IMPACT OF *TRICHODERMA PLEUROTUM* AND *T. PLEUROTICOLA* ISOLATES ON YIELDING OF *PLEUROTUS OSTREATUS* (FR.) KUMM.

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Abstract: The influence of substrate infestation with *Trichoderma pleurotum* and *T. pleuroticola* isolates on yielding of two commercial strains of *Pleurotus ostreatus* was investigated. The examined *P. ostreatus* strains growing on substrates infested with *Trichoderma* isolates showed a considerable yield decline. *T. pleuroticola* isolates were found to exert a greater unfavorable impact on *P. ostreatus* yields than *T. pleurotum* isolates. The performed experiments demonstrated that the response of the examined *P. ostreatus* strains to infestations with *T. pleurotum* and *T. pleuroticola* isolates was similar.

Key words: *Trichoderma* spp., *Pleurotus ostreatus*, strain, infested substrate, yield

INTRODUCTION

According to recent investigations, oyster mushroom cultivations are infested with two genetically closely related, though phenotypically quite different, *Trichoderma* species: *T. pleuroticola* and *T. pleurotum* (Kommon-Żelazowska *et al*. 2007). The above-mentioned species were found in mushroom cultivations and substrates in Europe, Iran and South America. *T. pleuroticola* species was found to occur in soil and wood samples obtained from different regions of Canada, the USA, Europe, Iran and New Zealand (Park *et al*. 2004a–c; Szekeres *et al*. 2005; Kommon-Żelazowska *et al*. 2007). A wide range of different species of green fungi were isolated from oyster mushroom cultivations in many countries including South America (Sharma and Vijay 1996), South Korea (Park *et al*. 2004, 2006), Italy (Woo *et al*. 2004), Hungary (Hatvani *et al*. 2007), Romania (Kredics *et al*. 2006) as well as in Spain (Gea 2009). Kommon-Żelazowska *et al*. (2007) demonstrated that, in the environment in which oyster mushroom occurs, *T. pleurotum* and *T. pleuroticola* isolates probably occupy various ecological and trophic niches. In the environment in which *Pleurotus ostreatus* occurs, a number of species of the *Trichoderma* genus were also identified. The most frequent of them included: *T. pleuroticola* as well as *T. harzianum*, *T. longibrachiatum* and *T. atroviride* (Kredics *et al*. 2009). The PCR marker developed in recent years, allows for a rapid method of identifying the two aggressive *Trichoderma* species found in oyster mushroom cultivations: *T. pleurotum* and *T. pleuroticola* (Park *et al*. 2006; Kredics *et al*. 2009).

The aim of our investigations was to determine the impact of substrate infestation with different *T. pleurotum* and *T. pleuroticola* isolates on yields of two commercial strains of *P. ostreatus*.

MATERIALS AND METHODS

The following two strains of *P. ostreatus* were used in the experiment: P80 and PX. The P80 strain is widely cultivated in Poland at the present time, whereas the PX strain was popular in the 1970s and 1980s. The *T. pleurotum* and *T. pleuroticola* isolates used in the experiments are shown in table 1.

The substrate employed in the trial was straw cut into 2–5 cm in length chaff. The experimental substrate was subjected to pasteurization with water steam of 90–95°C for a period of 1 hour, moistened with tap water to achieve a moisture content of 67 to 70% and then placed in perforated plastic foil bags. The bags were filled with 12 kg of substrate each. A hydraulic press was used for filling the bags. Substrate blocks in plastic foil bags measured 25x30x55 cm. Incubation took place in darkness, at a temperature of 18–21°C and with a relative air humidity of 80–85%.

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Table 1. List of *Trichoderma* isolates used in the studies

Species	Isolate designation	Identification
T. pleurotum *T. pleurotum*	E136 E139	Vienna University of Technology, Institute of Chemical Engineering, Division of Applied Biochemistry and Gene Technology
T. pleurotum	T12/B	Institute of Genetics Polish Academy of Science, Poznań
T. pleuroticola *T. pleuroticola*	M142 M143	Vienna University of Technology, Institute of Chemical Engineering, Division of Applied Biochemistry and Gene Technology
T. pleuroticola	T4/15/A	Institute of Genetics, Polish Academy of Science, Poznań

Mycelium of the examined oyster mushroom strains was prepared in the biological laboratory of the Department of Vegetable Science, Poznań University of Life Sciences and in the Cultivated Mushroom Spawn Farm in Łobez near Jarocin. The cultivation substrate was inoculated using grain mycelium prepared according to the recipe developed by Lemke (1971). Mycelium of the tested *T. pleuroticola* and *T. pleurotum* isolates was also prepared on wheat grain in the same way as the oyster mushroom mycelium. Infestation of cultivation substrates using the grain mycelium of *Trichoderma* isolates was carried out on day 12 of the oyster mushroom incubation. The cultivation substrate in plastic bags, was inoculated with the mycelium of the examined *Trichoderma* isolates. A 3 cm diameter tube with a piston was used to do the inoculating. Approximately 5 g of mycelium were introduced into the cultivation block to a depth of 9–11 cm. The mycelium was introduced in such a way as to achieve possible uniform distribution of the inoculum, by injecting it into 5 precisely defined places (corners and the centre) of the substrate block on each block side. A total of 50 g of grain mycelium of the above-mentioned *Trichoderma* isolates was used to inoculate each substrate block. Then, the blocks were incubated in darkness at a temperature of 21°C and 85–90% humidity. Incubation was conducted until the 21st day counting from the day of substrate inoculation with the oyster mushroom mycelium. After incubation, the substrate was transferred to the cultivation chamber where the temperature was kept at 13–15°C and relative air humidity – at 80–85%. The cultivation chamber was lit with fluorescent bulbs (Day-Light) with a lighting intensity of 500 l x for 10 hour a day periods.

In the described trials, only yields of the first flush were harvested because due to the infestation with fungi of the *Trichoderma* genus, no yields were harvested from the second flush in any of the experimental combinations. The control combinations were PX and P80 strains grown on non-infested substrate. Two cultivation cycles were carried out.

RESULTS

Yields of the PX strain growing on the non-infested substrate amounted to 173 g/kg fresh matter of substrate. The highest oyster mushroom yields on the substrate infected with *T. pleurotum* were recorded in the case of the E139 isolate (102 g/kg). The remaining two strains, *i.e.* T12/B and E136 caused significantly smaller but similar oyster mushroom yield losses. In the case of infestation with the above-mentioned isolates, oyster mushroom yields amounted to, respectively, 81 and 73 g/kg fresh matter of substrate. Yields obtained from the PX strain infested with the *T. pleuroticola* strain were considerably smaller. The highest oyster mushroom yield was observed in the case of infestation with M142 isolate (55 g/kg). Oyster mushroom yields obtained on substrates infested with M143 (40 g/kg) and T4/15/A (36 g/kg) isolates were similar (Fig. 1).

The performed *P. ostreatus* yield analysis of the P80 strain on substrates infested with *T. pleurotum* and *T. pleuroticola* isolates revealed that isolates of both species caused considerable yield losses. Yields of the P80 strain on the non-infested substrate reached the level of 195 g/kg. The highest yield of the P80 strain on the infested substrate was recorded in the case of infestation with the *T. pleurotum* E136 isolate (88 g/kg). Significantly lower yields of the P80 strain were achieved on substrates infested by T12/B (70 g/kg) and E139 isolates (59 g/kg) of the above-mentioned species. Significantly lower yields of the P80 strain were observed in the case of the infes-

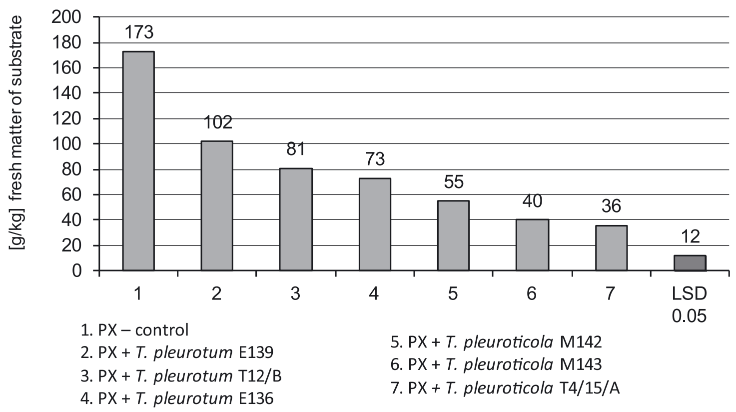

Fig. 1. Yield of *P. ostreatus* PX strain on substrate infested with *T. pleurotum* and *T. pleuroticola* isolates

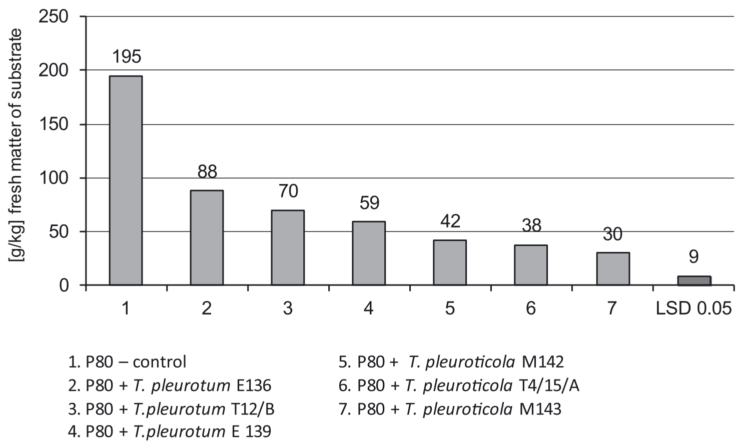

Fig. 2. Yield of *P. ostreatus* P80 strain on substrate infested with *T. pleurotum* and *T. pleuroticola* isolates

tation of the substrate with isolates of the *T. pleuroticola* species. The highest yield on the substrate infested with this species was recorded in the case of the M142 isolate (42 g/kg) while in the case of the T4/15/A isolate, the yield was lower but not significantly different (38 g/kg). The oyster mushroom yields on the substrate infested with the M143 isolate were similar to those recorded for the substrate attacked by the T4/15/A isolate but significantly lower in comparison to the substrate infestation with the M142 isolate (Fig. 2).

Yield drop percentage analysis of the PX strain of the *P. ostreatus* growing on substrates infested with *T. pleurotum* or *T. pleuroticola* showed that it varied significantly depending on the isolate used to infest the substrate. Substrate infestation with the *T. pleuroticola* isolate caused high yield losses of the PX strain which, in the case of the T4/15/A isolate were the highest (79.2%), while in the case of the M142 isolate – the lowest (68.2%). Yield reduction in the case of substrate infestation with two isolates of *T. pleurotum* were similar and amounted to 57.8% when the substrate was attacked by the E136 isolate and 53.2% in the case of the T12/B isolate. The smallest yield drop of the PX strain amounting to 41% was determined when the substrate was infested with the E139 isolate of this species (Table 2).

Table 2. Yield reduction [%] of *P. ostreatus* PX strain on substrate infested with *T. pleurotum* and *T. pleuroticola* isolates

Strain + *Trichoderma* sp. isolate	Yield reduction [%]
PX + *T. pleuroticola* T4/15/A	79.2
PX + *T. pleuroticola* M143	76.9
PX + *T. pleuroticola* M142	68.2
PX + *T. pleurotum* E136	57.8
PX + *T. pleurotum* T12/B	53.2
PX + *T. pleurotum* E139	41.0

The performed analysis of the percentage of *P. ostreatus* yield losses of the P80 strain cultivated on substrates infested with *T. pleurotum* and *T. pleuroticola*, revealed that infestation with the above-mentioned isolates caused very significant yield drops. Substrate infestation with *T. pleuroticola* isolates led to high yield losses. The highest yield loss of the P80 strain was determined in the case of the infestation of the substrate with the M143 isolate (84.6%) and the smallest – when the substrate was treated with the M142 isolate (78.5%). Substrate infestation with the *T. pleurotum* isolate caused yield losses of the P80 strain ranging from 69.7% in the case of substrate infestation with the E139 isolate, to 54.9% in the case of the E136 isolate (Table 3).

Table 3. Yield reduction [%] of *P. ostreatus* P80 strain on substrate infested with *T. pleurotum* and *T. pleuroticola* isolates

Strain + *Trichoderma* sp. isolate	Yield reduction [%]
P80 + *T. pleuroticola* M143	84.6
P80 + *T. pleuroticola* T4/15/A	80.5
P80 + *T. pleuroticola* M142	78.5
P80 + *T. pleurotum* E139	69.7
P80 + *T. pleurotum* T12/B	64.1
P80 + *T. pleurotum* E136	54.9

DISCUSSION

The performed trials showed that fungi of the *Trichoderma* genus, *i.e. T. pleurotum* and *T. pleuroticola* species caused significant losses of *P. ostreatus* yields. It can be said that *T. pleuroticola* isolates resulted in greater yield losses.

There is no information in the available literature regarding the influence of substrate infestation with *Trichoderma* isolates on *P. ostreatus* yields. Earlier investigations conducted by the authors concerning the impact of *T. aggressivum* f. *europaeum* showed that *Agaricus bisporus* strains are characterised by different resistance to the infestation with the above-mentioned *Trichoderma* species. We found that brown strains responded to the infestation with lower yield losses than white strains (Sobieralski *et al*. 2009). The performed experiments failed to demonstrate significant differences in the response to *T. pleurotum* and *T. pleuroticola* infestation between the two examined *P. ostreatus* strains. The P80 strain yield cultivated on the substrate not infested with *Trichoderma* isolates, was slightly higher than the yield of the PX strain. Nonetheless, yield losses in the case of both species infested with *T. pleurotum* and *T. pleuroticola* isolates were very similar.

The performed experiments confirmed the results of earlier studies regarding yield losses caused by aggressive forms of *Trichoderma* in mushroom cultivations. Investigations carried out in recent years revealed that fungi of the *Trichoderma* genus can significantly reduce yields of *Coprinus comatus* (Frużyńska-Jóźwiak *et al*. 2011). The performed experiments confirmed that like *P. eryngii*, *P. ostreatus* also show poor resistance to infestations of the cultivation substrate by *Trichoderma* isolates as evidenced by considerable yield losses (Sobieralski *et al*. 2010). The comparison of the earlier obtained results concerning the influence of substrate infestation with *Trichoderma* isolates on *P. eryngii* yielding, with those recorded in the current study, makes it possible to conclude that the response was similar in the case of both species.

CONCLUSIONS

1. Infestation of the cultivation substrate with *T. pleurotum* and *T. pleuroticola* isolates caused significant yield losses of the examined strains of *P. ostreatus*.
2. Substrate infestation with *T. pleuroticola* isolates exerted a stronger negative impact on *P. ostreatus* yields than with the *T. pleurotum* isolate.
3. The observed response of *P. ostreatus* strains to the infestation of growing substrates with *T. pleurotum* and *T. pleuroticola* isolates was similar.

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