Acta Biologica Szegediensis

Volume 66(2):100-105, 2022
Acta Biologica Szegediensis
http://abs.bibl.u-szeged.hu/index.php/abs
DOI:10.14232/abs.2022.2.100-105

ARTICLE

Investigation of the efficiency of some fungicides and disinfectants applied in *Agaricus bisporus* cultivation

András Misz[1],†, Henrietta Allaga[2],†, Rita Büchner[2], András Varga[2], Nóra Bakos-Barczi[1], Judit Bajzát[1], László Kredics[2], Csaba Csutorás[3], Csaba Vágvölgyi[2]*

[1]New Champignons Ltd., Budapest, Hungary
[2]University of Szeged, Faculty of Science and Informatics, Department of Microbiology, Szeged, Hungary
[3]Eszterházy Károly Catholic University, Eger, Hungary

ABSTRACT Pathogenic moulds (e.g., *Trichoderma, Hypomyces,* and *Lecanicillium*) cause huge problems in industrial scale production of *Agaricus bisporus*. It is important to choose the right control strategy, either chemical or biocontrol-based, to prevent substantial economic losses. The present study was carried out to reveal the *in vitro* efficacy of some fungicides and disinfectants against causative agents of wet bubble (*Hypomyces perniciosus*), dry bubble (*Lecanicillium fungicola* var. *fungicola*), cobweb disease (*Hypomyces odoratus*), and green mould (e.g., *Trichoderma aggressivum* f. *aggressivum*, *T. aggressivum* f. *europaeum*, *T. harzianum*), the four most devastating fungal infections in mushroom production. Prochloraz was efficient against *Trichoderma* and *H. perniciosus* isolates. Metrafenone did not cause complete inhibition for any of the isolates even at the highest concentration (5%) tested. In both cases *Lecanicillium* isolates displayed the lowest growth rate inhibition. Concerning the tested disinfectants, treatment with Sekusept Aktiv resulted in complete growth inhibition for all isolates in the concentration range of 1.25-5%. Terralin Protect, Disinflex and Formalin were also effective, except against the tested isolates of *T. agressivum* f. *agressivum* which proved to be the most resistant.
Acta Biol Szeged 66(2):100-105 (2022)

KEY WORDS
champignon
disinfectant
dry bubble
fungicides
green mould
cobweb disease
mushroom diseases
wet bubble

ARTICLE INFORMATION
Submitted
22 November 2022
Accepted
16 December 2022
***Corresponding author**
E-mail: csaba@bio.u-szeged.hu

Introduction

In 2013, world champignon (button mushroom, *Agaricus bisporus*) production was 4.43 million tons in Europe and North America, accounting for 15% of total mushroom production worldwide (Royse et al. 2017; Kredics et al. 2022). The industrial scale *Agaricus* production is based on a complex multistate process (Carrasco and Preston 2020). Mushrooms are growing on a special compost, which usually contains wheat straw, gypsum, and chicken manure. This mixture is processed through a composting process consisting of fermentation (Phase I), pasteurization (Phase II), conditioning and culturing with grains inoculated with *A. bisporus* mycelium (Phase III). When the compost is colonized, it is covered with a casing soil, which is required for maintaining moisture, developing a specific microbiota, and trapping several metabolites. Black peat is applied worldwide as casing material because of its outstanding properties, with an ideal pH of 7-8.

Mushroom production is affected by many biotic and abiotic factors. Among the several biotic factors associated with reduction in mushroom yield, fungal diseases are especially important. All the substrates and optimal conditions used in different production phases are favourable also for competing/pathogenic fungi. The most common fungal diseases of white button mushroom are (Berendsen et al. 2010, 2012; Zhang et al. 2017; Gea et al. 2021; Kredics et al. 2022):

- dry bubble, *Lecanicillium fungicola* (Preuss) Zare and Gams [syn.: *Verticillium fungicola* (Preuss) Hassebrauk, *Verticillium malthousei* (Preuss) Ware]
- wet bubble, *Hypomyces perniciosus* Magnus [syn.: *Mycogone perniciosa* (Magnus) Delacr.]
- cobweb disease, e.g., *Hypomyces odoratus* G.R.W. Arnold [syn.: *Cladobotryum mycophilum* (Oudem.) W. Gams & Hooz]
- green mold, e.g., *T. aggressivum* f. *aggressivum* (formerly known as *T. harzianum* Th4)

There are different approaches to prevent these fungal diseases. Maintaining technological rigor with proper hygienic measures is a basic requirement. Biocontrol strategies based on the application of selected microorganisms could be also effective; such successful experi-

†These authors contributed equally to this work and share first authorship.

Table 1. Fungal strains used in this study.

Strains	SZMC number*	Source / Country
Lecanicillium fungicola var. *fungicola*	SZMC 20790	*A. bisporus* / Serbia
L. fungicola var. *fungicola***	SZMC 23852	*A. bisporus* / Serbia
L. fungicola var. *fungicola*	SZMC 23856	*A. bisporus* / Ireland
L. fungicola var. *fungicola***	SZMC 23857	*A. bisporus* / Ireland
Hypomyces odoratus	SZMC 20795	*A. bisporus* / Serbia
H. odoratus	SZMC 23817	*A. bisporus* / Hungary
Hypomyces perniciosus	SZMC 20792	*A. bisporus* / Croatia
H. perniciosus	SZMC 20793	*A. bisporus* / Serbia
Trichoderma aggressivum f. *aggressivum*	SZMC 23035	*A. bisporus* / Canada
T. aggressivum f. *aggressivum*	SZMC 23834	*A. bisporus* / Hungary
T. aggressivum f. *aggressivum*	SZMC 26663	*A. bisporus* / Hungary
T. aggressivum f. *aggressivum*	SZMC 26664	*A. bisporus* / Hungary
T. aggressivum f. *aggressivum*	SZMC 26665	*A. bisporus* / Hungary
T. aggressivum f. *aggressivum*	SZMC 26666	*A. bisporus* / Hungary
T. aggressivum f. *europaeum*	SZMC 1811	*A. bisporus* / Ireland
T. aggressivum f. *europaeum*	SZMC 1746	*A. bisporus* / Hungary
T. decipiens	SZMC 24111	*A. bisporus* /Hungary
T. decipiens	SZMC 24112	*A. bisporus* / Hungary
T. harzianum	SZMC 1844	*A. bisporus* / Croatia
A. bisporus	SZMC 23395	- / Hungary

*SZMC: Szeged Microbiology Collection; **Contains mycoviruses (Kartali et al. 2017).

ments were carried out with *Bacillus velezensis* and *Bacillus subtilis* strains (Büchner et al. 2022; Potočnik et al. 2019). Challenging experiments were carried out to find disease resistant mushroom lines for the production, but this approach remained in the research phase until now (Dragt et al. 1995; Wuest and Harvey 1978). The most frequent approach is the use of chemical compounds or mixtures of various chemicals. Rarely these are of natural origin (Altaf et al. 2022), but traditionally mainly synthetic chemicals (fungicides or disinfectants) are used against these devastating moulds (Bhatt and Singh 2002).

Approved fungicides for mushroom production were not specifically developed against mushroom pathogens, therefore, the mushroom industry faces side problems such as reduced specificity, regulatory limitations for their use, as well as resistance outbreaks among pathogens. Currently the number of permitted active ingredients with fungicidal activity is limited, therefore, it is important to investigate the effectiveness of available fungicides and disinfectants which could promote the design of integrated disease management (IDM) programmes.

In this study the effect of two commercial fungicides (Harvinta and Sporgon 50 WP) and four disinfectants (Sekusept Active, Terralin Protect, Disinflex, and Formalin) were tested against 19 isolates of *Lecanicillium*, *Hypomyces*, and *Trichoderma*.

Materials and Methods

Fungal isolates

Nineteen strains of mushroom pathogens representing the genera *Lecanicillium* (4), *Hypomyces* (4), and *Trichoderma* (11) were involved in this study (Table 1). The isolates were obtained from the Szeged Microbiology Collection (SZMC, http://www.wfcc.info/ccinfo/collection/by_id/987). For experimental setups, cultures were maintained on commercial potato dextrose agar (PDA) medium (VWR, Debrecen, Hungary). An *Agaricus bisporus* isolate used for commercial scale mushroom production was also involved in the experiments. Species identification was carried out by nucleotide sequence analysis of characteristic markers as described in previous studies (Allaga et al. 2021; Luković et al. 2021).

Fungicide and disinfectant susceptibility tests

Conidial suspensions (10^7 cell/ml) were prepared from strains precultured on PDA media. Similarly, a mycelial suspension was prepared from the non-sporulating *Trichoderma decipiens* isolate.

Two commercial fungicides, approved for fungal cultivation in Europe, Harvinta (BASF, Ludwigshafen, Germany; active ingredient: metrafenone, 500 g/l) and Sporgon 50 WP (BASF Agro B.V, Zürich Branch, Switzerland; active ingredient: prochloraz, 461 g/kg) and four

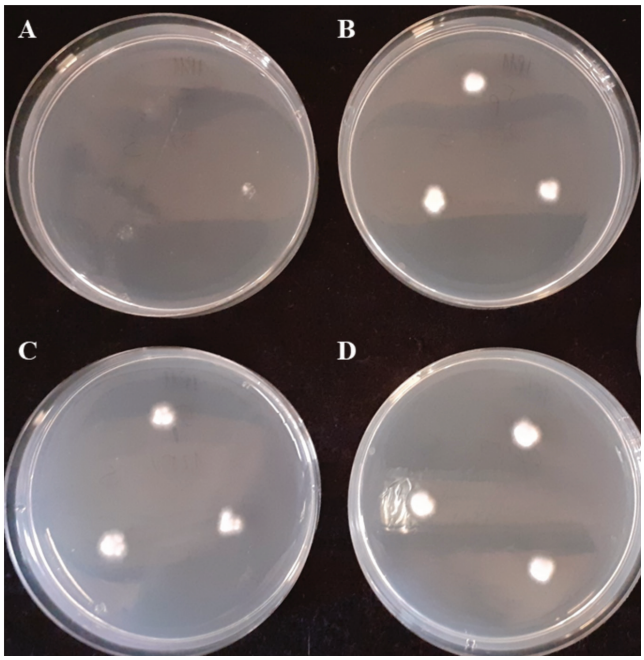

Figure 1. *Lecanicillium fungicola* var. *fungicola* SZMC 23852 strain on PDA plates with different prochloraz concentrations after 4 days of incubation. A: 5%, B: 2.5%, C: 1.25%, D: 0.625%.

disinfectants, Sekusept Active (Ecolab, Oer-Erkenschwick, Germany; active ingredient: peracetic acid), Terralin Protect (Schülke, Norderstedt, Germany; active ingredient: 22 g benzalkonium chloride; 17 g 2-phenoxy-ethanol; 0.9 g amino alkylglycine (amines, n-C10-16-alkyl-trimethylenedi-, reaction products with chloroacetic acid) /100 g), Disinflex (Hexachem, Bökény, Hungary; active ingredient: benzil-C12-16-alkyl-dimethyl-ammonium-chloride 15-25%, didecyl-dimethyl-ammonium-chloride 10-20%, glutaraldehyde 5-35%, isopropanol 1-5%, methanol < 0,05%), and formalin (VWR, Debrecen, Hungary, active ingredient: formaldehyde 35%).

The effect of these fungicides and disinfectants on mycelial growth was tested at concentrations of 5, 2.5, 1.25, 0.625%, compared to controls without fungicide and disinfectant treatment. Concentrations were adjusted and spores were treated with these chemicals on 96-well microtiter plates. After 5 min of spore and mycelium treatment, 10 µl of each suspension was inoculated on PDA plates in three replicates (Fig. 1). Untreated conidial/mycelial suspensions were used as controls and the effect of the treatment was expressed as rate of growth inhibition (GI%).

The antifungal of effect of the fungicides prochloraz and metrafenone were also tested with prolonged incubation time at concentrations of 32, 16, 8, 4, 2, and 1 mg/ml. Conidial/mycelial suspensions were drop-inoculated on the surface of PDA plates supplemented with fungicides.

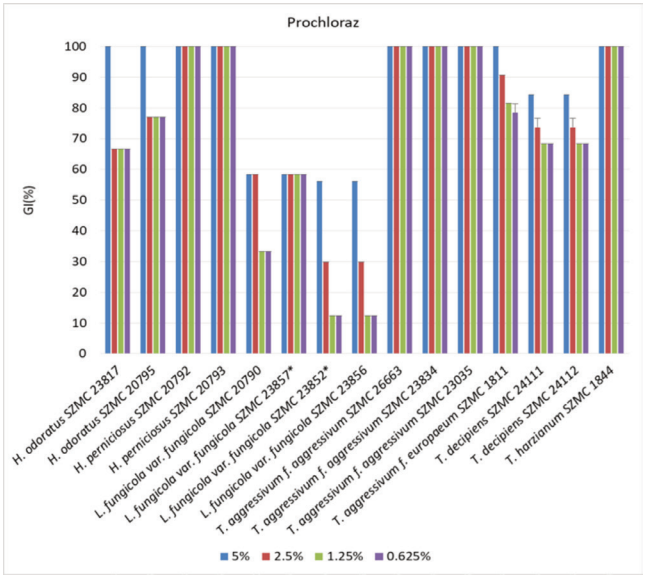

Figure 2. Effect of prochloraz (0.625%-5%) on the growth of the investigated mushroom-pathogenic moulds.

The colony diameters were measured after 2-5 days of incubation at 25 °C. The increase of colony diameters was expressed as growth inhibition (GI%) values compared to the control.

The rate of growth inhibition (GI, %) was calculated using the formula:

$$GI = 100 - (rf/rc \times 100)$$

where rf is the colony radius in the presence of the fun-

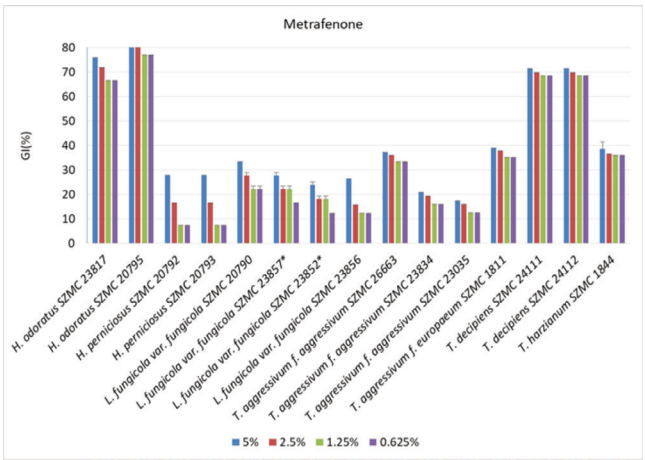

Figure 3. Effect of metrafenone (0.625%-5%) on the growth of the investigated mushroom-pathogenic moulds.

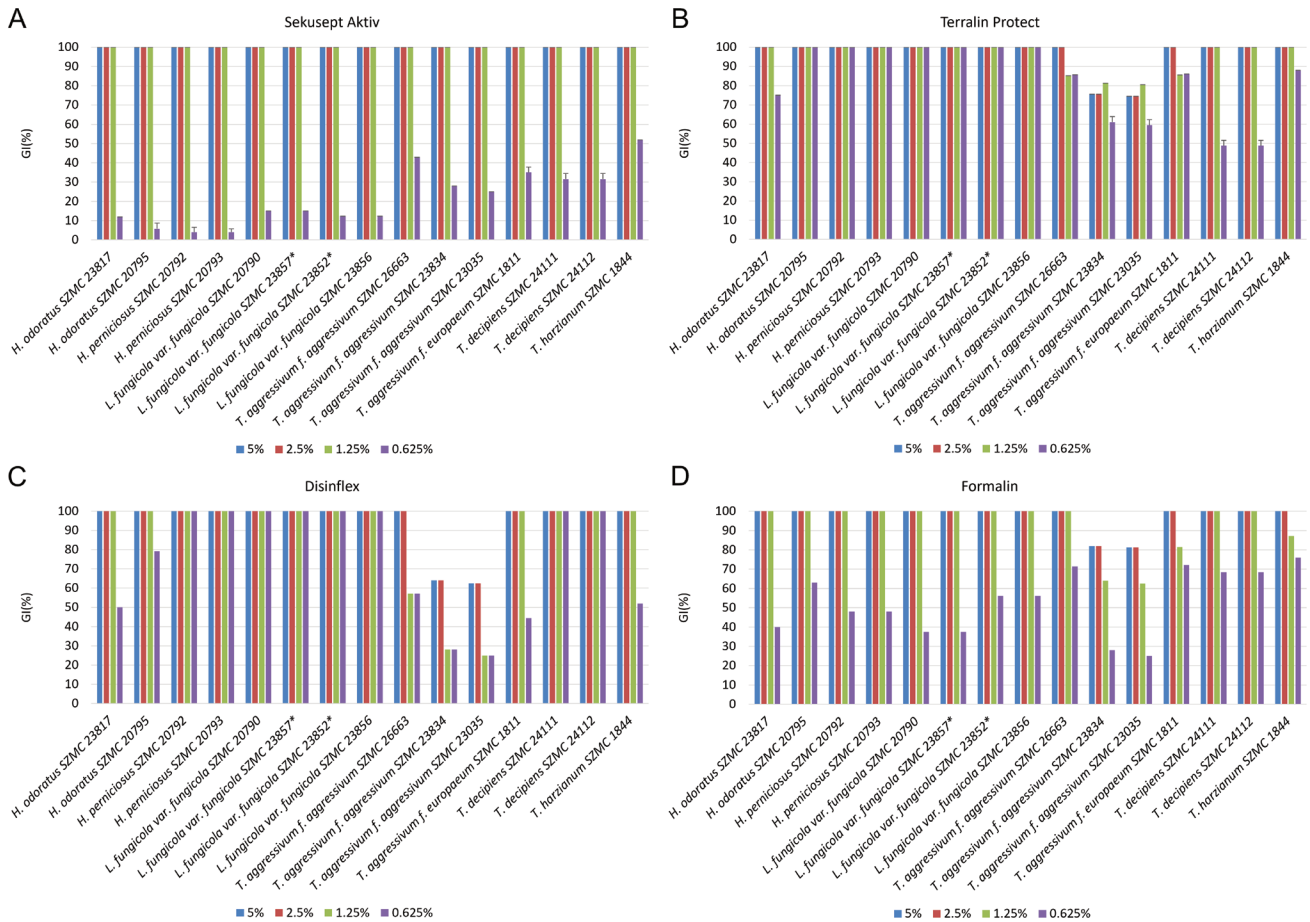

Figure 4. Effect of disinfectants (0.625%-5%) on the growth of the investigated mushroom-pathogenic moulds. A: Sekusept Aktiv. B: Terralin Protect. C: Disinflex. D: Formalin.

gicides at different concentrations, while rc is the colony radius value measured on the control plates (Allaga et al. 2021).

Results and discussions

The influence of two commercial fungicides (Harvinta and Sporgon 50 WP) and four disinfectants (Sekusept Active, Terralin Protect, Disinflex, Formalin) has been tested on 19 fungal isolates collected previously from infected *Agaricus bisporus* compost.

In the first experimental setup, when the interaction of the investigated chemicals and the conidia/mycelial fragments were relatively short (5 min), fungicides were less effective, though even in this case prochloraz (Sporgon 50 WP) was rather efficient against *Trichoderma* and *H. perniciosus* isolates (Fig. 2). Metrafenone did not cause complete inhibition of any of the isolates even at the highest concentration tested (Fig. 3.). In both cases *Lecanicillium* isolates displayed the lowest growth rate inhibition.

Prochloraz-Mn is an imidazole compound widely used in agriculture worldwide. This compound inhibits the enzyme lanosterol 14α-demethylase during ergosterol biosynthesis, thus it has an inhibitory effect on fungal growth and development. Since the compound affects the ergosterol biosynthesis of fungi, it also affects the biosynthesis of sterols found in human and could antagonize oestrogen and androgen receptors, leading to the development of human diseases (Vinggaard et al. 2005). Metrafenone is another widely used fungicide, a benzophenone compound which influences fungal development, including spore germination, appressorium formation, penetration, hyphal surface morphology and sporogenesis (Schmitt et al. 2006).

The short treatment time was more effective when the disinfectants were tested (Fig. 4A-D). Treatment with Sekusept Aktiv resulted in complete growth inhibition of all isolates in the 1.25-5% concentration range. Terralin Protect, Disinflex and Formalin were also effective, except

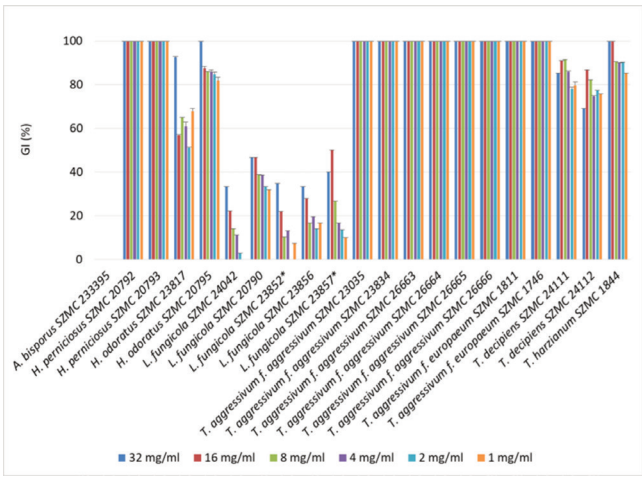

Figure 5. Effect of prochloraz (1-32 mg/ml) on the growth of the investigated mushroom-pathogenic moulds when they were surface-inoculated onto fungicide-supplemented PDA plates.

against the tested isolates of *T. agressivum* f. *agressivum* which proved to be the most resistant (Fig. 4A-D).

Grogan et al. (2015) examined the effect of aldehydes (e.g., Formalin), peroxides, phenol-synthetic phenols, oxidizing agents, halogens, and halogen-releasing compounds, organic acid-aromatic acid, against *T. aggressivum* at concentrations of 2% and 4%. They observed that formalin, aromatic acids, cationic surfactants, halogens, and oxidizing agents completely inhibited the fungal growth after 15- and 60-min treatments.

The experimental setup, when conidial/mycelial suspensions were inoculated onto the surface of fungicide-containing PDA plates, reinforced the above-mentioned observations: prochloraz caused complete inhibition

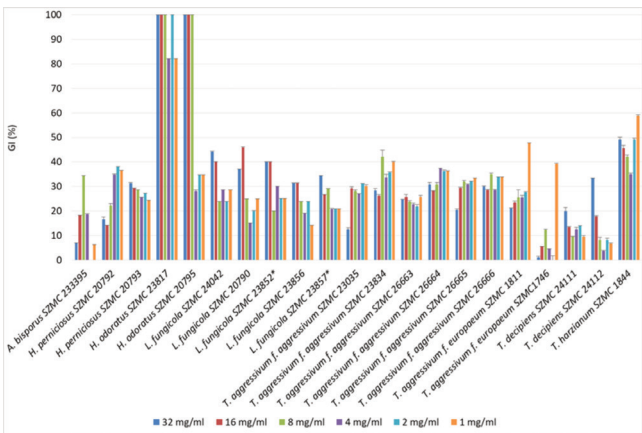

Figure 6. Effect of metrafenone (1-32 mg/ml) on the growth of the investigated mushroom-pathogenic moulds when they were surface-inoculated onto fungicide-supplemented PDA plates.

of *T. aggressivum* and *H. perniciosus* isolates. Substantial inhibition was achieved against *T. decipiens*, *T. harzianum* and *H. odoratus* isolates. *L. fungicola* isolates proved to be the least susceptible (Fig. 5).

As in the other experimental setup, metrafenone was substantially less effective than prochloraz with the greatest inhibition of *H. odoratus* strains. Luković et al. (2021) and Allaga et al. (2021) obtained similar results when the effects of prochloraz and metrafenone were tested on *T. aggressivum* isolates and members of the *T. harzianum* species complex, respectively.

These observations are important as the number of available chemicals for mushroom industry is very limited and even the available compounds have some well-defined side effects, therefore, it is crucial to optimize their use for a safe and economic mushroom production.

ACKNOWLEDGEMENTS

This study was carried out with the professional support of the Doctoral Student Scholarship Program of the Co-operative Doctoral Program of the Ministry of Innovation and Technology financed from the National Research, Development and Innovation Fund (KDP-2021-C1764158, to A. Misz). The authors thank Dr. Lóránt Hatvani for providing his collection of mushroom pathogenic moulds, as well as Dr. András Geösel for the *T. decipiens* strains.

References

Allaga H, Zhumakayev A, Büchner R, Kocsubé S, Szűcs A, Vágvölgyi Cs, Kredics L, Hatvani L (2021) Members of the *Trichoderma harzianum* species complex with mushroom pathogenic potential. Agronomy 11(12):2434.

Altaf S, Jan SK, Basu U, Ahanger SA, Dave A, Kakraliya SS, Baazeem A, Mishra AK, Kumar A, Shah IA, Mushtaq M (2022) Sustainable management of green mold disease of white button mushroom using botanicals and biocontrol agents under temperate conditions. Horticulturae 8:768.

Berendsen RL, Baars JJ, Kalkhove SI, Lugones LG, Wösten HA, Bakker PA (2010) *Lecanicillium fungicola*: Causal agent of dry bubble disease in white-button mushroom. Mol. Plant Pathol 11:585-595.

Berendsen RL, Kalkhove SIC, Lugones LG, Wösten HAB, Bakker PAHM (2012) Germination of *Lecanicillium fungicola* in the mycosphere of *Agaricus bisporus*. Environ Microbiol Rep 4(2):227-233.

Bhatt N, Singh RP (2002) Chemical control of mycoparasites of button mushroom. J Mycol Plant Pathol 32:38-45.

Büchner R, Vörös M, Allaga H, Varga A, Bartal A, Szekeres A, Varga S, Bajzát J, Bakos-Barczi N, Misz A, Csutorás

Cs, Hatvani L, Vágvölgyi Cs, Kredics L (2022) Selection and characterization of a *Bacillus* strain for potential application in industrial production of white button mushroom (*Agaricus bisporus*). Agronomy 12(2):467.

Carrasco J, Preston GM (2020) Growing edible mushrooms: A conversation between bacteria and fungi. Environ Microbiol 22:858-872.

Dragt JW, Geels FP, Rutjens AJ, Van Griensven LJLD (1995) Resistance in wild types of *Agaricus bisporus* to the mycoparasite *Verticillium fungicola* var. *fungicola*. Mushroom Sci 14:679-683.

Gea FJ, Navarro MJ, Santos M, Diánez F, Carrasco J (2021) Control of fungal diseases in mushroom crops while dealing with fungicide resistance: A review. Microorganisms 9(3):585.

Grogan H (2015) Use of chemical disinfectants in mushroom production. MushTV 01/15. https://www.teagasc.ie/publications/2015/use-of-chemical-disinfectants-in-mushroom-production.php

Kartali T, Shahab D, Nyilasi I, Hatvani L, Kredics L, Vágvölgyi Cs, Papp T (2017) Kettősszálú RNS-elemek kimutatása a csiperkegombát fertőző *Lecanicillium* és *Mycogone* törzsekben/Double-stranded RNA elements in *Lecanicillium* and *Mycogene* strains infecting *Agaricus bisporus.* Mikol Közl-Clusiana 56(1):103-105.

Kredics L, Hatvani L, Allaga H, Büchner R, Cai F, Vágvölgyi Cs, Druzhinina IS, Naeimi S (2022) *Trichoderma* green mould disease of cultivated mushrooms. In Amaresan N, Sankaranarayanan A, Dwivedi MK, Druzhinina IS, Eds, Advances in *Trichoderma* Biology for Agricultural Applications. Fungal Biology Series. Springer, Cham. pp 559-606.

Luković J, Milijašević-Marčić S, Hatvani L, Kredics L, Szűcs A, Vágvölgyi C, Duduk N, Vico I, Potočnik I (2021) Sensitivity of *Trichoderma* strains from edible mushrooms to the fungicides prochloraz and metrafenone. J Environ Sci Health B 56:54-63.

Potočnik I, Rekanović E, Todorović B, Luković J, Paunović D, Stanojević O, Milijašević-Marčić S (2019) The effects of casing soil treatment with *Bacillus subtilis* Ch-13 biofungicide on green mould control and mushroom yield. Pestic Phytomed 34:53-60.

Royse DJ, Baars J, Tan Q (2017) Current overview of mushroom production in the world. In Zied DC, Pardo-Giménez A, Eds. Edible and Medicinal Mushrooms: Technology and Applications. 1st Ed, Wiley-Blackwell, Hoboken, NJ, USA, pp 2-13.

Schmitt MR, Carzaniga R, Van T Cotter H, O'Connell R, Hollomon D (2006) Microscopy reveals disease control through novel effects on fungal development: a case study with and early-generation benzophenone fungicide. Pest Manag Sci 63:383-392.

Vinggaard MA, Hass U, Dalgaard M, Andersen HR, Bonefeld-Jørgensen E, Christiansen S, Laier P, Poulsen ME (2005) Prochloraz: an imidazole fungicide with multiple mechanisms of action. Int J Androl 29:186-192.

Wuest PJ, Harvey CL (1978) The nature of disease resistance in strains of the cultivated mushroom, *A. brunnescens*. Mushroom Sci 10:741-746.

Zhang C, Kakishima M, Xu J, Wang Q, Li Y (2017) The effect of *Hypomyces perniciosus* on the mycelia and basidiomes of *Agaricus bisporus*. Microbiology (Reading) 163(9):1273-1282.