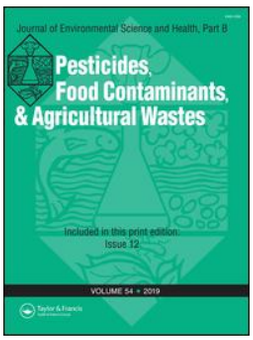

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Sensitivity of *Trichoderma* strains from edible mushrooms to the fungicides prochloraz and metrafenone

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ABSTRACT

Twenty-two strains of *Trichoderma* spp. (*T. harzianum* species complex [THSC], *Trichoderma aggressivum* f. *europaeum, Trichoderma pleuroti,* and *Trichoderma pleuroticola*) causing green mold disease on edible mushrooms (button mushroom, shiitake and oyster mushroom), collected during 2004–2018 from four countries (Serbia, North Macedonia, Croatia, and Hungary) were examined. Based on their ITS (internal transcribed spacer) sequences, strains from shiitake mushroom in Serbia were identified as members of the THSC, while in samples obtained from Serbian and North-Macedonian oyster mushroom farms THSC, *T. pleuroti* and *T. pleuroticola* were detected, which represent the first findings in the region. In fungicide susceptibility tests, all examined *Trichoderma* strains were found to be highly sensitive to prochloraz (ED_{50}<0.4 μg mL^{-1}) and considerably susceptible to metrafenone (ED_{50} < 4 μg mL^{-1}). The most sensitive taxon to both fungicides was THSC from oyster mushroom. The toxicity of metrafenone was satisfying and strains from oyster mushroom showed the highest sensitivity (ED_{50} < 1.43 μg mL^{-1}), while strains originating from button mushroom and shiitake displayed similar susceptibilities (ED_{50} < 3.64 μg mL^{-1}). After additional *in vivo* trials, metrafenone might also be recommended for the control of green mold disease in mushroom farms.

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Button mushrooms; oyster mushroom; shiitake; green mold disease; fungicide sensitivity test; disease control; *Trichoderma pleuroti*; *Trichoderma pleuroticola*; *Trichoderma harzianum*

Introduction

The most commonly cultivated mushrooms worldwide are button mushroom (*Agaricus bisporus* (Lange) Imbach), shiitake (*Lentinula edodes* (Berk.) Pegler) and oyster mushroom (*Pleurotus ostreatus* (Jasq.) P. Kummer). Fungal pathogens have a significant negative effect on mushroom yield and quality.[1] Green mold caused by *Trichoderma* spp. is the most devastating disease, accounting for mushroom yield losses between 60% and 100%.[2,3] The disease is characterized by the presence of white mycelia of fast-growing colonies that change color into dark green after extensive sporulation on the substrate of all three cultivated species. Brown necrotic spots and lesions may also appear on mushroom fruiting bodies as accompanying symptoms. Serious outbreaks appear as substrate areas without mushrooms are surrounded by red-pepper mites (*Pygmephorus* spp.) feeding on the pathogenic fungi.[2] Several *Trichoderma* species were identified as aggressive colonizers and predominant causal agents of green mold diseases of edible mushrooms: *Trichoderma harzianum sensu lato* (*T. harzianum* species complex, THSC), *Trichoderma aggressivum* f. *europaeum* Samuels & W. Gams and *T. aggressivum* f. *aggressivum* Samuels & W. Gams on button mushroom; *Trichoderma pleuroti* S.H. Yu & M.S. Park and *Trichoderma pleuroticola* S.H. Yu & M.S. Park on oyster mushroom and THSC on shiitake mushroom.[4–9] *Trichoderma* species are cosmopolitan in soils, playing a role primarily in the decomposition of wood and other plant materials. However, certain aggressive species like *T. aggressivum* and *T. pleuroti* have been found so far only in association with button mushroom and oyster mushroom substrate, respectively.[10]

Disease control usually includes a series of preventive measures in mushroom farms: strict hygiene, treatments with disinfectants and the application of fungicides. Only few chemical fungicides are approved, available and officially recommended in the mushroom industry, including prochloraz in the European Union, as well as chlorothalonil and thiabendazol in North America. [1,11,12] The most effective fungicide in mushroom disease control is prochloraz, which was found to be effective also against the main fungal pathogens in Serbian mushroom cultivation. [1,13–17] Nevertheless, decreased sensitivity of *Lecanicillium fungicola* (Preuss) Hassebrauk (dry bubble) and *Cladobotryum mycophylum* (Oudemans) W. Gams & Hoozemans (cobweb disease) to prochloraz was noted in many EU countries.[18–20] Recently, metrafenone was introduced to control fungal pathogens, *Cladobotryum* spp. and *L. fungicola* in Spain, France, Belgium, the UK and the USA.[12,21] However, for green mold disease control there is still no alternate option, and sensitivity data of *Trichoderma* strains to fungicides are

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scarce. Studies on the efficacy of new fungicides developed by agrochemical companies are rare and expensive, especially for small crops such as edible mushrooms.[22]

The objective of this study was the *in vitro* sensitivity testing of different *Trichoderma* taxa (THSC, *T. aggressivum* f. *europaeum*, *T. pleuroti*, and *T. pleuroticola*), causal agents of green mold diseases of cultivated mushrooms (button mushroom, shiitake, and oyster mushroom) to the fungicides prochloraz and metrafenone. *Trichoderma* strains from button mushroom, oyster mushroom and shiitake were derived from four neighboring countries (Serbia, North Macedonia, Croatia, and Hungary). Furthermore, the study presents the results of the first survey and molecular identification of oyster mushroom green molds in Serbia and North Macedonia, and of shiitake green molds in Serbia. Also, strains were characterized based on their morphological, pathogenic and ecological features. Strains from button mushroom (THSC and *T. aggressivum* f. *europaeum*) were tested against the fungicide metrafenone only, as their identification, virulence and sensitivity testing to prochloraz had been performed previously.[17]

Materials and methods

Trichoderma *strains*

A total set of 22 *Trichoderma* isolates collected from mushroom farms (button mushroom, oyster mushroom and shiitake) in four countries, Serbia, North Macedonia, Croatia and Hungary, during 2004–2018 (Table 1) was studied. Samples of substrate with symptoms resembling green mold disease were collected from farms growing oyster mushroom in North Macedonia and Serbia and shiitake farms from Serbia. Subsequently, twelve fungal strains were isolated from these samples by taking small pieces ($2 \times 2 \times 5$ mm), immersing them in a 1% sodium hypochlorite solution for 1 minute, and placing on Potato Dextrose Agar (PDA) medium. The plates were incubated for four days at 22 ± 1 °C after strain isolation. Six strains from *A. bisporus,* THSC T10, T52, and T54, as well as *T. aggressivum* f. *europaeum* T76, T77 and T85, identified and tested for sensitivity to prochloraz in previous studies by Kosanović et al. [17], were used in the current study for sensitivity testing to the fungicide metrafenone. The strains have been maintained in the culture collection of the Institute of Pesticides and Environmental Protection, Belgrade, Serbia. Four *Trichoderma* strains from Hungary and Croatia, obtained from the Szeged Microbiology Collection (SZMC, Department of Microbiology, Faculty of Science and Informatics, University of Szeged, Hungary), were also included in the survey (Table 1).[10,23]

Molecular identification of fungal strains from oyster mushroom and shiitake

Species identification of the novel *Trichoderma* strains from oyster mushroom and shiitake farms in Serbia and North Macedonia was performed by the PCR amplification and sequence analysis of the ITS (internal transcribed spacer) 1 and 2 regions, as described by Hatvani et al.[24,25] The obtained ITS sequences have been submitted to the NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/; accession numbers listed in Table 1).

Virulence test assay of fungal strains from oyster mushroom and shiitake

Virulence assay was performed by inoculating the harvested pilei of *P. ostreatus* and *L. edodes* with conidial suspensions (3×10^6 conidia mL^{-1}) prepared from four-day-old cultures of all tested strains from oyster mushroom and shiitake using the method of Bonnen and Hopkins.[26] Stipites were removed from all basidiomes and the top of the pilei were inoculated with 20 μL of conidial suspension. Three replicates per each strain were conducted. Sterile water was used as a negative control. Inoculated pilei were kept at room temperature (22 ± 2 °C), and after 2 days, the symptoms were rated as follows: 0 = no symptoms; 1 = faint rings around the inoculation site; 2 = light brown rings around the inoculation site; 3 = dark brown rings at the inoculation site; 4 = dark brown rings, some sporulation and some pitting of the pileus tissue at the inoculation site; and 5 = symptoms extended beyond the inoculation site, severe pitting of the tissue, and profuse sporulation.

Morphological studies and growth conditions of fungal strains from oyster mushroom and shiitake

Morphological characterization (colony color; conidial shape, color and size), growth rate and ecological features of the strains were investigated. Growth rate test was performed from day 2 to day 6 of cultivation on PDA in darkness at 22 °C. Descriptive statistics of conidial dimensions and length/width ratio were made based on 50 measurements per strain. Mean and range values were reported. An Olympus CX41RF microscope (Olympus Life and Material Science Europa GMBH, Hamburg, Germany), *Moticam 10+* digital camera and Motic Images Plus 3.0 software (LLG GmbH, Meckenheim, Germany) were used in the study. The 6-mm agar plugs of each tested strain were placed 1 cm from the edge of PDA plate. Radial growth rate of the strains was defined in mm h^{-1} by measuring colony diameters from the edge of mycelial agar plug after 72 h of incubation at 22 °C and calculated using the Eq. (1):

$$\text{Radial growth rate } (\text{mm h}^{-1}) = \text{Colony radius (mm)}/\text{Incubation period (h)} \quad (1)$$

[17]

The effect of temperature on growth was studied on strains growing on PDA in darkness at 17, 20, 22, 25, 30, and 35 °C by measuring colony diameter after 3 days. Four replicates per each strain were done.

Table 1. *Trichoderma* strains involved in the study.

Strain code	Species	Origin	Cultivated mushroom	Specimen, year of collection	GenBank ITS (reference)
T57[a]	*T. harzianum* species complex (THSC)	Ugrinovci, Serbia	Shiitake	Fruiting body, 2008	MT876593
T58		Ugrinovci, Serbia		Fruiting body, 2008	MT876594
T59		Ugrinovci, Serbia		Fruiting body, 2008	MT876595
T10		Požarevac, Serbia	Button mushroom	Fruiting body, 2006	KC555182 [17]
T52		Zemun, Serbia		Fruiting body, 2008	KC555177 [17]
T54		Kula, Serbia		Fruiting body, 2008	KC555183 [17]
T76	*T. aggressivum* f. *europaeum*	Lisovići, Barajevo, Serbia		Mushroom compost, 2010	KC555185 [17]
T77		Lisovići, Barajevo, Serbia		Mushroom compost, 2010	KC555186 [17]
T85		Lisovići, Barajevo, Serbia		Mushroom compost, 2010	KC555184 [17]
SZMC 24039 (MFBF 10386)	*T. pleuroti*	Croatia	Oyster mushroom	Mushroom substrate, 2011	MT876591 [10]
SZMC 24040 (MFBF 10387)	*T. pleuroticola*	Croatia		Mushroom substrate, 2011	MT876592 [10]
SZMC 12454	*T. pleuroti*	Hungary		Mushroom substrate, 2009	MT876590 [23]
SZMC 23033 (A37, CPK 2104)	*T. pleuroticola*	Hungary		Mushroom substrate, 2004	EF392794 [6,24]
KG6	*T. pleuroticola*	Kragujevac, Serbia		Mushroom substrate, 2018	MT876581
KG10	*T. harzianum* species complex (THSC)	Kragujevac, Serbia		Mushroom substrate, 2018	MT876582
KG13		Kragujevac, Serbia		Mushroom substrate, 2018	MT876583
KM4	*T. pleuroticola*	Kavadarci, N. Macedonia		Mushroom substrate, 2018	MT876584
KM5		Kavadarci, N. Macedonia		Mushroom substrate, 2018	MT876585
KM12		Kavadarci, N. Macedonia		Mushroom substrate, 2018	MT876589
KM6	*T. pleuroti*	Kavadarci, N. Macedonia		Mushroom substrate, 2018	MT876586
KM8		Kavadarci, N. Macedonia		Mushroom substrate, 2018	MT876587
KM11		Kavadarci, N. Macedonia		Mushroom substrate, 2018	MT876588

[a]Strains isolated within the frames of the present study are set in bold.

Fungicides

In vitro toxicity of two commercial fungicides: prochloraz (Mirage® 45 EC, 450 g L^{-1}; Adama Makhteshim Ltd, Beer Sheva, Israel) and metrafenone (Vivando® SC, 500 g L^{-1}; BASF SE, Ludwigshafen am Rhein, Germany), were tested against *Trichoderma* strains. Six strains from button mushroom, THSC T10, T52, and T54 as well as *T. aggressivum* f. *europaeum* T76, T77, and T85 were tested only against the fungicide metrafenone, as their sensitivity to prochloraz was previously reported by Kosanović et al.[17]

In vitro *antifungal activity*

Activity of the selected fungicides against *Trichoderma* strains was tested by macrodilution method on PDA amended with the tested fungicides, using the preliminary concentrations of 0.01, 0.1, 1, 10, 100, and 1000 µg mL^{-1}. Based on earlier observations,[17] concentrations of prochloraz and metrafenone selected for the study included 0.00243, 0.0081, 0.027, 0.09, and 0.30 µg mL^{-1}. The selected fungicide concentrations from 1% stock solutions were added to sterile molten medium (about 50 °C). The fungicide-amended media, and the fungicide-free media used as control were inoculated with agar disks (Ø 6 mm) covered by mycelium taken from the edge of four-day-old cultures of *Trichoderma* strains and incubated at 20 ± 1 °C. Colony diameters were measured after 72 h. Mycelial growth in the fungicide-amended media was expressed as percentage compared to control using the Eq. (2):

$$\text{Percentage of growth inhibition} = 100 - \left[\frac{(\text{Colony diameter in control plate} - \text{Colony diameter in tested plate})}{\text{Colony diameter in control plate}} \times 100\right] \quad (2)$$

Table 2. Colony diameter (mm) of *Trichoderma* strains on Potato Dextrose Agar at different temperatures, 72 h after inoculation.

Species	Cultivated mushroom	Strain code	Colony diameter (mm) Mean ± SE1						
			17 °C	20 °C	22 °C	25 °C	30 °C	35 °C	*F*
Trichoderma aggressivum f. *europaeum*	Button mushroom*	T76	23.4 ± 3.5^{e}	38.6 ± 2.1^{d}	84.5 ± 1.1^{b}	89.6 ± 0.6^{a}	65.4 ± 1.5^{c}	3.0 ± 2.1^{f}	485.4
		T77	26.0 ± 0.0^{d}	44.0 ± 2.7^{c}	69.5 ± 0.5^{b}	97.6 ± 2.1^{a}	74.0 ± 6.0^{b}	12.0 ± 0.0^{e}	244.0
		T85	21.4 ± 3.5^{e}	31.6 ± 1.2^{d}	90.0 ± 0.8^{b}	111.4 ± 1.0^{a}	56.0 ± 1.7^{c}	2.6 ± 0.6^{f}	2013.1
Trichoderma harzianum species complex (THSC)		T10	24.6 ± 1.8^{e}	50.0 ± 2.0^{d}	88.0 ± 0.0^{c}	108.6 ± 2.5^{b}	116.4 ± 3.8^{a}	1.0 ± 2.1^{f}	1171.5
		T52	24.6 ± 0.6^{e}	41.6 ± 0.6^{d}	84.0 ± 0.8^{c}	86.6 ± 0.6^{b}	139.0 ± 0.0^{a}	9.0 ± 0.0^{f}	496.3
		T54	38.6 ± 1.5^{d}	56.0 ± 0.6^{c}	72.0 ± 0.8^{b}	83.4 ± 4.0^{a}	81.4 ± 2.3^{a}	0.0 ± 3.5^{e}	569.7
	Shiitake	T57	47.0 ± 0.5^{f}	74.0 ± 1.4^{d}	107.0 ± 0.6^{c}	132.5 ± 1.4^{b}	140.0 ± 0.0^{a}	55.5 ± 7.8^{e}	306.1
		T58	33.0 ± 0.7^{d}	63.0 ± 2.1^{c}	88.0 ± 1.2^{b}	124.0 ± 0.0^{a}	120.5 ± 1.4^{a}	65.0 ± 1.4^{c}	588.3
		T59	32.5 ± 0.7^{e}	78.0 ± 0.7^{c}	105.5 ± 1.0^{b}	129.0 ± 0.0^{a}	128.0 ± 0.0^{a}	46.0 ± 3.5^{d}	943.5
	Oyster mushroom	KG13	35.0 ± 0.4^{e}	80.0 ± 0.0^{c}	102.5 ± 0.5^{b}	131.5 ± 0.7^{a}	134.0 ± 2.8^{a}	64.0 ± 2.8^{d}	564.7
		KG10	30.5 ± 0.7^{e}	80.0 ± 0.0^{c}	104.0 ± 1.2^{b}	133.5 ± 0.0^{a}	138.0 ± 1.4^{a}	71.0 ± 2.1^{d}	675.1
Trichoderma pleuroticola		KG6	46.5 ± 1.4^{f}	83.5 ± 1.4^{d}	109.5 ± 0.5^{c}	133.0 ± 0.7^{b}	138.5 ± 2.1^{a}	67.0 ± 3.5^{e}	790.2
		KM4	39.5 ± 0.7^{e}	76.5 ± 0.7^{d}	97.5 ± 1.5^{c}	123.5 ± 0.7^{b}	133.0 ± 0.7^{a}	28.5 ± 2.1^{f}	582.0
		KM5	50.0 ± 0.0^{d}	100.0 ± 0.0^{c}	127.5 ± 0.5^{b}	139.5 ± 0.7^{a}	140.5 ± 0.7^{a}	46.5 ± 3.5^{e}	527.2
		KM12	48.0 ± 0.7^{e}	100.0 ± 0.0^{c}	123.5 ± 0.5^{b}	140.0 ± 0.0^{a}	140.0 ± 0.0^{a}	61.0 ± 4.2^{d}	1111.6
		SZMC 24040	38.5 ± 2.1^{e}	66.5 ± 2.1^{d}	87.5 ± 2.5^{b}	105.0 ± 0.7^{a}	77.5 ± 0.0^{c}	25.0 ± 3.5^{f}	197.4
		SZMC 23033	34.0 ± 3.5^{e}	83.0 ± 2.8^{c}	102.5 ± 1.5^{b}	123.5 ± 1.4^{a}	121.5 ± 2.8^{a}	53.0 ± 4.2^{d}	237.3
Trichoderma pleuroti		KM6	42.5 ± 0.7^{e}	104.5 ± 0.0^{c}	123.5 ± 2.1^{b}	139.5 ± 0.0^{a}	140.0 ± 0.0^{a}	62.0 ± 0.0^{d}	1465.4
		KM8	41.0 ± 0.7^{e}	90.5 ± 0.0^{c}	106.0 ± 0.0^{b}	114.0 ± 1.4^{a}	82.0 ± 0.7^{d}	23.5 ± 0.7^{f}	600.1
		KM11	49.5 ± 0.0^{e}	99.0 ± 0.0^{c}	122.5 ± 0.5^{b}	139.5 ± 0.0^{a}	140.0 ± 0.0^{a}	67.5 ± 0.7^{d}	4332.0
		SZMC 24039	37.5 ± 0.0^{e}	80.0 ± 0.7^{c}	110.0 ± 0.0^{b}	137.5 ± 2.1^{a}	134.5 ± 1.4^{a}	69.0 ± 3.5^{d}	550.9
		SZMC 12454	45.5 ± 0.0^{e}	98.5 ± 2.1^{c}	119.5 ± 1.0^{b}	135.5 ± 0.0^{a}	141.0 ± 0.0^{a}	63.5 ± 6.3^{d}	423.8

[1]Data are means of four replicates ± SE, standard error of means; ^{a,b,c,d,e,f} Strains by same uppercase letter are not significantly different at $P < 0.05$.
*Kosanović et al.[17]

Three replicates per strain were used. The experiment was repeated twice (assays I and II). Fungicide effects were studied by regression analysis ($r^2 > 0.95$). The estimated effective fungicide concentrations inhibiting radial mycelial growth by 50% (ED_{50}) were determined for each strain by interpolation from computer-generated log-probit plots of fungicide concentration and relative inhibition. The strains were considered sensitive to a fungicide if their ED_{50} values were less than 5 μg mL^{-1}, weakly resistant if ED_{50} was in the range between 5 and 50 μg mL^{-1} and resistant if the ED_{50} value exceeded 50 μg mL^{-1}.[27] A resistance factor (RF) was also calculated for each strain according to the Eq. (3):

$$RF = ED_{50} \text{ of the observed strain} / ED_{50} \text{ of the most sensitive strain} \quad (3)$$ [28]

According to the RF values, strains were considered to be sensitive if the RF was less than 3, weakly resistant if it was between 3 and 20, resistant if it ranged between 20 and 80 and highly resistant if it was over 100.[29]

Results

Molecular identification of Trichoderma *strains from oyster mushroom and shiitake*

The obtained ITS 1 and 2 sequences were analyzed as described by Druzhinina et al.[30] Among the strains collected from the oyster mushroom farm in Serbia, *T. pleuroticola* (KG6) and the THSC (KG10, KG13) were detected, while *T. pleuroticola* (KM4, KM5, and KM12) and *T. pleuroti* (KM6, KM8, and KM11) were recovered from the North Macedonian samples. The agents causing green mold in the cultivation of shiitake (T57, T58, and T59) were identified as members of the THSC (Table 1).

Virulence assay of Trichoderma *strains from oyster mushroom and shiitake*

Five days after inoculation of the harvested pilei of *P. ostreatus* and *L. edodes*, the virulence assay showed that all tested *Trichoderma* strains (THSC, *T. pleuroti* and *T. pleuroticola*) from oyster mushroom displayed very low virulence levels on fruiting bodies (0 or 1 on the scale of 0–5), while all tested THSC strains from shiitake displayed high virulence levels (5 on the scale of 0–5) (Table 3; Figs. 1 and 2). Sterile water used as a negative control produced no symptoms.

Morphological and growth characteristics of Trichoderma *strains from oyster mushroom and shiitake*

During the first three days, all tested strains were characterized by white mycelium, which later became green as the result of sporulation. Colony diameter values of all tested strains at different temperatures after three days of incubation are shown in Table 2. The optimum temperature values for the mycelial growth of all tested strains were between 25 and 30 °C. The optimum temperature for THSC strains from shiitake, as well as for THSC and *T. pleuroti* strains from oyster mushroom were at 30 °C, while it was at 25 °C for *T. pleuroticola* strains from oyster mushroom. All strains produced green, subglobose, smooth-walled conidia on phialides after two to five days of incubation on PDA at 20 °C. Conidial dimensions were 2.13–3.04–3.62 × 1.88–2.68–4.08 for THSC isolates from shiitake; 2.41–3.15–4.41 × 2.05–2.93–4.53

Table 3. Phenotypical and pathogenic characters of *Trichoderma* strains from cultivated mushrooms.

Species	Cultivated mushroom	Strain code	Virulence[a]	Radial growth rate at 22 °C (mm h^{-1}) ±SD[b]	Conidial dimensions (µm) (length × width)[c]	Conidial length/ width ratio[c]
Trichoderma aggressivum f. *europaeum*	Button mushroom[d]	T76	4	0.59 ± 0.02	(2.5)–3.2–(4.0) × (2.2)–2.7–(3.4)	(1.0)–1.2–(1.4)
		T77	3	0.48 ± 0.01		
		T85	3	0.63 ± 0.01		
Trichoderma harzianum species complex (THSC)		T10	4	0.61 ± 0.00	(2.3)–3.0–(3.6) × (2.0)–2.5–(3.0)	(1.0)–1.2–(1.5)
		T52	3	0.58 ± 0.01		
		T54	5	0.50 ± 0.01		
	Shiitake	T57	5	0.74 ± 0.01	(2.6)–3.3–(3.6) × (1.9)–2.6–(4.1)	(1.4)–1.1–(0.9)
		T58	5	0.61 ± 0.02	(2.1)–2.7–(3.6) × (1.9)–2.6–(3.7)	(1.1)–1.1–(1.0)
		T59	5	0.73 ± 0.01	(2.4)–3.0–(3.6) × (2.0)–2.6–(3.6)	(1.2)–1.2–(1.1)
	Oyster mushroom	KG10	0	0.71 ± 0.01	(2.6)–3.3–(4.4) × (2.1)–3.0–(4.5)	(1.3)–1.1–(1.0)
		KG13	0	0.72 ± 0.02	(2.4)–3.0–(4.0) × (2.2)–2.9–(3.9)	(1.1)–1.0–(1.0)
Trichoderma pleuroticola		KG6	1	0.76 ± 0.01	(1.8)–2.6–(3.2) × (1.8)–2.3–(4.2)	(1.0)–1.1–(0.8)
		KM4	0	0.68 ± 0.02	(1.8)–2.9–(3.9) × (1.7)–2.8–(3.9)	(1.1)–1.0–(1.1)
		KM5	0	0.88 ± 0.01	(2.1)–2.7–(4.2) × (2.1)–2.7–(3.4)	(1.0)–1.0–(1.0)
		KM12	0	0.86 ± 0.01	(2.1)–2.7–(3.6) × (2.0)–2.8–(3.3)	(1.0)–1.0–(1.1)
		SZMC 24040	0	0.61 ± 0.03	(2.4)–3.1–(4.0) × (2.1)–2.9–(3.7)	(1.2)–1.1–(1.1)
		SZMC 23033	0	0.71 ± 0.02	(2.2)–3.3–(5.4) × (2.4)–3.0–(5.1)	(0.9)–1.1–(1.1)
Trichoderma pleuroti		KM6	0	0.87 ± 0.03	(1.8)–2.5–(3.3) × (1.8)–2.3–(3.1)	(1.0)–1.1–(1.1)
		KM8	0	0.74 ± 0.00	(2.4)–2.7–(3.5) × (1.9)–2.5–(3.2)	(1.3)–1.1–(1.2)
		KM11	0	0.85 ± 0.01	(2.2)–2.9–(3.5) × (1.7)–2.5–(3.0)	(1.3)–1.2–(1.2)
		SZMC 24039	0	0.76 ± 0.00	(2.4)–3.8–(5.5) × (2.6)–3.7–(5.4)	(0.9)–1.0–(1.0)
		SZMC 12454	1	0.83 ± 0.01	(2.0)–2.9–(4.7) × (1.9)–2.6–(4.1)	(1.0)–1.1–(1.3)

[a]Virulence based on a scale of 0–5 with 0 = no symptoms and 5 = severe symptoms;
[b]SD, standard deviation;
[c]Mean and value ranges (min and max) of conidial dimensions and conidial length/width ratio (50 measurements per strain), value ranges (min and max) are shown in brackets.
[d]Kosanović et al.[17]

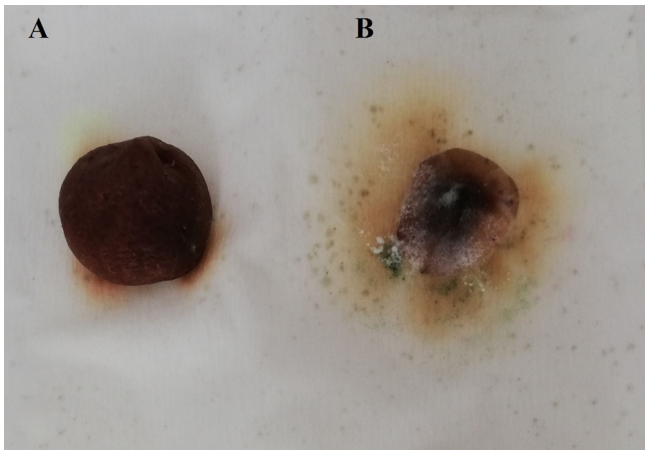

Figure 1. Artificial infection of *Lentinula edodes* fruiting bodies (fifth day); (A) control, (B) *Trichoderma harzianum* species complex [THSC] strain T58 (5 = profuse sporulation).

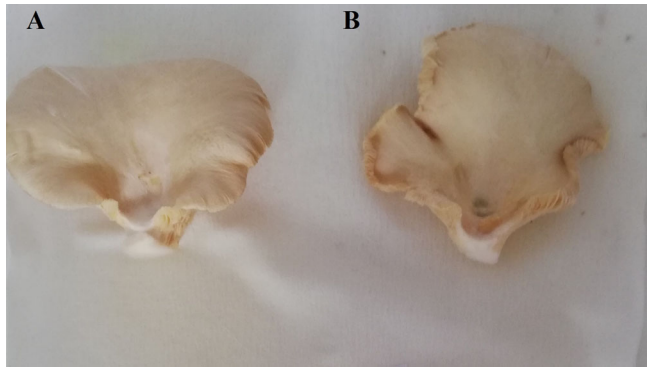

Figure 2. Artificial infection of *Pleurotus ostreatus* fruiting bodies (fifth day), (A) control, (B) *Trichoderma pleuroti* strain KM6 (0 = no symptoms).

for THSC from oyster mushroom; 1.82–2.86–5.39 × 1.72–2.6–5.05 for *T. pleuroti*; and 1.79–2.98–5.36 × 1.88–2.9–5.36 for *T. pleuroticola* strains. Conidium length/ width ratio for THSC isolates from shiitake as well as for *T. harzianum* and *T. pleuroti* from oyster mushroom were 1.1 in average, while it was 1.0 for *T. pleuroticola* (Table 3).

Sensitivity of Trichoderma strains to fungicides prochloraz and metrafenone

The sensitivity data of all tested *Trichoderma* strains from cultivated mushrooms (oyster mushroom, shiitake and button mushroom) to the fungicides metrafenone and prochloraz are presented in Tables 4 and 5, respectively; while the comparison of sensitivity of THSC strains from different cultivated mushrooms to both fungicides are shown in Table 6. The examined *Trichoderma* strains showed different levels of sensitivity to both tested fungicides *in vitro*, and their susceptibility was high. Sensitivity parameters (ED_{50} values) of metrafenone for *T. pleuroti* from oyster mushroom ranged from 0.03 to 0.65 µg mL^{-1} (assay I) and from 0.02 to 0.19 µg mL^{-1} (assay II). The highest RF values for metrafenone was shown by the strains SZMC 12454 (13) and KM6 (14), in assays I and II, respectively.

Sensitivity parameters of prochloraz for *T. pleuroti* were below 0.14 µg mL^{-1} in both assays, and strain SZMC 12454 showed the highest RF values, 140 and 28 in assays I and II,

Table 4. Effect of the fungicide metrafenone on the growth of *Trichoderma* strains from oyster mushroom, shiitake and button mushroom.

			Metrafenone					
			I			II		
			ED_{50} (μg mL^{-1})			ED_{50} (μg mL^{-1})		
Species	Cultivated mushroom	Code	Mean	Range	RF	Mean	Range	RF
Trichoderma pleuroti	Oyster mushroom	SZMC 12454	0.65	0.20–11.02	13.00	0.19	0.08–1.34	9.50
		SZMC 24039	0.09	0.05–0.27	1.80	0.02	0.01–0.03	1.00
		KM6	0.12	0.07–0.26	4.00	0.14	0.08–0.38	14.00
		KM8	0.07	0.04–0.12	2.33	0.02	0.01–0.03	2.00
		KM11	0.03	0.02–0.05	1.00	0.07	0.05–0.10	7.00
Trichoderma pleuroticola		SZMC 23033	0.17	0.08–0.68	5.67	1.43	0.29–301.08	143.00
		SZMC 24040	0.06	0.03–0.13	2.00	0.01	0.00–0.01	1.00
		KG6	0.03	0.02–0.04	1.00	0.12	0.08–0.018	12.00
		KM4	0.05	0.03–0.06	1.00	0.10	0.07–0.13	5.00
		KM5	0.06	0.04–0.08	1.20	0.20	0.09–0.88	10.00
		KM12	0.17	0.09–0.42	3.40	0.02	0.01–0.03	1.00
Trichoderma harzianum species complex (THSC)		KG10	0.05	0.04–0.06	1.25	0.01	0.00–0.01	1.00
		KG13	0.04	0.03–0.06	1.00	0.06	0.04–0.09	6.00
	Shiitake	T57	2.91	0.42–5679.04	18.19	3.63	0.52–4747.91	20.16
		T58	0.25	0.15–0.55	1.56	0.28	0.17–0.61	1.55
		T59	0.16	0.08–0.47	1.00	0.18	0.09–0.56	1.00
	Button mushroom	T10	0.03	0.01–0.05	1.00	0.21	0.09–1.13	5.25
		T52	0,09	0,06–0,17	3.00	0.04	0.03–0.05	1.00
		T54	1.26	0.41–12.63	42.00	3.64	0.76–186.60	91.00
Trichoderma aggressivum f. *europaeum*		T76	0.99	0.38–6.37	24.75	1.34	0.50–8.49	33.50
		T77	0.04	0.03–0.07	1.00	0.04	0.03–0.07	1.00
		T85	0.11	0.04–0.99	2.75	0.26	0.15–0.60	6.50

RF, resistance factor; ED_{50}, effective dose which inhibits mycelial growth to 50%.

respectively. The ED_{50} of metrafenone for *T. pleuroticola* from oyster mushroom ranged from 0.03 to 0.17 and from 0.01 to 1.43 μg mL^{-1} in assays I and II, respectively. The highest RF values (5.67 and 140) were shown by strain SZMC 23033. Prochloraz against *T. pleuroticola* showed respective ED_{50} values of 0.001–0.05 and 0.004–0.03 μg mL^{-1} in the two assays, while strain SZMC 23033 had the highest RF values (50 and 7.5, respectively). Both metrafenone and prochloraz displayed similar toxicity to THSC members from oyster mushroom in the two separate assays, with ED_{50} values of 0.01–0.06 and 0.01–0.02 μg mL^{-1}, respectively. THSC strain KG13 showed the highest RF values for both fungicides, i.e. 6 for metrafenone and 2 for prochloraz.

All tested Serbian THSC strains from shiitake were sensitive to both tested fungicides. ED_{50} values of metrafenone for THSC strains from shiitake fell into the ranges of 0.16–2.91 μg mL^{-1} (assay I) and 0.18–3.63 μg mL^{-1} (assay II). Sensitivity to metrafenone varied among the different strains, with THSC strain T57 showing the highest RF values (18.19 and 20.16 in assays I and II, respectively). All THSC strains from shiitake showed higher sensitivity to prochloraz than to metrafenone. ED_{50} values of prochloraz were lower than those of metrafenone, ranging from 0.03 to 0.07 μg mL^{-1} (assay I), and from 0.01 to 0.05 μg mL^{-1} (assay II). The highest RF value (5) for prochloraz was displayed by the THSC T59 strain from shiitake in assay II.

As for *Trichoderma* strains from button mushroom, in preliminary tests, their growth was good in the presence of 1 μg mL^{-1} metrafenone, while severely inhibited at 10 μg mL^{-1} and higher concentrations. ED_{50} values of metrafenone for *Trichoderma* strains from button mushroom ranged from 0.03 to 1.26 μg mL^{-1} (assay I) and from 0.04 to 3.64 μg mL^{-1} (assay II). The highest metrafenone factors of resistance was exhibited by *Trichoderma* strains from button mushroom, THSC T54 (42 and 91), and *T. aggressivum* f. *europaeum* T76 (24.75 and 33.5), rendering them the least sensitive. Nevertheless, all tested strains from button mushroom farms were sensitive to metrafenone with $ED_{50} < 5$ μg mL^{-1}, while the RF values indicated that differences existed between the strains.

Discussion

The results of molecular identification in the case of *Trichoderma* strains from Serbian and North-Macedonian oyster mushroom farms indicated that THSC, *T. pleuroti* and *T. pleuroticola* were the predominant causal agents of green mold disease. However, *T. pleuroti* was not found among the Serbian *Trichoderma* strains from oyster mushroom farms. Previous studies showed that *T. pleuroticola* was predominant in Italian *Pleurotus* farms, while the majority of strains from Hungary belonged to the species *T. pleuroti*.[6] The present study confirmed the findings of Hatvani[32] and Hatvani et al.[10,24] that *T. pleuroti* and *T. pleuroticola* were the main causal agents of green mold disease on oyster mushroom. On the basis of preliminary data obtained from morphological and genetic characterization, Woo et al.[33] had considered *T. harzianum* to be the causal agent of oyster mushroom green mold in Italy, however, Innocenti et al.[34] stated recently that the role of *T. harzianum* was unclear and this species was not responsible for disease in oyster mushroom farms. The results of molecular identification in the case of *Trichoderma* strains from Serbian shiitake farms indicated that THSC was the predominant *Trichoderma* species. Also, previous studies showed that THSC was the prevailing group on shiitake mushroom.[7–9]

Table 5. Effect of the fungicide prochloraz on the growth of *Trichoderma* strains from oyster mushroom, shiitake and button mushroom.

			Prochloraz					
			I			II		
			ED_{50} (μg mL^{-1})			ED_{50} (μg mL^{-1})		
Species	Cultivated mushroom	Code	Mean	Range	RF	Mean	Range	RF
Trichoderma pleuroti	Oyster mushroom	SZMC 12454	0.14	0.11–0.18	140.00	0.14	0.11–0.18	28.00
		SZMC 24039	0.02	0.01–0.03	20.00	0.02	0.01–0.03	4.00
		KM6	0.001	0.0001–0.001	1.00	0.01	0.01–0.01	2.50
		KM8	0.001	0.0001–0,001	1.00	0.004	0.002–0.006	1.00
		KM11	0.01	0.00–0.01	10.00	0.005	0,003–0.007	1.25
Trichoderma pleuroticola		SZMC 23033	0.05	0.04–0.06	50.00	0.03	0.03–0.05	7.50
		SZMC 24040	0.05	0.04–0.07	50.00	0.03	0.02–0.05	7.50
		KG6	0.002	0.001–0.003	2.00	0.004	0.002–0.006	1.00
		KM4	0.01	0.01–0.02	10.00	0.01	0.00–0.01	2.00
		KM5	0.001	0.0004–0.002	1.00	0.01	0.01–0.02	2.00
		KM12	0.004	0.003–0.005	4.00	0.005	0.003–0.009	1.00
Trichoderma harzianum species complex (THSC)		KG10	0.01	0.01–0.02	1.00	0.01	0.00–0.02	1.00
		KG13	0.01	0.00–0.01	1.00	0.02	0.01–0.03	2.00
	Shiitake	T57	0.07	0.05–0.1	2.33	0.01	0.01–0.01	1.00
		T58	0.04	0.03–0.06	1.33	0.02	0.02–0.03	2.00
		T59	0.03	0.02–0.04	1.00	0.05	0.04–0.07	5.00
	Button mushroom*	T10	0.21*	0.09–1.13*	5.25**	nd	nd	nd
		T52	0.04*	0.03–0.05*	1.00 **	nd	nd	nd
		T54	3.64*	0.76–186.60*	91.0**	nd	nd	nd
Trichoderma aggressivum f. *europaeum*		T76	1.34*	0.50–8.49*	33.5**	nd	nd	nd
		T77	0.04*	0.03–0.07*	1.00**	nd	nd	nd
		T85	0.26*	0.15–0.60*	6.50**	nd	nd	nd

RF, resistance factor; ED_{50}, effective dose which inhibits mycelial growth to 50%.
*Kosanović et al.[17]
**Kosanović.[31]
nd, no data.

Table 6. Sensitivity of *Trichoderma harzianum* species complex (THSC) strains from button mushroom, shiitake and oyster mushroom to the fungicides metrafenone and prochloraz.

		Metrafenone				Prochloraz			
		ED_{50} (μg mL^{-1}) mean and range		Resistance factor		ED_{50} (μg mL^{-1}) mean and range		Resistance factor	
Species	Code	I assay	II assay	RFI	RFII	I assay	II assay	RFI	RFII
THSC (button mushroom)	T10	0.03 (0.01–0.05)	0.21 (0.09–1.13)	1.00	5.25	0.72* (0.48–1.34)	nd	5.25**	nd
	T52	0.09 (0.06–0.17)	0.04 (0.03–0.05)	3.00	1.00	0.07* (0.03–0.11)	nd	1.00**	nd
	T54	1.26 (0.41–12.63)	3.64 (0.76–186.60)	42.00	91.00	0.10* (0.05–0.16)	nd	91.00**	nd
THSC (shiitake)	T57	2.91 (0.42–5679.04)	3.63 (0.52–4747.91)	18.19	20.16	0.07 (0.05–0.1)	0.01 (0.01–0.01)	2.33	1.00
	T58	0.25 (0.15–0.55)	0.28 (0.17–0.61)	1.56	1.55	0.04 (0.03–0.06)	0.02 (0.02–0.03)	1.33	2.00
	T59	0.16 (0.08–0.47)	0.18 (0.09–0.56)	1.00	1.00	0.03 (0.02–0.04)	0.05 (0.04–0.07	1.00	5.00
THSC (oyster mushroom)	KG10	0.05 (0.04–0.06)	0.01 (0.00–0.01)	1.25	1.00	0.01 (0.01–0.02)	0.01 (0.00–0.02)	1.00	1.00
	KG13	0.04 (0.03–0.06)	0.06 (0.04–0.09)	1.00	6.00	0.01 (0.00–0.01)	0.02 (0.01–0.03)	1.00	2.00

ED_{50}, effective dose which inhibits mycelial growth to 50%; RFI, resistance factor for first assay; RFII, resistance factor for second assay.
*Kosanović et al.[17] .
**Kosanović[31].
nd, no data.

T. pleuroti strains from oyster mushroom, as well as THSC strains from shiitake and oyster mushroom had their optimum growth temperature at 30 °C, which was in accordance with Kosanović et al.[17] for THSC strains from button mushroom. Moreover, 25 °C was the optimum temperature for *T. pleuroticola* strains from oyster mushroom in the current study and for *T. aggressivum* f. *europaeum* from button mushroom.[17]

Using the method of Bonnen and Hopkins[26] in this study, the virulence assay showed that *T. pleuroti, T. pleuroticola* and THSC strains from oyster mushroom displayed very low virulence levels on fruiting bodies (0 or 1), while THSC strains from shiitake displayed high virulence levels (5). Kosanović et al.[17] showed that virulence levels for *Trichoderma (T. aggressivum* f. *europaeum* and THSC) strains from button mushroom were between 3 and 5 on the same scale. The results of virulence assays indicated that THSC strains were more virulent to fruiting bodies than aggressive *Trichoderma* species from cultivated button mushrooms, accordingly to Kosanović et al.[17] The oyster

mushroom displayed weaker reaction to *Trichoderma* conidial inoculation on pilei than shiitake and button mushroom. Perhaps the source of isolation of *Trichoderma* strains indicated their level of virulence. Those originated from the growing substrate of oyster mushroom seriously reduced the crop yield, without producing green mold symptoms on fruiting bodies. On the contrary, *Trichoderma* strains from shiitake collected from fruiting bodies showed evident disease symptoms on edible mushroom basidiomata. According to the criteria of Gea et al.[27] and Grogan et al.[18], all tested *Trichoderma* strains from Serbia, North Macedonia, Croatia and Hungary were sensitive to the fungicides metrafenone and prochloraz, since their ED_{50} values were less than 3.6 and 0.8 μg mL^{-1}, respectively. Prochloraz was more toxic than metrafenone to all tested *Trichoderma* strains from cultivated mushrooms. Previously, Kosanović et al.[17] reported that prochloraz had a high toxicity to all *Trichoderma* strains from button mushroom farms, as its ED_{50} values were below 0.4 μg mL^{-1} for *T. aggressivum* f. *europaeum* and less than 0.7 μg mL^{-1} for *T. harzianum* (THSC). According to Kosanović et al.[17], prochloraz had the highest antifungal effect on causal agents of green mold disease in button mushroom farms. The tested *Trichoderma* strains were highly resistant to both tiophanate-methyl and trifloxystrobin. The fungicides carbendazim, iprodione, and chlorothalonil showed similar toxicity levels to that of prochloraz toward both *T. aggressivum* f. *europaeum* and *T. harzianum* strains. Nevertheless, Spanish and Serbian strains of *L. fungicola* were resistant to iprodione, while the benzimidazole carbendazim was withdrawn from the market, and chlorothalonil induced toxicity problem in the mycelial growth of *A. bisporus* strains F56, B62, B98, and U3.[15,27,35]

A few studies investigated the sensitivity of *Trichoderma* strains from oyster mushroom to fungicides, showing that prochloraz inhibited spore germination and mycelial growth of *Trichoderma* strains without any negative effect on oyster mushroom.[10,32–34] Innocenti et al.[34] reported that prochloraz was effective against *T. pleuroti* and *T. pleuroticola* at the concentrations of 0.25 and 1.25 μL mL^{-1} (field doses), respectively. In the present study, *T. pleuroticola* was less sensitive than *T. pleuroti* and THSC from oyster mushroom farms to both tested fungicides, similarly to the findings of Innocenti et al.[34], where *T. pleuroti* was more aggressive than *T. pleuroticola* against *P. ostreatus* in both *in vitro* and *in vivo* experiments. The sensitivities of all *T. pleuroti* strains from three different countries to metrafenone fell into the same range. As for *T. pleuroticola*, strains from Croatia and Serbia were more sensitive to metrafenone than those from Hungary and North Macedonia. Concerning prochloraz, the Hungarian *T. pleuroticola* strain was the least susceptible, followed by that from Croatia, and strains from North Macedonia were the most sensitive. Strains of *T. pleuroticola* were divided into two sensitivity groups: less sensitive from Hungary and Croatia, and more sensitive from Serbia and North Macedonia. According to the criteria given by Gouot[29], the tested strains from mushroom cultivation showed different levels of sensitivity to selected fungicides. The least sensitive strains to both fungicides were the Hungarian strains of *T. pleuroti* (SZMC 12454) and *T. pleuroticola* (SZMC 23033) from oyster mushroom cultivation. In addition, *Trichoderma* strains from oyster mushroom cultivation were more sensitive to prochloraz than to metrafenone. They were also more sensitive to both fungicides than the strains from button mushroom and shiitake. Strains of *T. pleuroti* were the least sensitive, followed by *T. pleuroticola,* while THSC strains were the most sensitive to prochloraz. The highest sensitivity to metrafenone was also shown by THSC, followed by *T. pleuroticola* and *T. pleuroti.* On the contrary, results of Hatvani et al.[10] and Innocenti and Montanari[36] showed that *T. pleuroti* and *T. pleuroticola* were more sensitive to prochloraz than *T. harzianum*. In addition, Innocenti et al.[34] reported that prochloraz at the highest doses of 0.25 and 1.25 μL mL^{-1} reduced colony growth rate of *Trichoderma* strains from oyster mushroom farms with 22.3–100% and 86.7–100%, respectively. Kredics et al. [3] found minimum inhibitory concentration (MIC) values of prochloraz <6 μg mL^{-1} for *T. aggressivum* f. *europaeum* (CBS 100525 and B1), *T. aggressivum* f. *aggressivum* (CBS 100527), *T. harzianum* (C8) and *T. pleuroti* (C15). Additionally, Hatvani et al.[10] reported MIC values of prochloraz for Croatian strains: 1.25 μg mL^{-1} for *T. pleuroti* and *T. pleuroticola* and 5 μg mL^{-1} for *T. harzianum* (THSC).

No reports are available in the literature about the fungicide sensitivity of *Trichoderma* species pathogenic to *L. edodes*. In the present study, all tested strains from shiitake cultivation were sensitive to the examined fungicides, with prochloraz being more toxic than metrafenone. The fungicide metrafenone was recently introduced for the control of fungal pathogens, namely, *Cladobotryum* spp. and *L. fungicola*, in many countries, but its effect on *Trichoderma* species was not known.[21] The results of this study indicated that metrafenone may be recommended for the control of green mold disease of edible mushrooms after further efficacy trials *in vivo*. The *Trichoderma* taxon most sensitive to both tested fungicides from the cultivated mushrooms (button mushroom, oyster mushroom and shiitake) was THSC. Hermosa et al.[37] stated that aggressive forms probably originated from *T. harzianum* sources with better adoptability to the conditions in button mushroom groves and that they are all phylogenetically not distant. The close phylogenetic relationship of *Trichoderma* species from oyster mushroom with aggressive *Trichoderma* species from button mushrooms was also noticed.[5,6]

Conclusions

The predominant causal agents of green mold disease on oyster mushroom in Serbia and North Macedonia are THSC, *T. pleuroti* and *T. pleuroticola,* yet *T. pleuroti* was not found in Serbia during this study. On shiitake, the prevalent causal agent of green mold disease in Serbia is THSC. Both tested fungicides, prochloraz and metrafenone, were effective against various taxa of the genus *Trichoderma* (THSC, *T. aggressivum* f. *europaeum*, *T. pleuroti,* and *T. pleuroticola*) isolated from the cultivation of edible

mushrooms (button mushroom, oyster mushroom, and shiitake), and THSC was the most sensitive taxon. Prochloraz, which is an officially recommended fungicide for mushroom disease control in EU countries, proved to be more effective to all tested *Trichoderma* strains than metrafenone. However, the toxicity of metrafenone was also satisfactory and might be recommended for the control of green mold diseases in mushroom farms.

Disclosure statement

We confirm that the research described in the manuscript is the original work of the authors that has not been previously published, in whole or in part, and that it is not under consideration by any other journal.

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References

[1] Grogan, H. M. Challenges Facing Mushroom Disease Control in the 21st Century. In *Proceeding of the Sixth International Conference on Mushroom Biology and Mushroom Products*, Lelley, J.I., Buswell, J.A., Eds.; Bonn, Germany: WSMBMP, **2008**; pp 120–127.

[2] Seaby, D. A. Investigation of the Epidemiology of Green Mold of Mushroom (*Agaricus bisporus*) Compost Caused by *Trichoderma harzianum*. *Plant Pathol.* **1996**, *45*, 913–923. DOI: 10.1111/j.1365-3059.1996.tb02902.x.

[3] Kredics, L.; Jimenez, L. G.; Naeimi, S.; Czifra, D.; Urbán, P.; Manczinger, L.; Vágvölgyi, C.; Hatvani, L. A Challenge to Mushroom Growers: The Green Mould Disease of Cultivated Champignons. In. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, Mendez-Vilas, A., Ed.; FORMATEX **2010**; 295–305

[4] Samuels, G. J.; Dodd, S. L.; Gams, W.; Castlebury, L. A.; Petrini, O. *Trichoderma* Species Associated with the Green Mold Epidemic of Commercially Grown *Agaricus bisporus*. *Mycologia* **2002**, *94*, 146–170. DOI: 10.2307/3761854.

[5] Park, M. S.; Bae, K. S.; Yu, S. H. Two New Species of *Trichoderma* Associated with Green Mold of Oyster Mushroom Cultivation in Korea. *Mycobiology* **2006**, *34*, 111–113. DOI: 10.4489/MYCO.2006.34.3.111.

[6] Komon-Zelazowska, M.; Bissett, J.; Zafari, D.; Hatvani, L.; Manczinger, L.; Woo, S.; Lorito, M.; Kredics, L.; Kubicek, C. P.; Druzhinina, I. S. Genetically Closely Related but Phenotypically Divergent *Trichoderma* Species Cause Green Mold Disease in Oyster Mushroom Farms Worldwide. Appl. Environ. Microbiol. **2007**, *73*, 7415–7426. DOI: 10.1128/AEM.01059-07.

[7] Lee, H. M.; Bak, W. C.; Lee, B. H.; Park, H.; Ka, K. H. Breeding and Screening of *Lentinula edodes* Species Resistance to *Trichoderma* spp. *Mycobiology* **2008**, *36*, 270–272. DOI: 10.4489/MYCO.2008.36.4.270.

[8] Kim, C. S.; Park, M. S.; Kim, S. C.; Maekawa, N.; Yu, S. H. Identification of *Trichoderma*, a Competitor of Shiitake Mushroom (*Lentinula edodes*), and Competition between *Lentinula edodes* and *Trichoderma* Species in Korea. *Plant Pathol. J.* **2012**, *28*, 137–148. DOI: 10.5423/PPJ.2012.28.2.137.

[9] Wang, G.; Cao, X.; Ma, X.; Guo, M.; Liu, C.; Yan, L.; Bian, Y. Diversity and Effect of *Trichoderma* spp. associated with Green Mold disease on *Lentinula edodes* in China. Microbiologyopen. **2016**, *5*, 709–718. DOI: 10.1002/mbo3.364.

[10] Hatvani, L.; Sabolić, P.; Kocsubé, S.; Kredics, L.; Czifra, D.; Vágvölgyi, C.; Kaliterna, J.; Ivić, D.; Đermić, E.; Kosalec, I. The First Report on Mushroom Green mould disease in Croatia. *Arh. Hig. Rada. Toksikol.* **2012**, *63*, 481–487. DOI: 10.2478/10004-1254-63-2012-2220.

[11] Beyer, D. M.; Kremser, I. J. Evaluation of Fungicide Tolerance and Control for Three Fungal Diseases of Mushrooms. In *Science and Cultivation of Edible and Medicinal Fungi, Mushroom Science XVI*, Romaine, C.P., Keil, C.B., Rinker, D.L., Royse, D.J., Eds.,; Philadelphia, PA: Penn State University, **2004**; pp 521–529.

[12] Anonymous. Grower Resources, Integrated Pest Management, Pesticides, Fungicides. American Mushroom Institute. https://www.americanmushroom.org/integrated-pest-management/fungicides/. (accessed May 14, 2020).

[13] Potočnik, I.; Vukojević, J.; Stajić, M.; Tanović, B.; Todorović, B. Fungicide Sensitivity of Selected *Verticillium fungicola* Isolates from *Agaricus bisporus* Farms. *Arch. Biol. Sci.* **2008**, *60*, 151–157.

[14] Potočnik, I.; Vukojević, J.; Stajić, M.; Rekanović, E.; Milijašević, S.; Todorović, B.; Stepanović, M. *In Vitro* Toxicity of Selected Fungicides from the Groups of Benzimidazoles and Demethylation Inhibitors to *Cladobotryum dendroides* and *Agaricus bisporus*. J. Environ. Sci. Health. B. **2009**a, *44*, 365–370. DOI: 10.1080/03601230902801059.

[15] Potočnik, I.; Vukojević, J.; Stajić, M.; Rekanović, E.; Milijašević, S.; Stepanović, M.; Todorović, B. Toxicity of Fungicides with Different Modes of Action to *Cladobotryum dendroides* and *Agaricus bisporus*. J. Environ. Sci. Health. B. **2009**b, *44*, 823–827. DOI: 10.1080/03601230903238699.

[16] Potočnik, I.; Vukojević, J.; Stajić, M.; Tanović, B.; Rekanović, E. Sensitivity of *Mycogone perniciosa*, Pathogen of Culinary-Medicinal Button Mushroom *Agaricus bisporus* (J. Lge) Imbach (Agaricomycetideae), to Selected Fungicides and Essential Oils. *Int. J. Med. Mushrooms* **2010**, *12*, 91–98.

[17] Kosanović, D.; Potočnik, I.; Duduk, B.; Vukojević, J.; Stajić, M.; Rekanovič, E.; Milijašević- Marčić, S. *Trichoderma* Species on *Agaricus bisporus* Farms in Serbia and Their Biocontrol. Ann. Appl. Biol. **2013**, *163*, 218–230. DOI: 10.1111/aab.12048.

[18] Grogan, H. M.; Keeling, C.; Jukes, A. A. In vitro Response of the Mushroom Pathogen Verticillium fungicola (Dry Bubble) to Prochloraz-Manganese. Presented at the Crop Protection Conference, in: Pests and Diseases, Brighton, UK; **2000**, pp 273–278.

[19] Gea, F. J.; Navarro, M. J.; Tello, J. C. Reduced Sensitivity of the Mushroom Pathogen *Verticillium fungicola* to Prochloraz-Manganese *in vitro*. Mycol. Res. **2005**, *109*, 741–745. DOI: 10.1017/s095375620500242x.

[20] Grogan, H. M. Fungicide Control of Mushroom Cobweb Disease Caused by *Cladobotryum* Strains with Different Benzimidazole Resistance Profiles. *Pest Manag. Sci.* **2006**, *62*, 153–161. DOI: 10.1002/ps.1133.

[21] Carrasco, J.; Navarro, M. J.; Santos, M.; Gea, F. J. Effect of Five Fungicides with Different Modes of Action on Mushroom Cobweb Disease (*Cladobotryum mycophilum*) and Mushroom Yield. Ann. Appl. Biol. **2017**, *171*, 62–69. DOI: 10.1111/aab.12352.

[22] Chrysayi-Tokousbalides, M.; Kastanias, M. A.; Philippoussis, A.; Diamantopoulou, P. Selective Fungitoxicity of Famaxadone, Tebuconazole and Trifloxystrobin between *Verticillium fungicola* and *Agaricus bisporus*. *Crop Prot.* **2007**, *26*, 469–475. DOI: 10.1016/j.cropro.2006.02.016.

[23] Urbán, P. Termesztett Laskagomba Trichoderma Közösségeinek Biodiverzitása és Ökofiziológiai Vizsgálata. MSc Thesis, University of Szeged, Szeged, Hungary, **2012**.

[24] Hatvani, L.; Antal, Z.; Manczinger, L.; Szekeres, A.; Druzhinina, I. S.; Kubicek, C. P.; Nagy, A.; Nagy, E.; Vágvölgyi, C.; Kredics, L. Green Mold Diseases of *Agaricus* and *Pleurotus* spp. Are Caused by Related but Phylogenetically Different *Trichoderma* Species. *Phytopathology* **2007**, *97*, 532–537. DOI: 10.1094/PHYTO-97-4-0532.

[25] Hatvani, L.; Homa, M.; Chenthamara, K.; Cai, F.; Kocsubé, S.; Atanasova, L.; Mlinaric-Missoni, E.; Manikandan, P.; Revathi, R.; Dóczi, I.; et al. Agricultural Systems as Potential Sources of Emerging Human Mycoses Caused by *Trichoderma*: A Successful, Common Phylotype of *Trichoderma longibrachiatum* in the Frontline. *FEMS Microbiol. Lett.* **2019**, *366*, fnz246. DOI: 10.1093/femsle/fnz246.

[26] Bonnen, A. M.; Hopkins, C. Fungicide Resistance and Population Variation in *Verticillium fungicola*, a Pathogen of the Button Mushroom, *Agaricus bisporus*. Mycol. Res. **1997**, *101*, 89–96. DOI: 10.1017/S0953756296002237.

[27] Gea, F. J.; Tello, J. C.; Honrubia, M. *In vitro* Sensitivity of *Verticillium fungicola* to Selected Fungicides. *Mycopathologia* **1996**, *136*, 133–137. DOI: 10.1007/BF00438918.

[28] Delp, C. J.; Dekker, J. Fungicide Resistance: definitions and Use of Terms. *EPPO Bull. Eur. Mediterranean Plant Protect. Organ.* **1985**, *15*, 333–335. DOI: 10.1111/j.1365-2338.1985.tb00237.x.

[29] Gouot, J. M. Characteristics and Population Dynamics of *Botrytis cinerea* and Other Pathogens Resistant to Dicarboximides. In *Fungicide Resistance in North America*. Delp, C.J. Ed.; St. Paul, MO: The American Phytopathological Society, **1994**; pp 53–55.

[30] Druzhinina, I. S.; Kopchinskiy, A. G.; Komoń, M.; Bissett, J.; Szakacs, G.; Kubicek, C. P. An Oligonucleotide Barcode for Species Identification in *Trichoderma* and *Hypocrea*. *Fungal. Genet. Biol.* **2005**, *42*, 813–828. DOI: 10.1016/j.fgb.2005.06.007.

[31] Kosanović, D. *Trichoderma* Species Causing Green Mould Diseases of Button Mushroom [*Agaricus bisporus* (Lange) Imbach] and Their Sensitivity to Fungicides and Biofungicides. PhD thesis, Faculty of Biology, University of Belgrade, Belgrade, Serbia; **2015**.

[32] Hatvani, L. Mushroom Pathogenic *Trichoderma* Species: Occurrence, Diagnosis and Extracellular Enzyme Production. PhD thesis, University of Szeged, Szeged, Hungary; **2008**,

[33] Woo, S. L.; Di Benedetto, P.; Senatore, M.; Gigante, S.; Soriente, I.; Ferraioli, S.; Scala, F.; Lorito, M. Identification and Characterization of *Trichoderma* Species Aggressive to *Pleurotus* in Italy. *J. Zhejiang Univ. (Agriculture and Life Sciences)* **2004**, *30*, 469–470.

[34] Innocenti, G.; Montanari, M.; Righini, H.; Roberti, R. *Trichoderma* Species Associated with Green Mould Disease of *Pleurotus ostreatus* and Their Sensitivity to Prochloraz. *Plant Pathol.* **2019**, *68*, 392–398. DOI: 10.1111/ppa.12953.

[35] Chalaux, N.; Savoie, J. M.; Olivier, J. M. Growth Inhibition of *Agaricus bisporus* and Associated *Thermophilic* Species by Fungicides Used in Wheat Cultivation. *Agronomie* **1993**, *13*, 407–412. DOI: 10.1051/agro:19930505.

[36] Innocenti, G.; Montanari, M. *Trichoderma* Green Mould Disease: A Case Study in a *Pleurotus ostreatus* Farm. Preliminary Data. *Micologia Italiana* **2014**, *1-2-3*, 49–57.

[37] Hermosa, M. R.; Grondona, I.; Iturriaga, E. A.; Diaz-Minguez, J. M.; Castro, C.; Monte, E.; Garcia-Acha, I. Molecular Characterization and Identification of Biocontrol Isolates of *Trichoderma* spp. Appl. Environ. Microbiol. **2000**, *66*, 1890–1898. DOI: 10.1128/aem.66.5.1890-1898.2000.