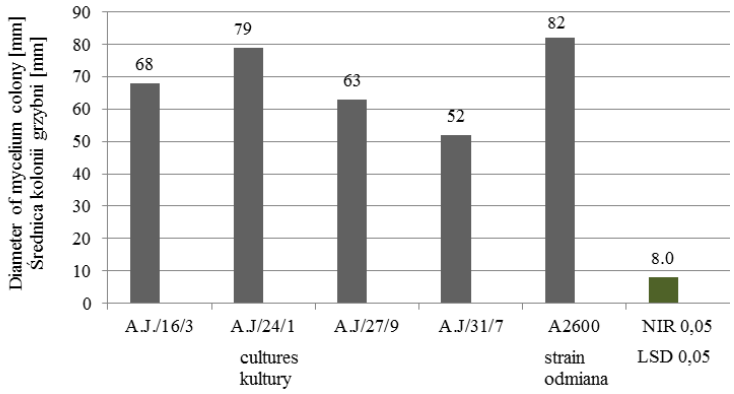

Fig. 2. Growth of four single-spore heterocaryotic cultures of *A. bisporus* and the strain Amycel 2600 on peat-manure medium, mm
Rys. 2. Wzrost czterech heteriokariotycznych kultur jednozarodnikowych i odmiany Amycel 2600 *A. bisporus* na pożywce obornikowo-torfowej, mm

Evaluation of the individual biotic effect (IEB) showed variation in interactions between the analysed isolates of *Trichoderma* and the cultures and the cultivated strain of *A. bisporus* (tab. 2). Values of IEB recorded in the course of the study ranged from +3 to +8. The highest IEB (+8) in relation to all the compared cultures and the strain of *A. bisporus* was found in case of isolate *T. aggressivum* f. *europaeum* T.11/5. In contrast, the weakest effect was observed for isolate *T. hamatum* T.3/36. The value of IEB in this case ranged from +3 to +4. Interactions between isolates of *T. harzianum* T.7/23 and *T. atroviride* T.10/4, and cultures and the strain of *A. bisporus* were similar. Here IEB ranged from +4 to +6. This confirmed results of studies by Mamoun et al. [2000a],

Table. 2. The value of the individual biotic effect (IEB) determining the effect of analysed isolates of *Trichoderma* on single-spore heterokaryotic cultures of *A. bisporus* and the strain Amycel 2600

Tabela 2. Wartość indywidualnego efektu biotycznego (IEB) określającego wpływ badanych izolatów *Trichoderma* na jednozarodnikowe heterokariotyczne kultury *A. bisporus* oraz rasę Amycel 2600

Trichoderma isolates Izolaty *Trichoderma*	Single-spore cultures – Kultury jednozarodnikowe				Strain Rasa
	A.J.16/3	A.J.24/1	A.J.27/9	A.J.31/7	A2600
T. harzianum T.7/23	+5	+3	+6	+4	+5
T. aggressivum f. *europaeum* T.11/5	+8	+8	+8	+8	+8
T. atroviride T.10/4	+6	+6	+5	+6	+5
T. hamatum T.3/36	+4	+3	+3	+4	+3

who showed that there is a chemical interaction between *A. bisporus* and *T. aggressivum,* as well as those reported by Williams et al. [2003], who showed that *T. aggressivum* may strongly inhibit growth and development of *A. bisporus*. Results of this study are also consistent with the claim by Krupke et al. [2003] that fungi from the genus *Trichoderma* produce metabolites inhibiting growth of *A. bisporus*. They also fully confirm results obtained by Mumpuni et al. [1998], who showed that secondary metabolites produced by the mycelium of *A. bisporus* stimulate growth of *T. aggressivum* f. *europaeum* and inhibit growth of *T. atroviride* and *T. harzianum*.

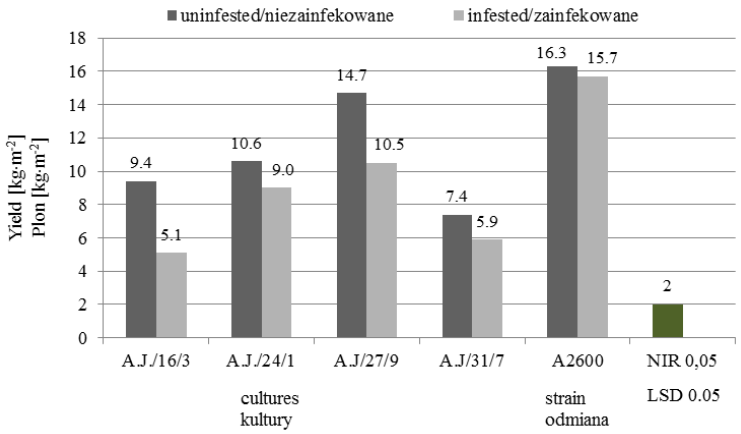

Fig. 3. Effect of substrate infestation with *T. harzianum* T.7/23 isolate on yield of four single-spore heterocaryotic cultures of *A. bisporus* and the strain Amycel 2600

Rys. 3. Wpływ infekcji podłoża izolatem *T. harzianum* T.7/23 na plon czterech jednozarodnikowych heterokariotycznych kultur oraz odmiany Amycel 2600 *A. bisporus*

As a result of the performed analyses it was found that the introduction of *Trichoderma* isolates to the growth medium has a significant effect on the yields of the tested cultures and a cultivation strain of *A. bisporus* (figs. 3–6). The response of the compared cultures and the strain of *A. bisporus* to substrate infestation was varied, although in all

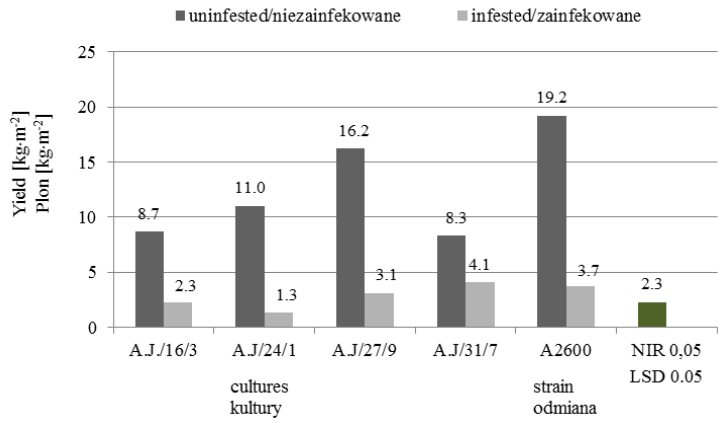

Fig. 4. Effect of substrate infestation with *T. aggressivum* T.11/5 isolate on yield of four single--spore heterocaryotic cultures of *A. bisporus* and the strain Amycel 2600

Rys. 4. Wpływ infekcji podłoża izolatem *T. aggressivum* T.11/5 na plon czterech jednozarodnikowych heterokariotycznych kultur oraz odmiany Amycel 2600 *A. bisporus*

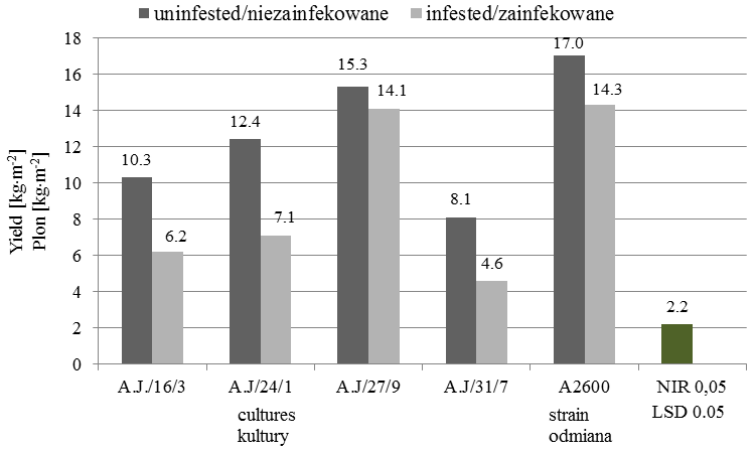

Fig. 5. Effect of substrate infestation with *T. atroviride* T.10/4 isolate on yield of four single--spore heterocaryotic cultures of *A. bisporus* and the strain Amycel 2600

Rys. 5. Wpływ infekcji podłoża izolatem *T. atroviride* T.10/4 na plon czterech jednozarodnikowych heterokariotycznych kultur oraz odmiany Amycel 2600 *A. bisporus*

cases the presence of *Trichoderma* isolates caused a reduction of yields. This confirmed the results of numerous studies which showed significant differences between strains of *A. bisporus* in their resistance to green mould [Mamoun et al. 2000b, Anderson et al. 2001, Chen et al. 2003, Sobieralski et al. 2009a].

It was found that the application of the isolate of *T. harzianum* T.7/23 significantly reduced yields in case of cultures *A. bisporus* A.J./16/3 and A.J./27/9 (fig. 3). In the other combinations the volume of yields from cultivation on the substrate infested with *T. harzianum* T.7/23 did not differ significantly from that on the substrate with no isolate.

The application of isolate *T. aggressivum* f. *europaeum* T.11/5 significantly reduced yields in all the compared cultures and the strain of *A. bisporus* (fig. 4). Differences in yields ranged from 4.2 to 15.5 kg/m^2. The weakest reaction to infestation of the substrate with *T. aggressivum* f. *europaeum* T.11/5 was found in case of culture A.J./31/7, while it was strongest in Amycel 2600. In the other combinations differences in yields ranged from 6.4 to 13.1 kg/m^2. Sharma et al. [1999] showed that a deterioration of the yield volume in *A. bisporus* grown on the substrate infested with *Trichoderma aggressivum* f. *europaeum* may amount to as much as 80%.

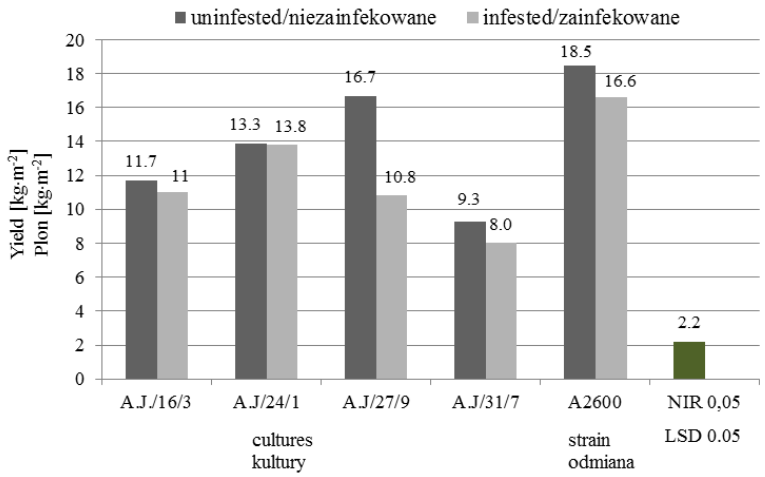

Fig. 6. Effect of substrate infestation with *T. hamatum* T.3/36 isolate on yield of four single-spore heterocaryotic cultures of *A. bisporus* and the strain Amycel 2600

Rys. 6. Wpływ infekcji podłoża izolatem *T. hamatum* T.3/36 na plon czterech jednozarodnikowych heterokariotycznych kultur oraz odmiany Amycel 2600 *A. bisporus*

A significant effect was also shown for the culture medium infestation with the isolate of *T. atroviride* T.10/4 on the level of yields in three compared cultures and the strain of *A. bisporus* (fig. 5). Only in case of culture A.J./27/9 no such effect was observed.

Lemmers [2010] mentioned e.g. *T. hamatum* among species pathogenic in relation to *A. bisporus*. Analyses conducted by the authors of this study showed that infestation of the culture medium with the isolate of *T. hamatum* T.3/36 had a significant effect on a reduction of yields only in case of *A. bisporus* A.J./27/9 (fig. 6).

As it was shown by the analyses, single-spore cultures of *A. bisporus* exhibit considerable variability in relation to the multi-spore cultures [Fritsche 1972, Kneebone et al. 1976]. Isolates of the genus *Trichoderma* applied in the analyses had a significant effect on mycelium growth and development as well as yields in *A. bisporus*. Response of the compared cultures and the strain to substrate infestation was markedly varied. This variability may be potentially used in breeding of new strains of *A. bisporus*, exhibiting low susceptibility to infestation with fungi from the genus *Trichoderma*.

CONCLUSIONS

1. Isolates of the analysed *Trichoderma* sp. differed in the mycelium growth rate on PDA medium. The fastest growth was recorded for the isolate of *T. aggressivum* f. *europaeum* T.11/5

2. Analysed single-spore cultures and the cultivated strain of *A. bisporus* differed in the mycelium growth rates. The fastest growth on the peat-manure substrate was found for mycelia of Amycel 2600.

3. Analysed isolates of *Trichoderma* to a varying degree limited mycelial growth in single-spore cultures and in the cultivated strain of *A. bisporus*. Mycelial growth of *A. bisporus* was inhibited most strongly by the isolate of *T. aggressivum* f. *europaeum* T.11/5.

4. Substrate infestation with *Trichoderma* isolates exhibited a varying negative effect on the yield of single-spore cultures and of the *A. bisporus* strain. The greatest yield reduction was caused by the isolate of *T. aggressivum* f. *europaeum* T.11/5.

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WPŁYW INFEKCJI IZOLATAMI *Trichoderma* sp. NA WZROST GRZYBNI I PLON JEDNOZARODNIKOWYCH HETEROKARIOTYCZNYCH KULTUR *Agaricus bisporus* (Lange) Imbach

Streszczenie. Występowanie grzybów rodzaju *Trichoderma* stanowi poważny problem w uprawie *Agaricus bisporus*, powodując znaczne straty. Badania miały na celu porównanie wzajemnego oddziaływania wybranych izolatów *Trichoderma* sp. i jednozarodnikowych heterokariotycznych kultur *A. bisporus* na pożywkach agarowych oraz w warunkach uprawy. W pierwszym etapie badań dokonano oceny wzrostu izolatów *T. harzianum*, *T. agrressivum*, *T. atroviride* i *T. hamatum* na pożywce PDA oraz wzrostu czterech heterokariotycznych kultur jednozarodnikowych i rasy Amycel 2600 *A. bisporus* na pożywce obornikowo-torfowej. Określono również indywidualny efekt biotyczny (IEB) pomiędzy izolatami *Trichoderma* oraz badanymi kulturami *A. bisporus*. W drugim etapie oceniono wielkość plonu *A. bisporus* na podłożu, do którego wprowadzono grzybnię izolatów *Trichoderma* sp. Stwierdzono, że badane kultury i rasa uprawna *A. bisporus* reagowały w sposób zróżnicowany na obecność izolatów *Trichoderma* sp. zarówno pod względem wzrostu grzybni, jak i wielkości plonu. Najsilniejszy hamujący wpływ na wzrost grzybni pieczarki miał izolat *T. aggressivum* f. *europaeum* T.11/5, który również powodował największą obniżkę plonu badanych kultur jednozarodnikowych i rasy uprawnej.

Słowa kluczowe: zielone pleśnie, pieczarka, efekt biotyczny, plon

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