

# Harmful Competitors and diseases of *P. leurotus ostreatus* and their control measures

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**Abstract.** Extended cultivation of Pleurotus ostreatus in the same location leads to increased infection by harmful organisms, which results in reduced yield and quality of the mushrooms. This, in turn, decreases the profitability of production and may render mushroom growing an unviable industry [1-4]. In blocks infected with harmful competing microorganisms, the growth of *P. ostreatus* mycelium is initially very slow, and the blocks lose their quality due to the influence of foreign microorganisms. Extended cultivation of Pleurotus ostreatus in the same location has been observed to result in an increase in the incidence of harmful organisms, leading to a decrease in the yield and quality of the mushrooms. Experiments were conducted to investigate the impact of foreign microorganisms on the growth, development, and yield of Pleurotus ostreatus using cotton bolls, a substrate that has proven to be effective in the Republic. According to the results, the appearance of Trichoderma fungus in the substrate blocks was observed 7 days after sowing *P. ostreatus*, with colonies forming that were 2-3 cm in diameter. These indicators were observed in 12.7% of the total number of planted blocks. After 14 days, the percentage of blocks with Trichoderma lesions increased to 18.4%, with moderate damage being the most common.

## 1 Introduction

In some instances, up to 60% of the blocks are unsuitable for cultivation, and growth may not be observed at all. These findings are supported by relevant literature sources [1-7]. These results highlight the importance of effective management strategies, including proper substrate selection and rotation, to maintain optimal growing conditions for *P. ostreatus* and maximize yield and quality [4-7].

*Pleurotus ostreatus*, also known as the oyster mushroom, is one of the most popular edible mushrooms worldwide. It is a saprophytic fungus that grows on a wide range of lignocellulosic substrates, making it an important crop for mushroom growers. However, the cultivation of *P. ostreatus* is often challenged by various diseases and harmful competitors that can reduce yield and quality of the crop, leading to economic losses [8-12].

Harmful competitors are microorganisms that compete with *P. ostreatus* for nutrients, and can colonize and infect the substrate, causing the growth of the mushroom to slow

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down or stop completely. Some of the common competitors that infect *P. ostreatus* include bacteria, other fungi, and insects. Diseases caused by bacteria and fungi are also major concerns in the cultivation of *P. ostreatus*. One of the most common diseases is the one caused by the bacterium *Pseudomonas tolaasii* Paine, which results in slimy patches on the mushroom caps, making them unmarketable [3-6]. Other diseases caused by fungi include *Trichoderma*, which causes green mold, and *Verticillium*, which causes yellowing of the substrate and brown spots on the mushroom caps. To control these harmful competitors and diseases, several measures can be taken. One common method is to sterilize the substrate before inoculation with *P. ostreatus* spores [4-8]. Sterilization can be done by heat treatment or chemical treatment, which can kill most of the harmful competitors and spores of diseases. However, this method is time-consuming and costly.

Another approach is to use biological control agents, such as antagonistic fungi, bacteria, and viruses that can control the growth of harmful competitors and diseases. For example, the use of *Trichoderma* spp. as a biocontrol agent has been shown to effectively control green mold disease [2-5, 7]. In addition, proper hygiene and sanitation practices should be implemented in the growing environment, including the disinfection of equipment and the removal of infected blocks. Proper ventilation and temperature control can also help to reduce the risk of infections. Overall, the control of harmful competitors and diseases is crucial for the successful cultivation of *P. ostreatus*. The use of a combination of control measures can help to minimize losses and ensure a high-quality and profitable crop [6-9].

## 2 Materials and methods

Experiments were conducted to investigate the impact of foreign microorganisms on the growth, development, and yield of *Pleurotus ostreatus* using cotton bolls, a substrate that has proven to be effective in the Republic [1-5]. The efficacy of hydrothermal treatment in suppressing harmful foreign microorganisms on substrate blocks was also studied, with the method described in paragraph 4.3 being applied [2-6].

The main method for controlling harmful competitors and diseases in *Pleurotus ostreatus* cultivation is through the implementation of proper hygiene and sanitation practices. This involves regular cleaning and disinfection of the cultivation area, tools, and equipment used in the cultivation process [6-8]. Another important method is the use of a sterilized substrate for growing the mushrooms, which can prevent the growth of harmful organisms in the substrate. The substrate should be prepared under sterile conditions and treated with appropriate methods to eliminate any potential contaminants [6-9]. Additionally, the use of biological control agents such as *Trichoderma* spp., *Bacillus* spp., and *Pseudomonas* spp. can help to suppress the growth of harmful competitors and pathogens in the cultivation environment [10-12].

Moreover, farmers should be vigilant and closely monitor their mushroom cultivation for any signs of disease or infection. Any infected blocks should be removed and disposed of properly to prevent the spread of disease to other blocks [12]. Lastly, proper environmental control measures such as maintaining optimal temperature and humidity levels, adequate ventilation, and sufficient air exchange can also help to prevent the growth and spread of harmful organisms.

## 3 Results and discussion

Table 1 presents the effectiveness of hydrothermal treatment based on the substrate and the mycelium of *P. ostreatus* as well as the presence of foreign microorganisms. The results

show that hydrothermal treatment of substrates led to a yield of 68.8-76.2% of the total number of blocks seeded with *P. ostreatus*. These findings demonstrate the potential of hydrothermal treatment as a useful tool in managing harmful microorganisms in substrate blocks for *P. ostreatus* cultivation.

Heat treatment of cotton bolls used as a substrate killed microorganisms in the vegetative state, but did not affect a certain part of heat-resistant thermophilic and mesophilic microorganisms with protected spores. They began to grow after the substrate cooled down and the seed mycelium was planted.

**Table 1.** Species composition and characteristics of fungi isolated from the substrate on which *P. ostreatus* was grown.

#	View	Occurrence	Place the selection of the organism in the block	Ecologotrophic characteristic
1	<i>Alternaria alternata</i> (Fr.) Keissler	few	On the surface of the substrate in the bag after puncture	aerobe, active cellulose-decomposing species
2	<i>Aspergillus fumigatus</i> F.	so many	On the surface of the substrate in a bag near the seed mycelium	“_”
3	<i>A. flavus</i> Lk	few	On the surface and inside the substrate bag	“_”
4	<i>A. niger</i> V.Tiegh .	so many	“_”	“_”
5	<i>Cladosporium herbarium</i> (Pers.) Lk ex Fr.	a lot of	“_”	“_”
6	<i>Coprinus</i> sp.	a lot of	On the surface of the substrate in the bag after puncture	“_”
7	<i>Fusarium oxysporum</i> Schlecht . f. betae Stewart.	few	On the surface of the substrate in the bag after puncture	“_”
8	<i>Gliocladium roseum</i> Bain.	few	“_”	“_”
9	<i>Hormiscium stilbosporum</i> ( Cda ) Sacc .	“_”	“_”	“_”
10	<i>Mucor mucedo</i> (L.)Fr.	a lot of	On the surface of the substrate in the bag after puncture	aerobe, active sugar-decomposing species
eleven	<i>Neurospora sphaerica</i> ( Sacc .) Mason	few	“_”	aerobe, active cellulose-decomposing species

12	<i>Penicillium hirsutum</i> Bain.	a lot of	On the surface and inside the substrate bag	“_”
13	<i>Rhizopus nigricans</i> Err.	“_”	On the surface of the substrate in the bag after puncture	aerobe, active sugar-decomposing species
14	<i>Stemphylium radicum</i> (MD et.R.)	a lot of	On the surface of the substrate in the bag after puncture	aerobe, active cellulose-decomposing species
15	<i>Trichoderma viride</i> Pers.	so many	On the surface and inside the substrate bag	“_”
16	<i>Trichotecium roseum</i> Lk	a lot of	“_”	“_”

Upon investigation of substrates used for cultivation of *P. ostreatus*, harmful microorganisms were observed to develop rapidly when up to 20% of the substrate was affected. Their growth may be attributed to the thermal hydrolysis of lignocellulosic materials, which leads to the formation of easily digestible nutrients in the substrate. After 4-6 days of sowing *P. ostreatus* mycelium, 18-20% of the substrates showed the presence of harmful micromycetes. Green mold was observed on some blocks on days 9-11. The temperature was identified as the main factor that inhibits the growth of foreign microorganisms during the growth and development of *P. ostreatus* in the substrate. The presence of high humidity and anaerobic conditions in areas not occupied by *P. ostreatus* mycelium prevented its development, leading to overheating of the substrate and death of the fungal mycelium. Such areas also became a breeding ground for foreign microorganisms. Homogeneity of the substrate is crucial for successful cultivation, as non-uniformity of the substrate can lead to reduced yield and quality, as well as an increased risk of infection by harmful microorganisms.

Various fungal species that coat seed mycelium and substrates have been identified, belonging to different ecological groups, and their presence is dependent on their biological characteristics. During cultivation of *P. ostreatus*, the presence of species from the Mucor and Rhizopus families has been observed, which grow rapidly on monosaccharide nutrient media. These species tend to appear first in the substrate blocks and disappear after 4-8 days, when the simple sugar supply is exhausted. Other fungal species, such as *Cladosporium herbarium* and *Gliocladium roseum*, were also isolated from the substrate, as they have the ability to assimilate carbon compounds with a relatively complex structure. However, *P. ostreatus* mycelium usually out-competes these fungi after 7-12 days. Although these fungi do not significantly affect the development of *P. ostreatus*, if they spread extensively over the substrate, the toxins they release during their metabolism can cause an increase in substrate temperature, which can have an adverse effect on *P. ostreatus* mycelium development.

When using seed mycelium sown on the substrate, various fungal species including *Alternaria alternata*, *Aspergillus fumigatus*, *Gliocladium roseum*, and *Neurospora sphaerica*, formed molds around the grain, but did not significantly impact the growth of *P. ostreatus* and were eventually pushed out of the block. *Coprinus* sp., a macromycete, was a competitor for nitrogen and carbon sources, but was also eventually replaced by *P. ostreatus* mycelium. However, *Aspergillus niger*, *A. flavus*, and *Trichoderma viride* posed a significant threat to the development of *P. ostreatus* on the substrate in blocks. *T. viride* was particularly dangerous, forming green mold around the seed mycelium 6-8 days after

sowing on substrates in blocks. The colonies of *T. viride* developed, merged, and extruded the *P. ostreatus* mycelium, resulting in the death of *P. ostreatus* mycelium due to an increase in temperature in the block. *T. viride* remained active even in conditions of high humidity (70%) and low oxygen content.

The *Trichoderma* fungus isolated from *P. ostreatus*-infected blocks demonstrated specific cultural and morphological traits. Initially, the colonies appeared green, which turned into dark green with spore pad formation. The spores were separated by septa, and the phialides were slightly curved and convex. The conidia were spherical or ovoid, measuring 2.5-3.8 microns in size, and were dark green in color when clustered. Based on the Litvinov identifier, this species was identified as *Trichoderma viride* Pers.

Table 2 displays the biological features of *T. viride*, frequently isolated from *P. ostreatus*-inoculated blocks. The fungi were cultured on beer wort agar medium to compare their biological traits with *P. ostreatus*. *T. viride* spores demonstrated germination after 15-20 hours. After 24-32 hours of growth and germination, spores began to form in the spore-forming organs. The colony growth rate was observed at 2.6 cm per day.

**Table 2.** Comparative biological characteristics of *T. viride* and *P. ostreatus* fungi.

Indicators	<i>T. viride</i>	<i>P. ostreatus</i>
The growth rate of mycelium on wort agar at temperature 24°C, cm/day	2.6	0.3
Optimal substrate moisture content, %	68-72	62-78
Optimum temperature for mycelium growth in the substrate, C	26-37	24-26
Optimum medium pH	5.5-6.5	4.8-6.7

In a study, the growth rate of *Trichoderma viride* was found to be 8.7 times faster than that of *Pleurotus ostreatus*, indicating its ability to occupy the substrate quickly. The researchers isolated *Trichoderma* strains from substrates in a block and examined their nutritional requirements and their ability to degrade plant biopolymers. They observed the growth of *T. viride* on different substrates and also infected *P. ostreatus* planting blocks with the *Trichoderma* fungus in a controlled environment at the FUNGI Center of ToshDAU. The substrates used in the experiment included wheat and rice straw, cotton seed husks, cotton bolls, and stalks. These substrates were heat-treated and then placed in plastic bags of size 30x60 cm. A suspension of *T. viride* spores was planted on the sterile substrates, and seed mycelium of *P. ostreatus* was planted on blocks with the same substrate as a control. The results are presented in Table 3.

The experiment involved monitoring and counting the growth of *T. viride* and *P. ostreatus* fungi on the substrate. The growth of *T. viride* mycelium was assessed by measuring the colony diameter after 8 and 14 days. After 8 days, no growth was observed in any option, except for the cotton husks blocks. On day 14, the diameter of the colonies formed on the substrates was found to be the largest on the husk of cotton bolls (8.2 cm), while no growth was observed on cotton stems, and on other substrates, the diameter ranged between 0.6-1.5 cm. The detailed results are presented in Table 3.

**Table 3.** Development of *T. viride* on waste media with cellulose and lignin.

#	Raw materials from waste	Trichoderma fungus colony diameter, cm	
		8 days	14 day
1	wheat straw	-	0.9
2	rice straw	-	0.6
3	Shredded cotton stalks	-	-

4	Cotton bolls	-	1.5
5	Cotton seed husk	0.7	8.2

The appearance of Trichoderma fungus in the substrate blocks was observed 7 days after sowing *P. ostreatus*, with colonies forming that were 2-3 cm in diameter. These indicators were observed in 12.7% of the total number of planted blocks. After 14 days, the percentage of blocks with Trichoderma lesions increased to 18.4%, with moderate damage being the most common. Trichoderma colonies were found in places with higher moisture content. After 21 days, the damage increased to 28.3%, and severely damaged blocks were observed, leading to their removal and destruction. The rapid development of Trichoderma fungus was influenced by relatively high temperatures. Detailed results are presented in Table 4.

**Table 4.** Infestation of blocks with *P.ostreatus* trichoderma (*T.viride*)

#	Days of counting	The total infection of blocks with <i>P. ostreatus</i> , %
1	7	12.7
2	14	18.4
3	21	28.3
EKF 05 1.78		

The study analyzed the effect of covering *P. ostreatus* mycelium mushroom blocks with *T. viride* contamination of varying degrees on yield. The results presented in Table 5 showed that compared to blocks completely covered with fungal mycelium (control), yield losses with strain NK35 ranged from 34.5% to 94.1%, with R77 ranging from 34.6% to 87.7%, and with local strain 12 ranging from 41.5% to 90.2%. Partially covered blocks with fungal mycelium experienced delayed formation of primordia of fruiting bodies by 7-12 days compared to the control. The first harvest was collected on days 29-32, and the mass harvesting of the first crop began 5 weeks after planting the mycelium in blocks.

**Table 5.** Impact on the yield of oyster mushrooms contamination of the substrate *T.viride*

<i>T.viride</i> substrate contamination	<i>P.ostreatus</i> yield (per 10 kg of substrate), kg					
	H-35	Lost yield compared to control, %	R77	Lost yield compared to control, %	12	Lost yield compared to control, %
Healthy blocks completely overgrown with oyster mushroom (control)	8.4	-	8.1	-	8.2	-
Strongly ( more than 20% )	0.5	94.1	1.0	87.7	0.8	90.2
Medium (5-20%)	2.9	65.5	3.5	56.8	2.9	64.6
little (5% gacha)	5.5	34.5	5.3	34.6	4.8	41.5
		ECF 05 1.42 0.94 1.12				

## 4 Conclusions

It is evident that *T.viride* fungus develops in substrates containing cellulose and lignin, and requires easily digestible nutrients for growth. On the other hand, *P.ostreatus* fungus grows well in conditions of substrate decomposition with the formation of easily decomposing organic compounds and is adaptable to a wide range of temperatures and pH levels.

However, infections caused by bacteria and fungi are common in the cultivation of *P. ostreatus*, and the disease caused by the bacterium *Pseudomonas tolaasii* Paine is the most common. This suggests that proper sanitation and hygiene practices should be followed to prevent the spread of disease in *P. ostreatus* cultivation. Overall, understanding the growth requirements and potential diseases of these fungi can aid in their successful cultivation for various purposes.

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