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# *Trichoderma* species associated with the green mould disease of *Pleurotus ostreatus* and their sensitivity to prochloraz

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**Abstract**

The green mould disease causes serious economic losses in *Pleurotus ostreatus* crop worldwide and also in Italy, where prochloraz is the only chemical fungicide allowed to control the disease. The effectiveness of the doses 0.01, 0.05, 0.25 and 1.25 μL $L^{-1}$ (field dose) of prochloraz against colony growth rate and spore germination of *Trichoderma pleuroti*, *T. pleuroticola* and *T. guizhouense* strains on wheat straw extract agar plates were evaluated. Complete inhibition of *Trichoderma pleuroti* and *T. pleuroticola* growth was showed by field dose of prochloraz, and for *T. pleuroti* also by 0.25 μL $L^{-1}$. Complete inhibition of spore germination occurred for all *Trichoderma* strains at field dose, and at 0.25 μL $L^{-1}$ for *T. pleuroti* strains. In *in vivo* assay, the effect of prochloraz doses 0.05, 0.25 and 1.25 μL $L^{-1}$ on colonisation of straw substrate by *T. pleuroti*, *T. pleuroticola* and *T. guizhouense* inoculated at two spore density ($1\times10^2$ and $1\times10^5$ spores $mL^{-1}$) immediately after *P. ostreatus* spawn was studied. *Trichoderma pleuroti* and *T. pleuroticola* were both responsible of green mould disease, whereas *T. guizhouense* was not pathogenic. The highest colonisation rate was showed by *T. pleuroti*, and only the field dose reduced its spread. *Trichoderma pleuroticola* colonisation was reduced by both doses 0.25 and 1.25 μL $L^{-1}$ of prochloraz. The study of *Trichoderma–Pleurotus* interaction type showed that *Trichoderma* species were active against the mycelial growth of *P. ostreatus* by competition for space and nutrients, and no parasitism and antibiosis occurred.

## Introduction

*Pleurotus ostreatus* (Jacq.) Kumm, commonly known as oyster mushroom, is one of the most commercially important edible mushroom worldwide. Italy, Hungary and Poland are the main producers in Europe (Błaszczyk e*t al*., 2013) . Many biotic diseases may cause significant yield losses in *P. ostreatus* farms. The green mould disease caused by *Trichoderma* species is one of the most serious problem in *P. ostreatus* crop. Typical symptoms of the disease are green sporulation

areas on the surface of cultivation substrate that is exposed to green mould infection mostly during spawn run. Massive attacks of the disease were reported in South Korea, where first significant losses were observed (Park *et al.*, 2006), Sri Lanka (Jayalal & Adikaram, 2007), Hungary (Hatvani, 2008), Croatia (Hatvani *et al.*, 2012), Romania (Kredics *et al.*, 2006), Spain (Gea, 2009), Poland (Sobieralski *et al.*, 2012). The disease was also reported in North America (Sharma & Vijay, 1996). Serious cases of the green mould were detected in *P. ostreatus* farms in Italy (Woo *et al.*, 2004; Alfonzo *et al.*, 2008; Woo *et al.*, 2009; Innocenti & Montanari, 2014). Studies by Park *et al.* (2006) and Komon-Zelazowska *et al.*, (2007) showed that two species of *Trichoderma*, *T. pleuroti* S. H. Yu & M.S. Park (2006; previously *T. pleurotum*) and *T. pleuroticola* S. H. Yu & M. S. Park (2006), are the predominant causal agents of *Pleurotus* green mould disease. They both belong to the *Harzianum* clade of *Hypocrea/Trichoderma,* which also includes *T. aggressivum* Samuels & W. Gams, the responsible of the green mould disease of *Agaricus bisporus* (Lange) Imbach (Hatvani *et al.*, 2007; Komon-Zelazowska *et al.*, 2007). Morphological studies have revealed that *T. pleuroticola* shows pachybasidium-like properties, characteristic of the *Harzianum* clade, while *T. pleuroti* possesses *Gliocladium*-like conidiophore morphology (Komon-Zelazowska *et al.*, 2007). *Trichoderma pleuroti* has been found only in the area of *P. ostreatus* cultivation (Kredics *et al*., 2009), in contrast, *T. pleuroticola* has been found in soil, plant debris or decaying wood in USA, Canada, New Zealand, Europe, India (Hatvani, 2008), and on basidioma surface of wild *P. ostreatus* (Kredics *et al.*, 2009). This suggests that the two species may occupy different ecological and trophic niches in nature (Hatvani, 2008). *Trichoderma pleuroti* and *T. pleuroticola* usually co-occurred in the cultivation substrate with no clear dominance of one or the other species (Hatvani, 2008), the latter was found to be more aggressive than *T. pleuroti* (Hatvani, 2008). The role of *T. harzianum* in the *P. ostreatus* green mould disease is still not clear. Woo *et al.* (2009) identified the majority of isolates pathogenic to *P. ostreatus* from Italian mushroom farms as *T. pleuroticola* and *T. harzianum* and less commonly as *T. pleuroti*. Hatvani *et al.* (2012) identified *T. pleuroti* and *T. pleuroticola* as causal agents of green mould disease from samples obtained from a Croatian farms.

In the study of Innocenti & Montanari (2014), *T. pleuroti* and *T. pleuroticola* were isolated from symptomatic areas of cultivation substrate, whereas *T. harzianum* was isolated only from disease symptomless areas.

The disease control is commonly based on the application of fungicides. Since studies on fungicide efficacy on cultivated mushrooms by agrochemical companies are rare, only few fungicides have been officially recommended. Currently, only prochloraz is allowed for use in mushroom farms in Italy. The few studies carried out on the sensitivity of *T. harzianum*, *T. pleuroti* and *T. pleuroticola* to fungicides, specifically to prochloraz, showed that *T. pleuroti* and *T. pleuroticola* were more sensitive than *T. harzianum* (Hatvani *et al.*, 2012; Innocenti & Montanari, 2014).

The objectives of the present work are to study: i) the role of *T. harzianum* in green mould disease; ii) the sensitivity to prochloraz of *Trichoderma* species pathogenic to *P. ostreatus* on wheat straw agarised medium; iii) the effect of prochloraz on green mould disease under conditions similar to those of mushroom farm; and iiii) *Trichoderma-P. ostreatus* interaction type.

## Materials and methods

### *Trichoderma* strains: origin and molecular identification

*Trichoderma* strains were isolated from wheat straw substrate of *P. ostreatus* from a mushroom farm located in Emilia Romagna, Italy, maintained in potato dextrose broth (PDB, Difco) added with 15% glycerol at -80 °C, and deposited in the culture collection of the Department of Agricultural and Food Sciences, University of Bologna under AFS codes.

A multiplex PCR assay with tef1 sequence-based primers FPforw1, FPrev1, PSrev1 was carried out under the conditions described by Kredics *et al.* (2009) to assess the belonging to the *T. pleuroticola/T. pleurotum* complex. Amplification of the fragments of tef1 gene was performed as follows: initial denaturation 5 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at 63 °C, 1 min at 72 °C, with the final extension of 10 min at 72 °C. Amplification products were separated by electrophoresis on 1.5% agarose gel in 1× TAE buffer at 100 V/cm for about 45 min, stained with

ethidium bromide and visualized under UV light. The presence of two bands of 447 pb and 218 pb respectively, indicates positivity to *T. pleurotum,* while a single band of 447 pb indicates positivity to *T. pleuroticola*. *Trichoderma pleurotum* was re-named *T. pleuroti* S.H. Yu & M.S. Park.

For the no *T. pleurotum/ T. pleuroticola* strains, DNA was extracted using the NucleoSpin Plant II kit (MACHEREY-NAGEL, Düren, Germany) from the mycelium of the mono-conidial colonies of *Trichoderma* following the manufacturer protocol. ITS region of ribosomal DNA was amplified by Polimerase Chain Reaction (PCR) using ITS1f and ITS4 as fungal primers (White *et al.*, 1990, Gardes & Bruns, 1993) . Sequencing of amplified ITS was performed at Macrogen Inc. (Korea) . Taxonomic identifications were performed comparing retrieved sequences with those available in the online databases provided by the National Centre for Biotechnology Information (NCBI) using the BLAST search program (Altschul *et al.*, 1997) . *Trichoderma harzianum* strains were thus identified. Since this species is now identified by sequencing the translation elongation factor 1-α (TEF1) (Chaverri *et al.*, 2015) a new characterization of the *T. harzianum* strains was carried out by sequencing a different fragment of TEF1 using the primers TEF1LLErev (Jaklitsch *et al.,* 2005) and EF1-728 F (Carbone & Kohn 1999) . Thus, our *T. harzianum* strains were identified as *T. guizhouense* Q.R. Li, McKenzie & Yong Wang bis.

The commercial *P. ostreatus* Spoppo (Sylvan Inc., Kittanning, PA, USA) widely cultivated in Italy was utilised. The fungi used and their symbols are reported in Table 1.

**Effect of prochloraz on *Trichoderma* colony growth and spore germination**

The experiment was conducted on wheat straw extract agar (WSEA, Rajendran *et al.*, 1991) amended with a range of five concentrations of fungicide, 0.01, 0.05, 0.25, 1.25 µL $L^{-1}$, poured into 9 cm plates (Da Silva & Neves, 2005) . The concentration range, dilution factor 1:5, was chosen considering the field dose 1.25 µL $L^{-1}$ as the maximum concentration. The fungicide was added to the medium after autoclaving at a temperature of approximately 40 °C. A 0.5 mm diam. plug from malt extract agar (MEA, Difco) actively growing colony of each fungus was inoculated in the

centrum of each plate. Untreated WSEA plates inoculated with each fungus acted as controls. Three plates (replicates) were used for each dose, and for the control. Plates were incubated at 25 °C in the dark in a completely randomised experimental design. Colony diameters were measured along two perpendicular axes two, four and six days after inoculation. Daily radial growth was then calculated (Reeslev & Kjoller, 1995).

In spore-germination studies, conidia were removed from 7-d-old colony of each Trichoderma isolate growing on MEA, by adding sterile water with Tween 80 to obtain the final concentration of $10^3$ spores $ml^{-1}$. An aliquot of 0.1 ml was spread on the surface of each 9 cm diam. WSEA plate amended with the fungicide as reported above. The percentage of spore germination was estimated 12 h after inoculation by using a Nikon ECLIPSE TE2000-E microscope (Nikon Instruments Europe BV, Amsterdam, Netherlands) at 600 × magnification. Spores were considered germinated when the germ tube length was equal to or greater than the spore width. Four replicates were performed.

***In vivo* assay**

In this assay PoSp, Tg 501, Tpa 432 and Tpi 492 inocula were used. PoSp commercial spawn consisted in sterile millet seeds colonised by the mycelium of the fungus. For *Trichoderma* inoculum, each isolate was cultured separately on MEA plates at 25 °C for four days in the dark, then under natural light, to obtain an abundant sporulation. The growing substrate was prepared by using the following technique similar to that used in mushroom farm. Chopped wheat straw (2-5 cm) was steam pasteurised at 90 °C for 1 h (Sobieralski *et al.*, 2012) and inoculated with the PoSp spawn (2% v/w). Immediately after spawn, prochloraz was applied to the substrate by adding water solutions of the fungicide corresponding to 0.05, 0.025 and 1.25 µL $L^{-1}$ doses. An amount of 100 g of substrate + PoSp was then distributed in each perforated transparent plastic food container (15×10×8 cm), and a water spore suspension of each *Trichoderma* strain was immediately added. Two density of *Trichoderma* ($1\times10^2$ and $1\times10^5$ µg $L^{-1}$) were used separately to simulate a low and a

massive attack by the pathogen. Each container was then wrapped in a plastic bag closed by a cotton plug. Infected controls consisted in containers with PoSp + *Trichoderma,* and uninfected controls consisted in containers with PoSp inoculum alone. The time of prochloraz treatment we used is comparable with that of the farm, where the fungicide is applied to substrate at spawn. Three replicates were performed for each treatment for a total of 84 containers which were located in a growth chamber following a complete randomized design at 23-25 °C and relative humidity at 80%. Four weeks later, during the spawn run phase, the colonisation of substrate by *Trichoderma* was visually assessed for each container using a six-point scale where: 0, no colonisation; 1, sporadic growth, few small green areas; 2, light growth, less than 20% of substrate colonized by green mould; 3, medium growth, 20-50% of colonized substrate; 4, heavy growth, more than 50% colonisation; 4, full substrate colonisation by green mould. The colonisation rate (index) was then calculated as the mean of the value of the replicates. At the end of the experiment, *Trichoderm*a was re-isolated from substrate and molecularly identified.

**Interaction between *Trichoderma* and *Pleurotus ostreatus***

To verify the mechanism of interaction between *Trichoderma* spp. and PoSp, dual cultures and volatile and non volatile metabolite techniques were utilised (Dennis & Webster, 1971a and 1971b; Innocenti *et al.*, 2015) . In dual culture assay, 5-mm diam. plugs from actively MEA growing colonies of PoSp were inoculated on WSEA plates, and 5-mm diam. plugs of *Trichoderma* were inoculated 40 mm apart in the same plates 48 h later. All combinations *Trichoderma*- PoSp were performed in triplicate. All plates were maintained at 25 °C in the dark. For measuring the competitive capacity of *Trichoderm*a *vs* PoSp, the colonisation of substrate by *Trichoderma* was evaluated 24, 48 and 72 h from inoculation using the method of Camporota (1985) with the formula (D1 × 100) /D2, where D1 is the distance covered by *Trichoderma* along the line which connects the two inoculated plugs, and D2 is the distance between the two plugs (40 mm) .

The hyphal interactions were studied on WSEA plates covered by a sterile cellophane layer (Safta, Piacenza, Italy) and inoculated as specified above with both fungi. After 24 h of incubation, the cellophane was removed and a portion of 1 $cm^2$ from interaction area was stained with 0.5% Trypan blu (1:1:1; lactic acid:glycerol:water), then inspected by light microscopy. The types of hyphal interaction were classified as coiling, penetration, vacuolation and coagulation of cytoplasm (Dennis & Webster, 1971c).

The ability of *Trichoderma* to produce non-volatile metabolite/s against Po Sp colony growth was determined by the method of Dennis & Webster (1971a). A 5 mm diam. plug of each *Trichoderma* strain was inoculated in the centrum of a sterile cellophane disc laying on 9 cm WSEA plate. After 24 h, the cellophane with the colony was removed and a 5 mm diam. plug of PoSp was inoculated in the centrum of the plate. Diameter of the PoSp colonies was determined after 48, 72 and 96 h, and compared with that of the PoSp grown on not inoculated *Trichoderma* plates. Four replicates for each treatment were considered.

The ability of *Trichoderma* to produce volatile metabolite/s against on PoSp mycelial growth was determined by the apparatus of Camporota (1985) constituted by two bottoms of WSEA plates overlapped and separated by an inox lid with a central hole closed by a sliding flap. One bottom was previously inoculated with a 5-mm plug of each *Trichoderma* strain separately, and the other with a 5 mm plug of PoSp. After 72 h of incubation in the dark at 24-25 °C, the hole was opened to allow volatile metabolites by *Trichoderma* to act against PoSp mycelium growth. Then, after 48, 72 and 96 h, the diameter of PoSp colonies was measured as specified above.

All *in vitro* and *in vivo* experiments were repeated once with similar results. The data of one experiment are reported.

## Statistical analysis

Data of colony growth rate at different prochloraz doses were analysed by two-way (two factors: fungal strain and fungicide dose) ANOVA, and compared by Student Neuman Keuls (SNK) test.

Data of *Trichoderma* colonisation rate were analysed by three-way (three factors fungal species, fungal density and prochloraz dose) ANOVA, and compared by SNK test. Statistical procedure was carried out with the software Statgraphic Plus version 2.1 (Statistical Graphics Corp., USA 1996).

## Results

### Effect of prochloraz on *Trichoderma* colony growth and spore germination

The colony growth rate of Tg, Tpa and Tpi strains at different prochloraz doses are reported in Table 2, 3 and 4, respectively. Two-way ANOVA indicated that for all *Trichoderma* the dose factor was significant ($P < 0.05$), whereas strain factor and the interaction between the two factors were not significant. For Tg 446 and Tg 501 the highest doses (0.25 and 1.25 μL $L^{-1}$) significantly reduced the mean colony growth rate by 22.3% (0.25 μL $L^{-1}$) and 86.7% (1.25 μL $L^{-1}$) respectively compared with untreated control (Table 2). All doses significantly reduced the mean colony growth rate of Tpa 432 and Tpa 488 strains, the field dose (1.25 μL $L^{-1}$) completely inhibited colony growth rate and 0.25 μL $L^{-1}$ dose reduced by 89.5%. The two lowest doses (0.05 and 0.01 μL $L^{-1}$) decreased colony growth rate of Tpa 432 and 488 strains by 45.4 and 19.0%, respectively (Table 3). For Tpi 492 and 497 all doses significantly reduced the mean colony growth rate. The field (1.25 μL $L^{-1}$) and 0.25 μL $L^{-1}$ doses completely inhibited colony growth rate. The two lowest doses (0.05 and 0.01 μL $L^{-1}$) decreased colony growth rate by 69.3 and 15.1%, respectively (Table 4). The effect of prochloraz on spore germination of *Trichoderma* strains is reported in Table 5. Complete inhibition of all *Trichoderma* strains occurred at field dose (1.25 μL $L^{-1}$) and at 0.25 μL $L^{-1}$ for Tpi strains. The spore germination of both Tpa strains gradually decreased from 0.05 to 0.25 μL $L^{-1}$, and of Tpi strains from 0.01 to 0.5 μL $L^{-1}$.

### *In vivo* assay

The *in vivo* assay showed that no symptoms of green mould disease were observed in all containers inoculated with Tg 501. Also in infected control containers where the fungus was inoculated at the highest dose and no treatment with prochloraz was carried out, no symptoms of disease were

observed. On the contrary, the growth of PoSp mycelium was optimal. However, colonies of the fungus developed from samples of infected control substrate plated on *Trichoderma* semi selective medium (Smith et al. 1990) at the end of the experiment. Molecular identification confirmed that these colonies belong to Tg species, indicating that this fungus is present, but it was not responsible of the disease. For this reason, Tg 501 data were not considered for statistical analysis. Multifactor ANOVA (Table 6) indicated that for Tpa 432 and Tpi 492 species (TS), concentration (TC), prochloraz dose (D) factors, and the interaction TS × D were significant ($P \leq 0.001$). Figure 1 shows the effect of any single factor on colonisation growth rate by *Trichoderma* species. As indicated in Fig. 1A, Tpi 492 strain colonized the substrate more efficiently than Tpa 432, and $1 \times 10^5$ spore $mL^{-1}$ concentration determined the highest colonisation (Fig. 1B). Independently of *Trichoderma* species and their spore concentration, 1.25 and 0.25 μL $L^{-1}$ fungicide doses significantly reduced substrate colonisation (Fig. 1C). Data of interaction between TS × D were compared in Table 7. The two *Trichoderma* species colonized the untreated substrate with different growth rate, 2.2 for Tpa 432 and 4.0 for Tpi 492. Upon prochloraz treatment, the growth of Tpi 492 was significantly inhibited only at field dose (1.25 μL $L^{-1}$), whereas that of Tpa 432 was inhibited both at 0.25 and 1.25 μL $L^{-1}$.

***Trichoderma –Pleurotus* interaction**

The substrate colonisation values of Tg 501, Tpa 432 and Tpi 492 in dual culture with PoSp are reported in Table 8. Tpi 492 was the most and the Tg 501 the less competitive strain. After four days, Tpa 432 and Tpi 492 colonies were able to overgrowth completely PoSp colony with an intense conidiation, whereas Tg 501 did not overgrow PoSp after colony contact. No *Trichoderma* strain caused any inhibitory effect by volatile or not volatile metabolites on Po Sp growth (data not shown). Regarding hyphal interactions, most of *Trichoderma* hyphae grew parallel to PoSp hyphae, and numerous hyphal tips of PoSp were dichotomous. Coiling and hyphal penetration did not occur.

## Conclusions

*Pleurotus ostreatus* is one of the most cultivated mushroom worldwide, its production is increasing, however it is susceptible to a variety of disease. One of the most serious disease is the green mould caused by some *Trichoderma* species i.e. *T. harzianum, T. pleuroticola and T. pleuroti*. The role of *T. harzianum* is not still clear. Our *T. harzianum* isolate, re- identified as *T. guizhouense* (Chiaverri *et al*., 2015), was not responsible of the disease, also when it was inoculated in the growing substrate at a high concentration. This finding is in accordance with Komon-Zelazowska *et al.* (2007), Hatvani (2008) and Hatvani *et al.* (2012), who stated that *T. pleuroticola* and *T. pleuroti* were the causal agents for the oyster mushroom green mould disease, and it is in contrast with Woo *et al.* (2009) that considered *T. harzianum* problematic to *P. ostreatus*. Hatvani (2008) and Hatvani *et al.* (2012) reported that *T. harzianum* was not detected from green mould-affected samples of the growing substrate collected from oyster mushroom farms. We found that *T. pleuroti* both *in vitro* and *in vivo* experiments was more aggressive than *T. pleuroticola* against *P. ostreatus*.

Prochloraz is the only fungicide registered for the application in mushroom farms. Few studies investigated the sensitivity to fungicides of *Trichoderma* species pathogenic to *P. ostreatus* (Hatvani, 2008; Woo *et al.*, 2009; Hatvani *et al.*, 2012). In these studies prochloraz controlled spore germination and mycelium growth of *Trichoderma* isolates without negative effects to *P. ostreatus* (Hatvani, 2008; Woo *et al.*, 2009). Our study confirmed, as expected, that prochloraz was not toxic to *P. ostreatus* (data not shown), and that the fungicide was very effective against both *Trichoderma* pathogenic species. *Trichoderma pleuroti* and *T. pleuroticola* strains on WSEA plates showed a similar sensitivity to prochloraz at field dose, consistently to Hatvani *et al.* (2012) on yeast extract-glucose medium. These *in vitro* results were confirmed in the *in vivo* assay. Indeed, when the fungicide was applied at field dose in a small-scale experiment reproducing the spawn run phase of cultivation cycle, no green mould disease symptoms by both *Trichoderma* pathogenic species were observed.

However, despite of the evident prochloraz efficacy and its diffuse use in mushroom farms, the disease is increasing. Based on our data, we could hypothesize that prochloraz, is able to control the

primary, but not the secondary infections by air borne spores of *Trichoderma*, that occur when the fungicide was no longer to be effective, because of the decline of its concentration (Potočnik *et al.*, 2015).

It is well known that *Trichoderma* are important biocontrol agents against several plant pathogens, and that they utilize several mechanisms such as mycoparasitism, antibiosis, competition for carbon, nitrogen and other growth factors, together with competition for space or specific infection sites (Harman, 2006). To our knowledge, the pathogenic mechanism/s of *Trichoderma* against *P. ostreatus* have not been investigated. We found that the mycelium of *Trichoderma* was more competitive than oyster mushroom mycelium for space and nutrients, whereas we did not observe neither parasitism nor antibiosis.

Finally, this study has provided evidence that, under our experimental conditions, T. *guizhouense* was not responsible of the green mould disease, and that prochloraz is effective against both *T. pleuroticola* and *T. pleuroti*. However, the treatment at spawn is not sufficient to ensure protection during the whole oyster cultivation cycle. Unfortunately, only one fungicide application is possible, because the substrate is bagged immediately after spawn. Therefore, it is important to prevent contaminations by *Trichoderma* spores by improving farm hygiene conditions.

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## Legends to Tables

Table 1. Fungal strains, their codes and symbols utilised in the experiments.

Table 2. Colony growth rate (mm/day) of *Trichoderma guizhouense* (Tg) 446 and 501 at different prochloraz doses.

Table 3. Colony growth rate (mm/day) of *Trichoderma pleuroticola* (Tpa) 432 and 488 at different prochloraz doses.

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Table 8. Colonisation rate (0-100) of WSEA substrate by *Trichoderma guizhouense* (Tg), *T. pleuroticola* (Tpa) and *T. pleuroti* (Tpi) in dual culture with *P. ostreatus* Sp measured 24, 48 and 72 h after *Trichoderma* inoculation.

Table 1. Fungal strains, their codes and symbols utilised in the experiments.

| Fungal species | Isolate code | Symbol |
|---|---|---|
| *Trichoderma guizhouense* | AFS 446 | Tg 446 |
| *Trichoderma guizhouense* | AFS 501 | Tg 501 |
| *Trichoderma pleuroti* | AFS 492 | Tpi 492 |
| *Trichoderma pleuroti* | AFS 497 | Tpi 497 |
| *Trichoderma pleuroticola* | AFS 432 | Tpa 432 |
| *Trichoderma pleuroticola* | AFS 488 | Tpa 488 |
| *Pleurotus ostreatus* Spoppo | - | PoSp |

Table 2. Colony growth rate (mm/day) of *Trichoderma guizhouense* (Tg) 446 and 501 at different prochloraz doses.

| Dose (μg/L $L^{-1}$) | Tg 446 | Tg 501 | Mean ± SD |
|---|---|---|---|
| 0 | 32.5 ± 2.1 | 34.8 ± 1.4 | 33.6 ± 2.0 c |
| 0.01 | 32.8 ± 1.9 | 34.8 ± 2.6 | 33.8 ± 2.3 c |
| 0.05 | 32.8 ± 2.9 | 35.8 ± 1.6 | 34.3 ± 2.7 c |
| 0.25 | 27.3 ± 1.3 | 24.8 ± 1.2 | 26.1 ± 1.8 b |
| 1.25 | 3.9 ± 1.4 | 5.2 ± 1.4 | 4.5 ± 1.4 a |

The dose factor was significant, whereas the strain factor and the dose × strain interaction factor were not significant according to two way ANOVA ($P < 0.05$). Data are mean ± SD. Data in the column followed by the same letter did not differ significantly according to SNK test ($P < 0.05$).

Table 3. Colony growth rate (mm/day) of *Trichoderma pleuroticola* (Tpa) 432 and 488 at different prochloraz doses.

| Dose (μgL $L^{-1}$) | Tpa 432 | Tpa 488 | Mean ± SD |
|---|---|---|---|
| 0 | 37.4 ± 1.9 | 38.8 ± 1.9 | 38.1 ± 1.9 e |
| 0.01 | 31.3 ± 1.3 | 30.3 ± 1.5 | 30.8 ± 1.4 d |
| 0.05 | 21.4 ± 2.8 | 20.3 ± 1.9 | 20.8 ± 5.2 c |
| 0.25 | 4.3 ± 1.6 | 3.7 ± 1.1 | 4.0 ± 8.7 b |
| 1.25 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 a |

The dose factor was significant, whereas the strain factor and the dose x strain interaction factor were not significant according to two way ANOVA ($P < 0.05$). Data are mean ± SD. Data in the column followed by the same letter did not differ significantly according to SNK test ($P < 0.05$).

Table 4. Colony growth rate (mm/day) of *Trichoderma pleuroti* (Tpi) 492 and 497 at different prochloraz doses.

| Dose (µL $L^{-1}$) | Tpi 492 | Tpi 497 | Mean ± SD |
|---|---|---|---|
| 0 | 26.1 ± 1.3 | 26.8 ± 1.9 | 26.4 ± 2.8 d |
| 0.01 | 22.4 ± 1.4 | 22.3 ± 1.0 | 22.4 ± 1.1 c |
| 0.05 | 8.1 ± 2.1 | 8.1 ± 1.8 | 8.1 ± 1.8 b |
| 0.25 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 a |
| 1.25 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 a |

The dose factor was significant, whereas the strain factor and the dose x strain interaction factor were not significant according to two way ANOVA ($P < 0.05$). Data are mean ± SD. Data in the column followed by the same letter did not differ significantly according to SNK test ($P < 0.05$).

Table 5. Effect of different prochloraz doses on spore germination (%) of *Trichoderma guizhouense* (Tg), *T. pleuroticola* (Tpa) and *T. pleuroti* (Tpi), 12 h after inoculation.

| Dose (μg L $L^{-1}$) | Tg | | Tpa | | Tpi | |
|---|---|---|---|---|---|---|
| | 446 | 501 | 432 | 488 | 492 | 497 |
| 0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 |
| 0.01 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 100 ± 0.0 | 80.0 ± 5.0 | 70.3 ± 2.1 |
| 0.05 | 100 ± 0.0 | 100 ± 0.0 | 59.7 ± 4.5 | 67.3 ± 8.7 | 35.0 ± 7.0 | 36.7 ± 2.9 |
| 0.25 | 60.3 ± 6.5 | 72.6 ± 7.8 | 25.3 ± 2.1 | 22.7 ± 3.5 | 0.0 ± 0.0 | 0.0 ± 0.0 |
| 1.25 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ±0.0 |

Data are mean ± SD.

Table 6. Multifactor ANOVA for substrate colonisation by *Trichoderma pleuroticola* 432 and *T. pleuroti* 492 during spawn run phase of *Pleurotus ostreatus* Sp cultivation cycle.

| **Source** | **Df** | **Sum of squares** | **Mean square** | ***F* values** |
|---|---|---|---|---|
| *Trichoderma* species (TS) | 1 | 36.75 | 36.75 | 190.56 *** |
| *Trichoderma* concentration (TC) | 1 | 3.0 | 3.0 | 15.56 *** |
| Prochloraz dose (D) | 3 | 79.1667 | 26.3889 | 136.83 *** |
| TS x TC | 1 | 0.75 | 0.75 | 3.89 |
| TS x D | 3 | 18.4167 | 6.13889 | 31.83 *** |
| TC x D | 3 | 1.16667 | 0.388889 | 2.02 |
| Error | 35 | 6.75 | 0.192857 | |
| Corr. total | 47 | 146 | | |

*** = $P \leq 0.001$; ** = $P \leq 0.01$; * = $P \leq 0.05$

Table 7. Effect of different doses of prochloraz on substrate colonisation (0-4) by *Trichoderma pleuroticola* (Tpa 432) and *T. pleuroti* (Tpi 492) three weeks after prochloraz treatment.

| | Prochloraz dose (μL $L^{-1}$) | | | |
|---|---|---|---|---|
| Species | 0 | 0.025 | 0.25 | 1.25 |
| Tpa 432 | 2.2 ± 1.0 aB | 2.3 ± 0.8 aB | 0.0 ± 0.0 bA | 0.0 ± 0.0 aA |
| Tpi 492 | 4.0 ± 0.0 bB | 4.0 ± 0.0 bB | 3.5 ± 0.8 bB | 0.0 ± 0.0 aA |

Mean values ± SD followed by the same upper case letter in a line and by the same lower case letter in a column are not significantly different according to SNK test ($P < 0.01$).

Table 8. Colonisation rate (0-100) of WSEA substrate by *Trichoderma guizhouense* (Tg), *T. pleuroticola* (Tpa) and *T. pleuroti* (Tpi) in dual culture with *P. ostreatus* Sp measured 24, 48 and 72 h after *Trichoderma* inoculation.

| Time (h) | Tg 501 | Tpa 432 | Tpi 492 |
|---|---|---|---|
| 24 | 16.7 ± 2.9 a | 24.2 ± 1.4 b | 40.8 ± 3.8 c |
| 48 | 34.2 ± 2.9 a | 55.8 ± 1.4 b | 66.7 ± 2.9 c |
| 72 | contact | overgrowth | overgrowth |

Mean values ± SD followed by the same letter in a line are not significantly different according to SNK test ($P < 0.05$).

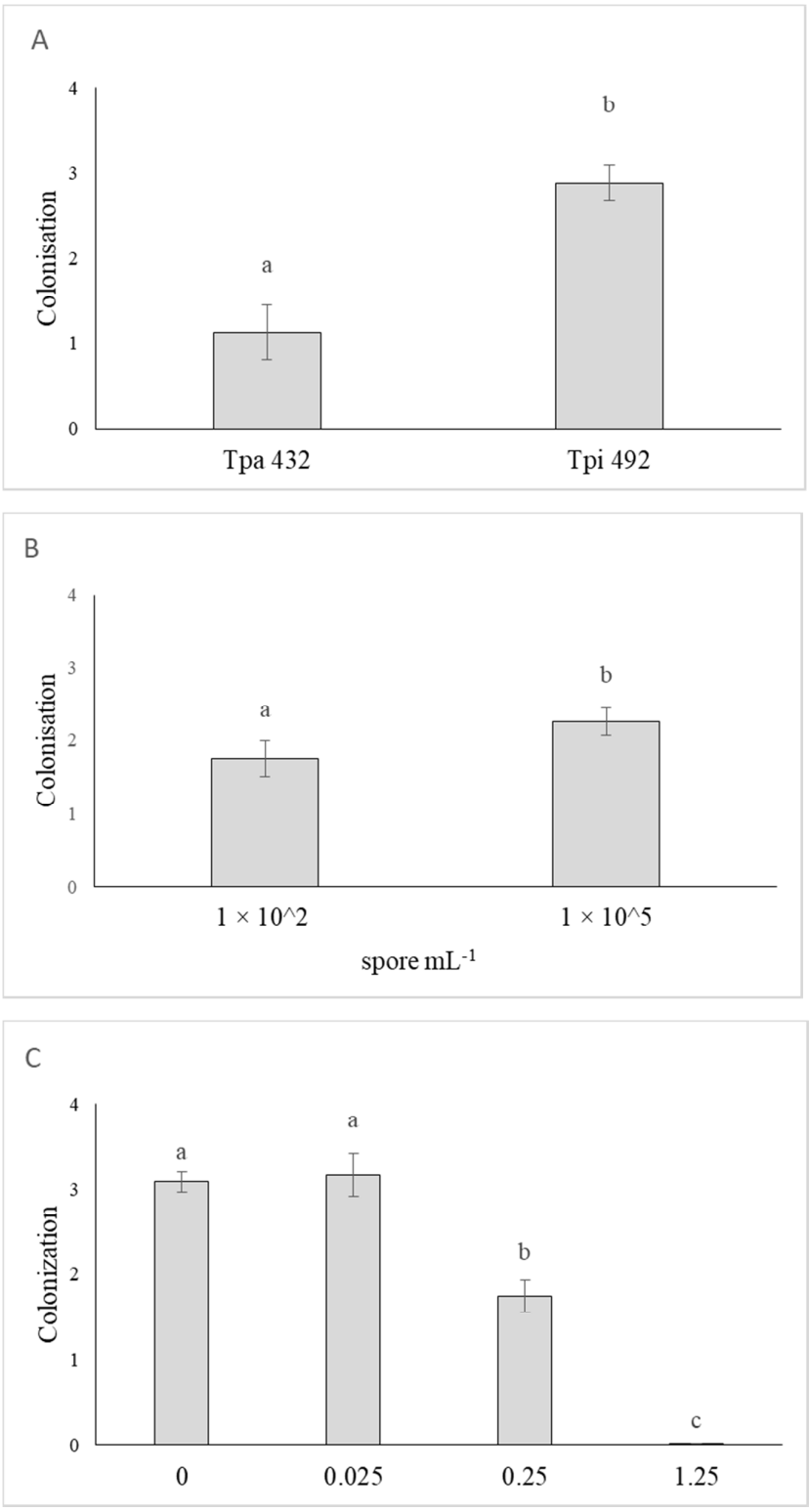


Figure 1. Effect of *Trichoderma pleuroticola* 432 and *T. pleuroti* 492 (A), their concentration (B) and prochloraz doses (C) on substrate colonisation rate (0-4) by *Pleurotus ostreatus* during spawn run. Each error bar represents SD. Different letters indicate significant differences according to SNK test ($P < 0.05$).