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THE EFFECT OF SUBSTRATE INFESTATION WITH *Trichoderma* ISOLATES ON YIELDING OF *Pholiota nameko* (T. Ito) S. Ito et Imai

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Abstract. Green moulds caused by *Trichoderma* result in considerable losses in the growing of several species of cultivated and medicinal mushrooms. *Pholiota nameko* is a mushroom species well-known in many Asian countries. The aim of the studies was to determine the effect of substrate infestation with *Trichoderma* isolates on yielding of *P. nameko*. The experiment was conducted on four strains of *P. nameko*, i.e. PN01, PN04, PN05 and PN06. The substrate was infested with isolate T361, belonging to the species *T. aggressivum* f. *europaeum* and isolate Th14/5, belonging to the species *T. harzianum*. Infestation of the substrate with both isolates of *Trichoderma* resulted in a reduction of yield in the analysed strains of *P. nameko*. A markedly greater yield reduction was caused by infestation with isolate T361 than isolate Th14/5. Infestation of the substrate with the analysed *Trichoderma* isolates to a slight extent influenced mean weight of fruiting bodies in *P. nameko* and their morphological traits, such as cap diameter, and stem length and diameter. Substrate infestation with *Trichoderma* isolates had no effect on dry matter content of carpophores.

Key words: green moulds, carpophores, morphological traits, dry matter

INTRODUCTION

Pholiota nameko is a mushroom species well-known and highly praised by consumers in many countries of Asia [Royse 1996]. It is one of the most popular species grown in Japan [Yamanaka 2011]. In the wild it is found in certain regions of China, Japan and Taiwan [Stamets 2000]. The nutritive value of *P. nameko* is similar to that of other

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cultivated mushrooms. Its fruiting bodies contain considerable amounts of vitamins and minerals, particularly magnesium [Florczak et al. 2009] and phytosterol [Cho et al. 2009], as well as polysaccharides exhibiting anti-inflammatory action [Li et al. 2008]. These polysaccharides also reduce blood cholesterol concentration [Li et al. 2010]. Compounds contained in the fruiting bodies of *P. nameko* also exhibit a bactericidal and antitumour activity [Dulger 2004, Zhang et al. 2009]. Yielding of this mushroom species depends on many factors, e.g. the applied cultivation method and growing conditions, as well as the used culture medium [Chen et al. 2000, Oei 2003].

Studies conducted in research centres in different countries worldwide aim at the development of cultivation technology for this mushroom, which would ensure high yields of fruiting bodies while utilising waste produced by agriculture and food processing, as well as other waste materials [Krishna and Sharma 1989, Pawlak and Siwulski 1999, Cha et al. 2010, Siwulski et al. 2010]. In order to obtain high yields of *P. nameko* it is necessary to maintain high moisture content of the culture medium [Stamets 2000, Sanchez 2010, Siwulski and Pawlak 2000]. Optimal moisture content of the culture medium in the cultivation of *P. nameko* should be 60–65% [Siwulski and Pawlak 2000], while humidity in the yielding period should amount to 90–95% [Stamets 2000]. The above-mentioned author stated that high humidity in the mushroom culture results in *P. nameko* being frequently infested by *Trichoderma* moulds. Green moulds cause considerable losses in the growing of several species of both cultivated and medicinal mushrooms [Sobieralski et al. 2009, 2010a, 2010b, Frużyńska-Jóźwiak et al. 2011]. Błaszczyk et al. [2011] characterised in detail 170 isolates of *Trichoderma* fungi found in Poland using morphological as well as molecular and phylogenetic methods.

Aggressive strains of *T. harzianum* were classified for the first time and described by Samuels et al. [2002]. That author classified an aggressive strain Th2 as *T. aggressivum* f. *europaeum* and Th4 as *T. aggressivum* f. *aggressivum*, respectively. The two above-mentioned strains exceed in terms of aggressiveness any other known strain of *T. harzianum* [Williams et al. 2003]. Szczech et al. [2008] in Polish mushroom farms identified the following isolates of *Trichoderma* fungi: *T. harzianum*, *T. artroviride*, *T. aggressivum* f. *europaeum* and *T. longibrachiatum*. Sharma and Vijay [1996] showed that the species *T. viride* causes losses in the cultures of *Pleurotus ostreatus*. The most recent studies indicated that two new fungal species from the genus *Trichoderma*, i.e. *T. pleuroticola* and *T. pleurotum*, have been found in the cultures of oyster mushrooms [Park et al. 2004a-c, Komoń-Żelazowska et al. 2007]. Investigations conducted in Poland confirmed that these species are also present in our country [Siwulski et al. 2011].

MATERIAL AND METHODS

The experiments were conducted on four strains of *P. nameko*, i.e. PN01, PN04, PN05 and PN06, coming from the mycological collection of the Department of Vegetable Crops, the Poznań University of Life Sciences as well as two isolates of *Trichoderma* denoted as T361 and Th14/5, which were isolated from the substrate used in the cultivation of *A. bisporus*. Identification of isolate T361, belonging to the species *T. aggressivum* f. *europaeum*, was performed at the Vienna University of Technology.

Isolate Th14/5, belonging to the species *T. harzianum* came from the mycological collection of the Department of Vegetable Crops, the Poznań University of Life Sciences and it was identified at the Institute of Plant Genetics, the Polish Academy of Sciences in Poznań. Identification was performed using morphological observations and evaluations of mycelial culture growth, as well as molecular analyses using the ITS1 and ITS2 sequences of rRNA coding genes.

Experiments were conducted on the beech sawdust substrate enriched with a 25% addition of wheat bran in relation to sawdust dry weight. The substrate was wetted with distilled water to a moisture content of 70% and placed in polypropylene bottles of 1 dm^3. Each bottle contained 600 g substrate. A hole of 1 cm in diameter and depth of 12 cm was made in the substrate in the central point of its upper surface in order to place the mycelium of *P. nameko*. Bottles after being sealed with caps with a polypropylene filter were sterilised for 1.5 h in an autoclave at a temperature of 121°C. Inoculation was run when the substrate temperature decreased to approx. 24°C.

In the first stage of the experiments the substrate was spawned with granular mycelium of the analysed strains of *P. nameko.* Granular mycelium was produced on wheat grain using a method presented by Lemke [1971]. Mycelium was placed in a previously prepared hole in the substrate. Approximately 5g mycelium were used per 1 bottle. Incubation was run at a temperature of 25°C and relative humidity of 85–90%, with no access of light. After 14 days of incubation the substrate was spawned with mycelia of the analysed *Trichoderma* isolates, for each isolate separately. For this purpose two holes of 0.5 cm in diameter and 4 cm in depth, spaced at 4 cm, were made in the upper surface of the substrate. A total of 10 wheat kernels overgrown with mycelia of the tested isolates were introduced into each of the holes. Granular mycelia of *Trichoderma* isolates were produced in a similar manner as in case of *P. nameko*. Infested substrates were subjected to further incubation under the conditions given above. After the substrate was completely overgrown with the mycelium of *P. nameko* bottles for the fruiting period were placed in a conditioned chamber, in which a temperature of 15–16°C and humidity of 90–95% were maintained. The culture was aired so that the concentration of CO_2 in the air did not exceed 0.1%, while lighting was provided for 10 h a day. Fluorescent Day-Light lamps were used and light intensity was 500 lx.

Fruiting bodies were harvested successively as they were ripening, at the time the membrane under the cap was breaking and the cap margin was straightening. Fully grown fruiting bodies were cut off with a narrow knife directly at the substrate surface. Yield weight comprised fruiting bodies with stems. Fruiting bodies from the substrates uninfested with *Trichoderma* isolates, constituting the control, were harvested for a period of four weeks. In turn, mushrooms from infested substrates were harvested until the time fruiting bodies appeared. The yield volume was determined and biometric measurements were taken on fruiting bodies. Determinations were conducted on a sample comprising 10 randomly selected fruiting bodies. Cap diameter, the length and diameter of the stem as well as mean weight of fruiting bodies were determined. Moreover, dry matter content was determined in fruiting bodies by gravimetry. Initially fruiting bodies were dried at a temperature of 50°C for 8 h and next they were forced dried to constant mass at 80°C.

The experiment was established in a random block design in eight replications, with two culture cycles. Experimental results were analysed as mean values from two yielding cycles. The analysis of variance for factorial experiments was applied at $\alpha = 0.05$.

RESULTS AND DISCUSSION

When analysing the effect of infestation with isolate Th14/5 *T. harzianum* on yielding in *P. nameko* strains it may be stated that infestation in case of all the strains caused a significant reduction of yields. The greatest decrease in yield, by over 50% in relation to the control, was found in case of strain PN05. In contrast, in the other strains the reduction of yield was much smaller (fig. 1). A markedly greater reduction of yield was recorded when analysing yielding of *P. nameko* on the substrate infested by isolate T361 of *T. aggressivum* f. *europaeum*. In case of strains PN04 and PN06 yields obtained on the infested substrate amounted to only approx. 30% of the yield produced on the control substrate, while in case of strain PN01 the yield was by 50% lower. The smallest reduction of yield on the substrate infested by this isolate was observed for strain PN05 (fig. 2).

There is practically no data in available literature on the effect of *Trichoderma* fungi on yielding and morphological traits of fruiting bodies in *P. nameko*. Thus analyses of recorded results may be based on studies by other authors concerning interactions between species of cultivated mushrooms and *Trichoderma* fungi infesting them. A sig-

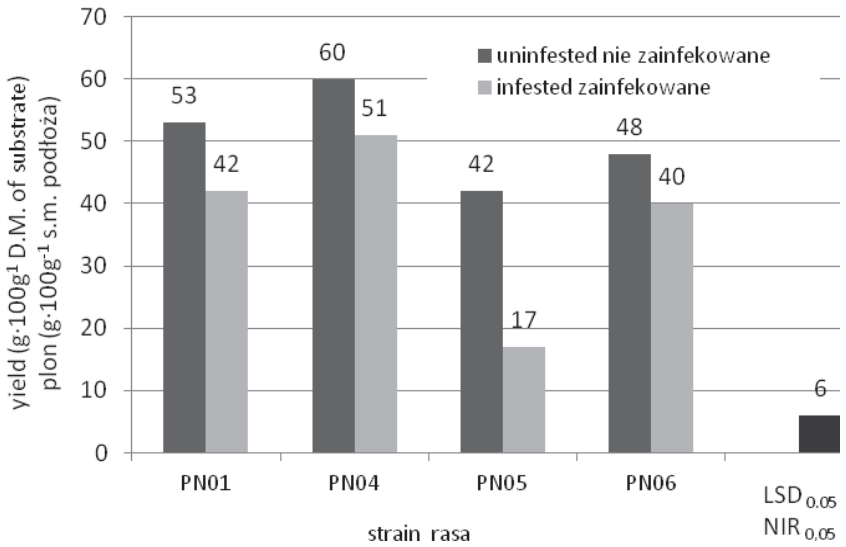

Fig. 1. Yield of *P. nameko* on the substrate infested with *T. harzianum* Th14/5
Ryc. 1. Plon łuskwiaka nameko na podłożu zainfekowanym *T. harzianum* Th14/5

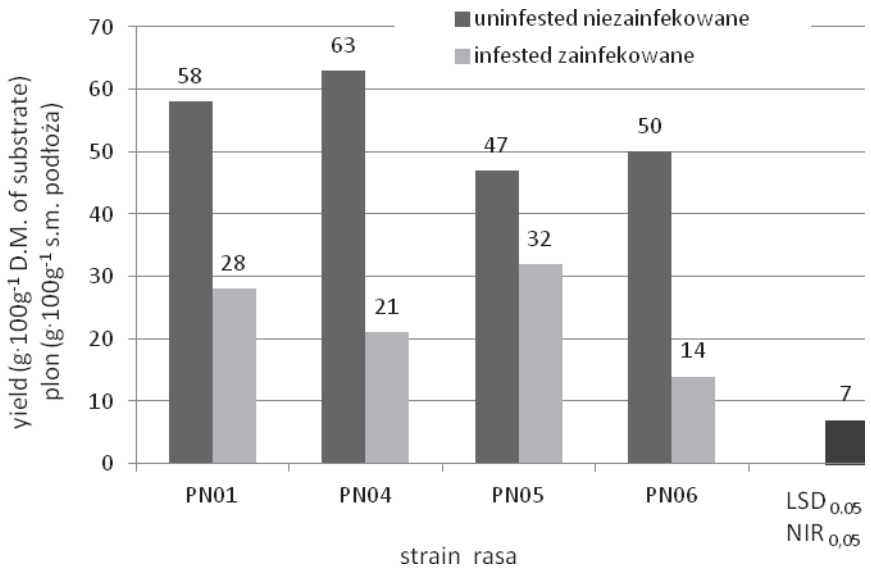

Fig. 2. Yield of *P. nameko* on the substrate infested with *T. aggresivum* f. *eurpaeum* T361
Ryc. 2. Plon łuskwiaka nameko na podłożu zainfekowanym *T. aggresivum* f. *eurpaeum* T361

nificant biotic reaction was shown between mycelium of *Agaricus bisporus* and *T. aggressivum* [Mamoun et al. 2000]. Williams et al. [2003] stated that a limitation of mycelium growth and development in *A. bisporus* by *Trichoderma* fungi occurs as a result of the their formation of pathogenic metabolites inhibiting growth of *A. bisporus* mycelium. Such a thesis was confirmed by the results of other studies [Mumpuni et al. 1998]. Investigations conducted by the above mentioned authors showed that secondary metabolites produced by mycelium of *A. bisporus* stimulated growth of *T. aggressivum* f. *europaeum*, while they inhibited growth of *T. atroviride* and *T. harzianum*. The authors of this study stated that *P. nameko* responds in a varied manner to two *Trichoderma* isolates. A much greater reduction of yield was caused by *T. aggressivum* f. *europaeum* in comparison to *T. harzianum*. Moreover, it was shown that the tested strains of *P. nameko* differed in their response to substrate infestation with the above mentioned *Trichoderma* species. In studies conducted by other authors differences were found in the resistance of *A. bisporus* strains to different *Trichoderma* species [Mamoun et al. 2000, Sobieralski et al. 2009]. Investigations conducted recently by Sobieralski et al. [2010c] showed that in all the tested *A. bisporus* strains infestation of the culture medium with isolates of *T. aggressivum* caused a reduction of fruiting body setting or led to their complete absence. Earlier studies conducted by Frużyńska-Jóźwiak et al. [2011] showed an effect of *Trichoderma* isolates on the development of mycelia in wild strains of *Coprinus comatus*. It was stated that *T. aggressivum* f. *europaeum* to a much greater extent limited the development of mycelia in *C. comatus* than *T. longibrachia-*

tum or *T. atroviride*. Mycelium of *C. comatus* showed an antagonistic reaction towards two isolates of *T. aggressivum* f. *europaeum*, i.e. CBS 115901 and T361. Information may be found in literature indicating that certain species of cultivated mushrooms, such as e.g. *Lentinula edodes*, *Pleurotus ostreatus* and *Pleurotus eryngii* [Savoie et al. 2001], may to a certain degree exhibit an antagonistic reaction towards different *Trichoderma* species.

Analyses showed that infestation with *Trichoderma* isolates to a limited degree influenced mean weight of fruiting bodies in *P. nameko* (figs 3, 4). Only in case of strains PN05 and PN06 a reduction was shown in the mean weight of fruiting bodies grown on the substrate infested with *T. aggressivum* f. *europaeum* isolate T361 in comparison to fruiting bodies from the control substrate. The mean weight of fruiting bodies in *P. nameko* on the uninfested substrate and substrate infested with *T. harzianum* isolate Th14/5 was similar.

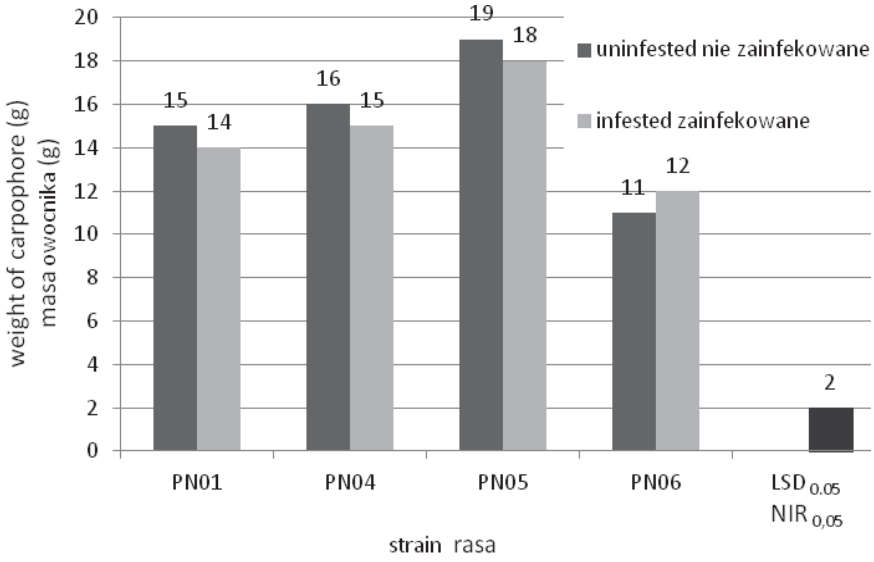

Fig. 3. Mean weight of *P. nameko* carpophore on the substrate infested with *T. harzianum* Th14/5

Ryc. 3. Średnia masa owocnika łuskwiaka nameko na podłożu zainfekowanym *T. harzianum* Th14/5

When analysing biometric measurements of fruiting bodies (figs 5, 6) it may be stated that infestation of the substrate with *Trichoderma* isolates to a limited extent influenced morphological characteristics of fruiting bodies. Fruiting bodies of *P. nameko* harvested from the substrate infested by *T. harzianum* isolate Th14/5 did not differ in cap diameter from fruiting bodies coming from the uninfested substrate. The response of the analysed *P. nameko* strains was similar in this respect. It may also be

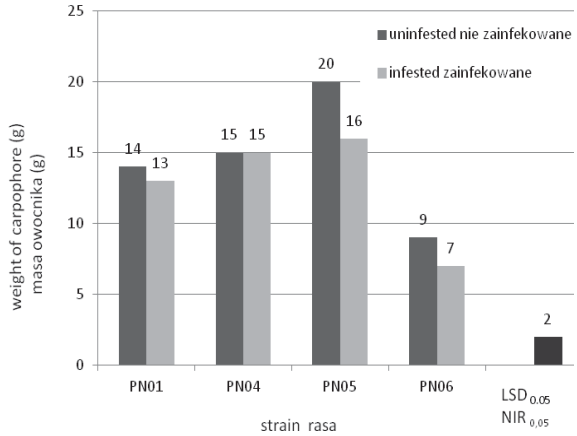

Fig. 4. Mean weight of *P. nameko* carpophore on the substrate infested with *T. aggresivum* f. *eurpaeum* T361

Ryc. 4. Średnia masa owocnika łuskwiaka nameko na podłożu zainfekowanym *T. aggresivum* f. *eurpaeum* T361

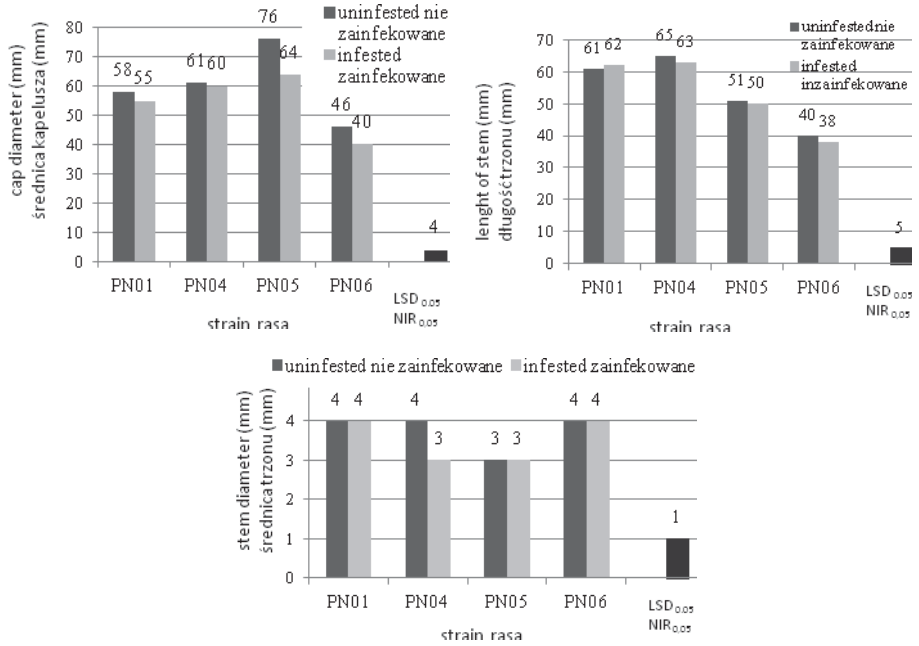

Fig. 5. Morphological features of *P. nameko* carpophores on the substrate infested with *T. harzianum* Th14/5

Ryc. 5. Cechy morfologiczne owocników łuskwiaka nameko na podłożu zainfekowanym *T. harzianum* Th14/5

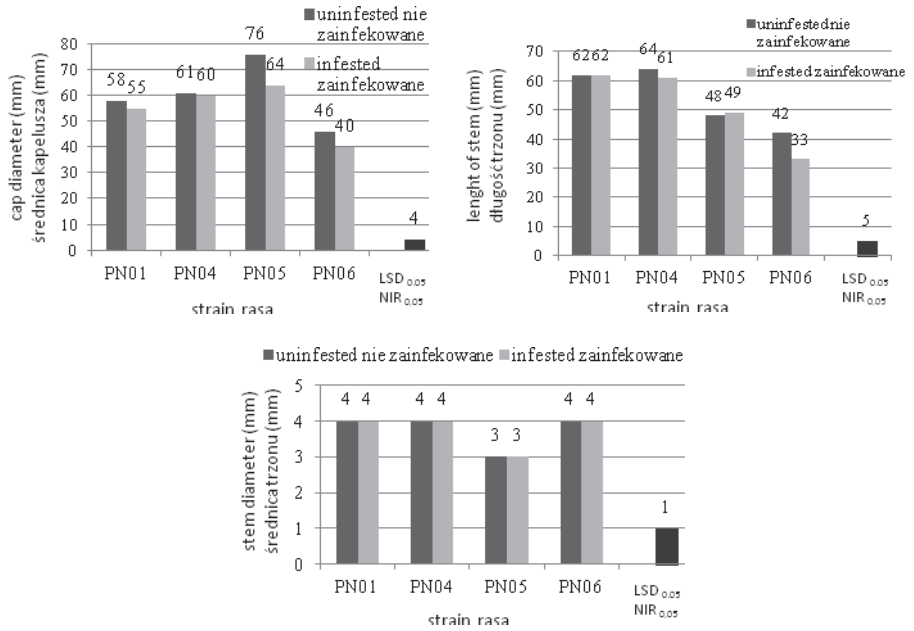

Fig. 6. Morphological features of *P. nameko* carpophores on the substrate infested with *T. aggressivum* f. *europaeum* T361

Ryc. 6. Cechy morfologiczne owocników łuskwiaka nameko na podłożu zainfekowanym *T. aggressivum* f. *europaeum* T361

stated that fruiting bodies of strain PN05 were characterised by a greater cap diameter in comparison to the other strains, irrespective of the substrate from which they were harvested (infested or unifested). The other strains in each case had similar cap diameters of their fruiting bodies. Substrate infestation by *T. aggressivum* f. *europaeum* isolate T361 influenced cap diameter of fruiting bodies in strains PN05 and PN06. Fruiting bodies harvested from the uninfested substrate were characterised by a greater cap diameter in comparison to fruiting bodies from the substrate infested by the above mentioned isolate. In the two other strains no such effect was found. Differences between strains in terms of cap diameter in fruiting bodies, irrespective of the effect of infestation, were similar as in the previous case. Infestation of the substrate with *T. harzianum* isolate Th14/5 did not influence the length of stem in fruiting bodies of the analysed *P. nameko* strains. However, it was found that strains PN01 and PN04 produced fruiting bodies with longer stems than strain PN05, irrespective of substrate infestation. In turn, strain PN06 was characterised by the shortest fruiting body stem. Substrate infestation by *T. aggressivum* f. *europaeum* isolate T361 did not influence stem length in fruiting bodies of the analysed *P. nameko* strains, except for strain PN06. On the infested substrate strain PN06 produced fruiting bodies with shorter stems than on the uninfested substrate. Irrespective of substrate infestation strains PN01 and PN04 produced fruiting bodies with longer stems than the other strains. Substrate infestation with both *Tricho-*

derma isolates had no effect on stem diameter in fruiting bodies of analysed *P. nameko* strains, except for isolate Th14/5 and strain PN04. In the latter case fruiting bodies harvested from the substrate infested with this isolate had smaller diameters than fruiting bodies from the uninfested substrate.

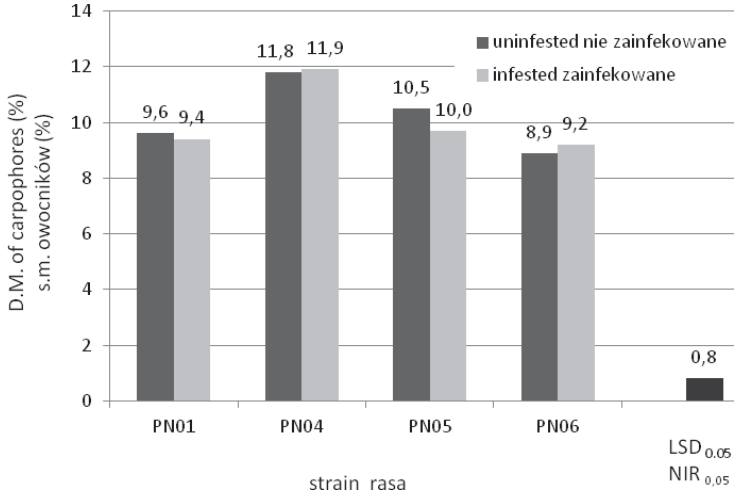

Fig. 7. Dry matter of *P. nameko* carpophores on the substrate infested with *T. harzianum* T14/5
Ryc. 7. Sucha masa owocników łuskwiaka nameko na podłożu zainfekowanym *T. harzianum* 14/5

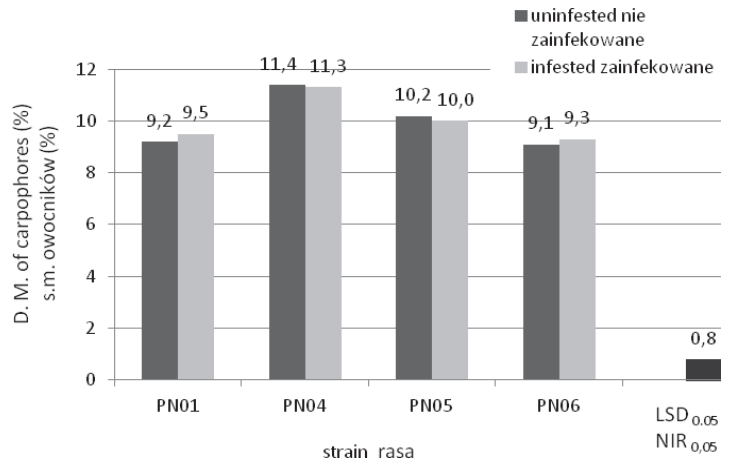

Fig. 8. Dry matter of *P. nameko* carpophores on the substrate infested with *T. aggressivum* f. *europaeum* T361
Ryc. 8. Sucha masa owocników łuskwiaka nameko na podłożu zainfekowanym *T. aggressivum* f. *europaeum* T361

Substrate infestation with *T. harzianum* isolate Th14/5 as well as *T. aggressivum* f. *europaeum* isolate T361 did not influence dry matter of *P. nameko* fruiting bodies. Dry matter contents of fruiting bodies harvested from infested substrate and from the uninfested substrate were similar. In contrast, it was stated that the analysed strains of *P. nameko* differed significantly in terms of dry matter content in their fruiting bodies, which fell within a wide range of values from 8.9 to 11.8% (figs 7, 8). The highest dry matter content was found for strain PN04, while it was lower in PN01 and PN05 and the lowest in PN06.

CONCLUSIONS

1. Infestation of the substrate with *T. harzianum* isolate Th14/5 and *T. aggressivum* f. *europaeum* isolate T361 resulted in a reduction of yield in the analysed strains of *P. nameko*. A markedly greater yield reduction was caused by infestation with isolate T361 rather than isolate Th14/5.

2. Infestation of the substrate with the analysed *Trichoderma* isolates to a slight extent influenced mean weight of fruiting bodies in *P. nameko* and their morphological traits, such as cap diameter, and stem length and diameter.

3. The analysed strains of *P. nameko* differed in dry matter content of their fruiting bodies and substrate infestation with *Trichoderma* isolates had no effect on this trait.

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WPŁYW INFEKCJI PODŁOŻA UPRAWOWEGO IZOLATAMI *Trichoderma* NA PLONOWANIE *Pholiota nameko* (T. Ito) S. Ito et Imai

Streszczenie. Zielone pleśnie powodowane przez *Trichoderma* są przyczyną znacznych strat w uprawie gatunków grzybów uprawnych i leczniczych. *Pholiota nameko* jest gatunkiem cenionym w wielu krajach azjatyckich. Celem badań było określenie wpływu infekcji podłoża uprawowego izolatami *Trichoderma* na plonowanie *Pholiota nameko*. W doświadczeniach użyto czterech ras *P. nameko*, tj. PN01, PN04, PN05 i PN06. Podłoże infekowano dwoma izolatami *Trichoderma*: T361 należącym do gatunku *T. aggressivum* f. *europaeum* oraz Th14/5 należącym do gatunku *T. harzianum*. Infekcja podłoża uprawowego izolatami Th14/5 oraz T361 powodowała zmniejszenie plonu badanych ras *P nameko*. Zdecydowanie większą obniżkę plonu powodowała infekcja izolatem T361 niż izolatem Th14/5. Infekcja podłoża uprawowego badanymi izolatami *Trichoderma* w nieznacznym stopniu wpływała na średnią masę owocników *P. nameko* oraz ich cechy morfologiczne, takie jak średnica kapelusza oraz długość i grubość trzonu. Infekcja podłoża uprawowego izolatami *Trichoderma* nie miała wpływu na suchą masę owocników.

Słowa kluczowe: zielone pleśnie, owocniki, cechy morfologiczne, sucha masa

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