Pestic. Phytomed. (Belgrade), 40(2-3), 2025, 63–71

Efficacy of biofungicide *Bacillus subtilis* Ch-13 in the suppression of *Hypomyces odoratus* (cobweb disease) in a large-scale white button mushroom production

Biljana Todorović, Svetlana Milijašević-Marčić, Ljiljana Šantrić, Jelena Luković, Emil Rekanović and Ivana Potočnik*

Institute of Pesticides and Environmental Protection, Banatska 31b, POB 163, 11080 Belgrade-Zemun, Serbia

Article info

Original scientific paper

Received: 14 November 2025

Accepted: 3 December 2025

Publication is free of charge.

*Corresponding author: ivana.potocnik@pesting.org.rs

DOI: https://doi.org/10.2298/PIF2503063T

SUMMARY

More frequent application of the biofungicide *Bacillus subtilis* Ch-13 enhanced its efficacy against natural infestation of *Hypomyces odoratus* (cobweb disease) in white button mushrooms (*Agaricus bisporus*), as well as its positive impact on mushroom production. In two different application procedures, strain *B. subtilis* Ch-13 was used at a final concentration of 60 ml per m^2 of casing layer (1×10^8 CFU ml^{-1}). In comparison to the low efficacy of the fungicide prochloraz (53%), optimal efficacy of *B. subtilis* Ch-13 in the suppression of *H. odoratus* was recorded. Substantially higher efficacy of this biofungicide in cobweb disease control was achieved when it was applied in three split doses (42%), rather than two (30%), in the large-scale production of white button mushrooms. The greatest improvement in white button mushroom production, in comparison to the untreated control, was achieved when *B. subtilis* Ch-13 was applied in three split doses (biological efficiency, BE=15%), rather than two (BE=7%). For suppression of the mycopathogen *H. odoratus* on white button mushrooms, this study supports the application of the biofungicide *B. subtilis* Ch-13 in three split doses of 30 + 15 + 15 ml m^{-2} on the second day after casing, two weeks after casing, and after the first fruiting flush, respectively.

Keywords: edible mushrooms; biocontrol agent; disease control

INTRODUCTION

The causal agent of cobweb disease, *Hypomyces odoratus* (G.R.W. Arnold) [formerly *Cladobotryum mycophilum* (Oudemans) W. Gams & Hooz.], is one of the major fungal pathogens of the white button mushroom [*Agaricus bisporus* (Lange) Imbach] in mainland Europe (Gea et al., 2012; Tamm & Põldmaa, 2013; Carrasco et al., 2016; 2017a; Luković et al., 2021), North America (McKay et al., 1999), Asia (Back et al., 2010; Muhammad et al., 2019), Australia (McKay et al., 1999; Tamm & Põldmaa, 2013), and Africa (Chakwiya et al., 2015; 2019), while *Hypomyces rosellus* (Alb. & Schwein.) Tul. [formerly *Cladobotryum dendroides* (Bull.: Fr.) W. Gams & Hooz.] is detected in the

British Isles (McKay et al., 1999; Fletcher, 2002; Grogan, 2006) and North America (McKay et al., 1999; Tamm & Põldmaa, 2013). These pathogens are soil-borne fungi that are spread by black peat soil, the main component of casing soil in the mushroom substrate. Cobweb disease agents induce crop losses of 28-40%, which recently reduced the number of white button mushroom producers (Grogan, 2006; Carrasco et al., 2016). Cobweb disease agents may also appear mixed with other substantial mycopathogens, such as *Zarea fungicola* (Preuss) Khons., Thanakitp. and Luangsa-ard. [formerly *Lecanicillium fungicola* (Preuss) Zare & W. Gams], *Hypomyces perniciosus* Magnus [formerly *Mycogone perniciosus* (Magnus) Delacroix], and *Trichoderma aggressivum* Samuels & W. Gams. Symptoms of cobweb disease manifest as white fluffy colonies that quickly cover the casing soil and mushroom fruiting bodies, changing colour with sporulation into yellow or pink. Mild symptoms include cap spotting and sunken lesions in mushrooms. Conidia of *Hypomyces odoratus* are dry and easily spread by air flow throughout the growing chamber (Carrasco et al., 2016).

Cobweb disease agents are difficult to suppress, as the genus *Hypomyces* is characterized by a lack of recombinations, resulting in the quick spread of fungicide resistance after single point mutations occur (Tamm & Põldmaa, 2013). For example, development of resistance in *H. odoratus* (Grogan, 2006) and *Z. fungicola* (Grogan et al., 2000; Gea et al., 2005) to the fungicide prochloraz resulted in its withdrawal from the EU market in June 2023 (Clarke et al., 2024), whereas it is still used in China (Shi et al., 2020) and Australia (Australian Pesticides and Veterinary Medicines Authority [APVMA], 2025). Strains of *Hypomyces* tolerant to the fungicide metrafenone were detected shortly after its introduction (Clarke et al., 2024) as the only chemical fungicide registered in the EU and North America for the control of *Hypomyces* spp. and *Z. fungicola* in cultivated mushrooms (Carrasco et al., 2017b). Chlorothalonil is authorized in some EU countries (France, Poland, Spain) and North America for use in mushrooms (Carrasco et al., 2017b; United States Environmental Protection Agency [US EPA], 2020). However, tolerance development to chlorothalonil in *H. odoratus* (Beyer & Kremser, 2004), as well as fungicide toxicity in mushroom mycelia were recorded (Challen & Elliot, 1985; Fletcher, 2002).

Thus, the development of pathogen resistance, in addition to the negative impacts of fungicides on humans, non-target organisms, and the environment, has shifted disease control towards the broader use of biological agents. Mushroom substrate is composed of various beneficial and harmful microorganisms, and its supplementation with suitable microbials helps mushrooms to resist pathogen infestation (Marčić et al., 2025). Different *Bacillus* species produce a multitude of biomolecules with remarkable pesticidal properties (Stanojević et al., 2019). Furthermore, *B. subtilis* (Ehrenberg) Cohn is considered a safe (Generally Recognized as Safe [GRAS]) microorganism for humans and the environment (Food and Drug Administration [FDA], 1999), and is also the growth promoter of cultivated plants and mushrooms (Liu et al., 2015). Though many studies concerning the suppression of various mycoparasites with biofungicides, such as *Bacillus* spp., are available, few studies and industrial-scale experiments have focused on the control of *H. odoratus*. For example, a large-scale study conducted by Regnier and Combrinck (2010) evaluated the application rate (40 µl l^{-1}) of essential oils of lemon verbena, thyme, and lemongrass, as well as their main components nerol and thymol, against natural infestation of *H. perniciosus*.

The aim of this study was to use two different procedures to (*1*) compare the efficacy of strain *B. subtilis* Ch-13 to that of the fungicide prochloraz in cobweb disease control in natural infestation conditions, and (*2*) compare the effects of *B. subtilis* Ch-13 and prochloraz on white button mushroom production. In these two procedures, the same final concentration of *B. subtilis* Ch-13 was applied either in two or three split doses.

MATERIAL AND METHODS

Antifungal agents

Efficacy of the biofungicide *Bacillus subtilis* Ch-13 (Ekstrasol F SC, BioGenesis d.o.o., Belgrade, Serbia; content of active ingredient [a.i.] 1×10^8 CFU ml^{-1}) was evaluated in the suppression of *H. odoratus* (cobweb disease agent) on naturally infected white button mushrooms (*A. bisporus*). The experiment was organized in a mushroom growing room (A2) of the mushroom factory Delta Danube d.o.o., Kovin, Serbia. Efficacy in pathogen control and biological efficiency (impact on mushroom yield) of *B. subtilis* Ch-13 were compared to those of the chemical fungicide prochloraz (Mirage® EC, ADAMA Agricultural Solutions UK Ltd., UK; content of a.i. 450 ml l^{-1}).

Large-scale experiment in mushroom growing room

The mushroom substrate used in this study consisted of mushroom compost and casing soil. The mushroom compost (Phase III) was produced from fermented and

pasteurized straw and chicken manure (Champicomp d.o.o., Pločica, Kovin, Serbia). Plastic briquettes sized 0.6 × 0.4 × 0.25 m (*l* x *w* x *h*) contained 18 kg of mushroom compost inoculated with 0.7% of grain spawn of *A. bisporus* F56 (Italspawn, Onigo di Pederobba, Italy). The surface area of five briquettes was estimated at 1 m^2. Casing soil made of black peat soil (Pešter peat soil, Dallas Company d.o.o., Tutin, Serbia) was layered onto the briquettes at a thickness of 40-50 mm (7 kg per briquette), and disinfected using 90 ml of 0.02% peracetic acid (Peral-S 15%, Vetprom, Belgrade, Serbia) per m^2 of casing layer. Mushroom mycelia were incubated at 25°C in the substrate for 16 days. After casing time (day one), the air temperature was gradually lowered to 17°C for 7 days for the case run. The experiment was conducted according to the standard PP 1/270(1) methodology (European and Mediterranean Plant Protection Organization [EPPO], 2010). Bio/fungicides were applied on the casing soil as a drench application. The fungicide prochloraz was used at the standard product application rate of 3 ml per m^2 of casing layer, in two split applications (2 × 1.5 ml in 1 l of water per m^2, four days after casing and after the first fruiting flush). The biofungicide *B. subtilis* Ch-13 was used at a rate of 60 ml m^{-2} in two protocols: (*1*) three split applications (30 + 15 + 15 ml in 1 l of water per m^2, on the second and 15th day after casing, and after the first flush, respectively); (*2*) two split applications (2 × 30 ml in 1 l of water per m^2, on the second day after casing and after the first flush). Bio/fungicides were applied with an automatic "fir" sprayer with 10 full cone nozzles, in the amount of 1 l per m^2 of casing layer. The untreated mushroom control was sprayed with tap water.

Each treatment was repeated twice (repetitions I and II) in a randomized block design, on 43 m^2 of casing surface per block. Each block consisted of 214 briquettes. Mushrooms were picked from the 14th to the 21st day of the first fruiting flush, and from the 24th to the 34th day of the second flush. The harvested mushrooms were classified as healthy and diseased, and then weighed. The effect of bio/fungicides on mushroom yield was calculated via biological efficiency (BE) (Chrysayi-Tokousbalides et al., 2007). Bio/fungicide efficacy (E) was evaluated using Abbott's formula (Abbott, 1925) based on disease incidence (DI) (proportion of diseased mushrooms and total yield) in the untreated control and each treated sample (Gea et al., 2010).

Statistical analyses

Data concerning the efficacy of bio/fungicides (E) in the suppression of *H. odoratus* and their effect on yield (biological efficiency, BE) of white button mushrooms were analyzed using the one-way analysis of variance (ANOVA). Average values for E and BE, obtained in two repetitions of bio/fungicide treatments against *H. odoratus*, were compared and any statistically significant differences were detected using the *F*-test. The level of significance was determined at $p<0.05$ (Sokal & Rohlf, 1995). Statistical data analysis was conducted using the software Statistica for Windows 7.0 (StatSoft Inc., 2004).

RESULTS

Cobweb disease symptoms in white button mushrooms, including white fluffy colonies on casing soil and mushroom fruiting bodies, were detected from the 20th to the 34th day of casing time.

Table 1. Efficacy of bio/fungicides in relation to the untreated control or standard fungicide treatment against *Hypomyces odoratus* on naturally infected white button mushrooms

Treatments	Fungicide application rate	$^1E_{st}$ (%)	^2E_c (%)	SE
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1} (II)	2 × 30 ml m^{-2}	56.78 b[3]	30.13 c	0.75
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1} (III)	30 ml m^{-2} + 2 × 15 ml m^{-2}	79.12 a	41.98 b	0.58
Prochloraz 450 ml l^{-1}	2 × 1.5 ml m^{-2}	100.00	53.06 a	0.61
Untreated Control	–	1.88 c	–	1.25

The data represent means (of two repetitions, each including 214 experimental units) ± SE, standard error of the mean; efficacy in relation to the fungicide prochloraz (standard) set to 100% $^1(E_{st})$, or untreated control $^2(E_c)$; SED, standard error of difference = 32.64; df, degrees of freedom = 3; $F = 69.54$; p-value = 0.001. [3]Values marked with same letter within each repetition series are not significantly different according to the *F*-test ($p<0.05$).

The incidence of cobweb disease in mushrooms varied depending on different treatments (Figure 1). Mushrooms in the untreated control had the highest disease incidence (12.62-12.8%). Reduction of disease symptoms was recorded in briquettes treated with *B. subtilis* Ch-13 in two (DI=8.84-8.92%) or three (DI=7.27-7.48%) split doses. The lowest disease incidence was observed after treatment of mushrooms with the fungicide prochloraz (DI=5.81-6.12%). Efficacy of each bio/fungicide in disease control was estimated in two ways: in relation to standard prochloraz treatment set to 100% efficacy (E_{st}), or in relation to the untreated control (E_c) (Table 1). A statistically significant difference in the efficacy of bio/fungicides in cobweb disease control was detected between prochloraz and the biofungicide *B. subtilis* Ch-13 used in both application protocols (two or three split doses). In relation to the untreated control (E_c), prochloraz showed the highest efficacy against *H. odoratus* (53.1%), followed by the biofungicide *B. subtilis* Ch-13 applied three times (42%) (Table 1). The biofungicide *B. subtilis* Ch-13 used twice exhibited the lowest efficacy against *H. odoratus* (30.1%). In relation to the fungicide prochloraz when its efficacy was set to 100% (E_{st}), the biofungicide *B. subtilis* Ch-13 used three times showed 28% higher efficacy in pathogen suppression (79.1%), than when applied twice (56.8%) (Table 1). Furthermore, when the biofungicide *B. subtilis* Ch-13 was used three times, it considerably diminished the incidence of cobweb disease of white button mushrooms, by up to 58% compared to the untreated control.

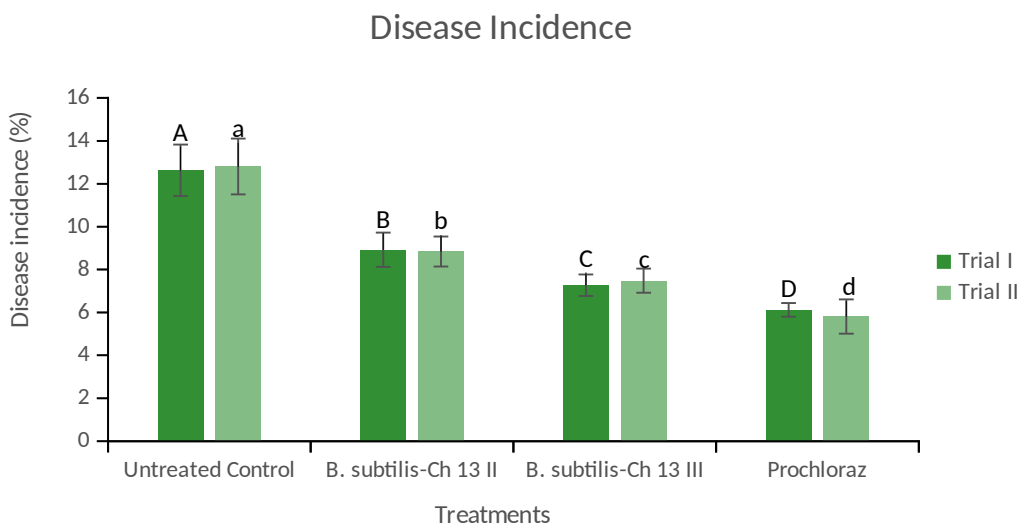

Figure 1. Incidence of cobweb disease (*Hypomyces odoratus*) after using bio/fungicides on white button mushrooms. The biofungicide *Bacillus subtilis* Ch-13 was used in two (II) or three (III) split doses. Data represent means of repetition trials I and II (each performed on a set of 214 experimental briquettes) ± SE, standard error of the mean; SED, standard error of difference = 0.8 (I); 0.9 (II); df, degrees of freedom = 3; $F = 62.52$ (I); 73.64 (II); p-value = 0.001. Values marked with the same letter within each repetition series (I – capital letters; II – lowercase letters) are not significantly different according to the F-test ($p<0.05$).

Table 2. Biological efficiency (BE%) of bio/fungicides in relation to the untreated control or standard fungicide treatment of white button mushrooms naturally infected with *Hypomyces odoratus*

Treatments	Fungicide application rate	$^1BE_{st}$ (%)	^2BE_c (%)	SE
Bacillus subtilis Ch-13 1×10^8 CFU ml^{-1} (II)	2×30 ml m^{-2}	104.82 b[3]	107.41 b	2.05
Bacillus subtilis Ch-13 1×10^8 CFU ml^{-1} (III)	30 ml m^{-2} + 2×15 ml m^{-2}	112.66 a	115.44 a	2.22
Prochloraz 450 ml l^{-1}	2×1.5 ml m^{-2}	100.00	102.47 b	2.02
Untreated Control	–	97.57 c	100.00	2.42

The data represent means (of two repetitions, each including 214 experimental units) ± SE, standard error of the mean; fungicide prochloraz (standard) impact [1](BE_{st}) or untreated control impact [2](BE_c) value is set to 100%; SED, standard error of difference = 32; df, degrees of freedom = 3; $F = 28.32$; p-value = 0.001. [3]Values marked with same letter within each repetition series are not significantly different according to the F-test ($p<0.05$).

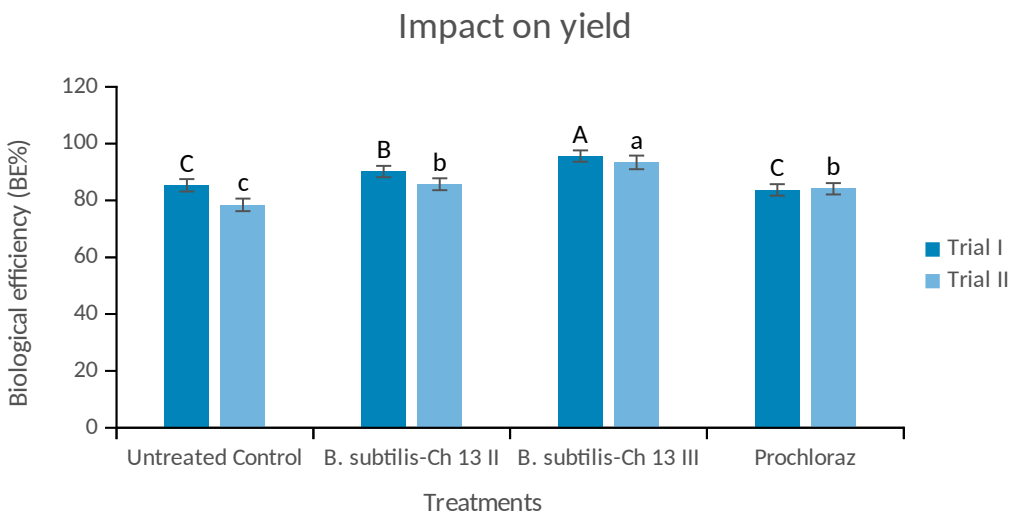

Figure 2. The impact of bio/fungicides on yield (biological efficiency – BE%) of white button mushrooms naturally infected with *Hypomyces odoratus*. The biofungicide *Bacillus subtilis* Ch-13 was used in two (II) or three (III) split doses. Data represent means of repetition trials I and II (each including 214 experimental briquettes) ± SE, standard error of the mean; SED, standard error of difference = 2.1 (I); 2.2 (II); df, degrees of freedom = 3; $F = 30.2$ (I); 28.8 (II); p-value = 0.001. Values marked with the same letter within each repetition series (I – capital letters; II – lowercase letters) are not significantly different according to the F-test ($p<0.05$).

The effect of bio/fungicides on white button mushroom production was estimated with biological efficiency (BE) (Table 2; Figure 2). In comparison to the untreated control (BE=78.4-85.3%), application of *B. subtilis* Ch-13 significantly improved mushroom yield, whether used in three (BE=93.3-95.6%) or two split doses (BE=85.7-90.1%). No significant difference in biological efficiency was recorded between the untreated control (BE=78.4-85.3%) and prochloraz treatment (BE=75.4-78.4%) (Figure 2). The effect of bio/fungicides on mushroom production (biological efficiency) was evaluated in two ways: in relation to the untreated control (BE_c) or standard prochloraz treatment (BE_{st}), when the value of each was set to 100% biological efficiency (Figure 2). Compared to the untreated control set at 100% (BE_c), *B. subtilis* Ch-13 applied in three split doses significantly increased mushroom production (BE=115.4%), whereas *B. subtilis* Ch-13 applied in two split doses and prochloraz treatment did not significantly affect mushroom production (BE=107.4; 102.5%, respectively) (Table 2). When prochloraz treatment was set to 100% biological efficiency (BE_{st}), three split doses of the biofungicide *B. subtilis* Ch-13 promoted a mushroom yield (BE=112.7%) that was 7% higher than the yield obtained with two split doses (BE=104.8%) (Table 2). Additionally, in relation to the fungicide prochloraz (BE_{st}), mushroom yield obtained with the biofungicide *B. subtilis* Ch-13 applied in two split doses significantly differed from that of the untreated control (BE=97.6%) (Table 2). The use of the biofungicide in three split doses significantly increased mushroom production, in contrast to its application in two split doses, the fungicide prochloraz treatment, or the untreated control. In comparison to the untreated control and prochloraz treatment, the application of strain *B. subtilis* Ch-13 in three split doses significantly increased white button mushroom production: up to 15% and 13%, respectively.

DISCUSSION

Symptoms found in all experimental briquettes with white button mushrooms resembled those of cobweb disease caused by *H. odoratus* (Luković et al., 2021). Tested for the suppression of natural infestation of cobweb disease agent *H. odoratus*, the biofungicide *B. subtilis* Ch-13 was applied at a concentration of 60 ml (1×10^8 CFU ml^{-1}) per m^2 of casing layer in two procedures – using two (2×30 ml m^{-2}) or three (30 ml $m^{-2} + 2 \times 15$ ml m^{-2}) split doses – whereas the fungicide prochloraz was applied at the standard application rate used in industrial-scale conditions of white button mushroom cultivation. The effects of *B. subtilis* Ch-13 and prochloraz on mushroom production were also estimated.

Strain *B. subtilis* Ch-13 has been registered as a microbiological fertilizer, fungicide, and wheat seed disinfectant in several countries (Chebotar et al., 2009; Kayin et al., 2015; Potočnik et al., 2019). Concerning the protection of naturally infected white button mushrooms, *B. subtilis* Ch-13 was previously tested only against the mycopathogen *T. aggressivum* in industrial-scale cultivation (Potočnik et al., 2021). The biofungicide *B. subtilis* Ch-13 displayed optimal efficacy in the suppression of *H. odoratus* when used in three split doses (≈42%) and to a certain extent when applied twice (≈30%), compared to the fungicide prochloraz (≈53%). The efficacy of *B. subtilis* Ch-13 against *H. odoratus* was substantially higher when applied three times, rather than twice, despite the same total concentration of *B. subtilis* Ch-13 used in both applications. The efficacy of three split doses of *B. subtilis* Ch-13 (≈42%) against *H. odoratus* was comparable to the very low efficacy of the fungicide prochloraz (≈53%).

When the strain *B. subtilis* Ch-13 was previously tested against *T. aggressivum* in an industrial-scale experiment, Potočnik et al. (2021) found that it displayed higher efficacy when applied in three (53.6%) or two split doses (46.4%), than when used in the same manner to control *H. odoratus* in the present study. Additionally, the fungicide prochloraz showed much lower efficacy against *H. odoratus* (53%) in the current study than found by Potočnik et al. (2021) in the suppression of *T. aggressivum* (71%). The decreased efficacy of prochloraz in cobweb disease control implies the importance of replacing it with effective beneficial microorganisms to suppress *H. odoratus*.

Strains *B. velezensis* QST 713 (formerly *B. subtilis*), QST 713 (Ehrenberg) Cohn, *B. amyloliquefaciens* subsp. *plantarum* D747, and *B. amyloliquefaciens* MBI 600 were authorized for use against *Trichoderma harzianum* Rifai and *T. aggressivum* on white button mushrooms worldwide (Marčić et al., 2025). Strain *B. velezensis* QST 713 (5.13×10^{10} CFU g^{-1}) used at the standard application rate showed distinct efficacies in the suppression of various mushroom disease agents: 45-62% against *Z. fungicola* (Stanojević et al., 2019), 48-58% against *T. aggressivum* (Milijašević-Marčić et al., 2017; Potočnik et al., 2018; 2019; Stanojević et al., 2019), 44% against *T. harzianum* (Kosanović et al., 2013; Milijašević-Marčić et al., 2017), and 17-21% against *H. perniciosus* (Navarro et al., 2023). Strain *B. amyloliquefaciens* subsp. *plantarum* D747 (5×10^{13} CFU g^{-1}) used at the standard application rate exhibited an efficacy of 15-19% against *H. perniciosus* (Navarro et al., 2023). On the other hand, *B. subtilis* QST 713 failed to control cobweb disease (*H. rosellus*) in the experiment conducted by Ślusarski et al. (2012).

Various native *Bacillus* spp. strains also showed different efficacies against white button mushroom pathogens: *B. velezensis* Kos, 30-40% against *H. odoratus* (Clarke et al., 2024), similar to *B. subtilis* Ch-13 in the current study (30-42%); *B. subtilis* B-38, 50% against *T. harzianum* (Milijašević-Marčić et al., 2017); *B. subtilis* B-38, 36% (Milijašević-Marčić et al., 2017) and *B. amyloliquefaciens* B-241, 53-68% against *T. aggressivum* (Stanojević et al., 2019); and *B. amyloliquefaciens* B-241, 46-58% against *Z. fungicola* (Stanojević et al., 2019).

Furthermore, various *Bacillus* spp. strains distinctly improved mushroom yield: *B. velezensis* QST 713, 15% (Milijašević-Marčić et al., 2017; Navarro et al., 2023); *B. velezensis,* 18-26% (Büchner et al., 2021); *B. amyloliquefaciens* subsp. *plantarum* D747, 4-8% (Navarro et al., 2023); and *B. subtilis* Ch-13, 8-12% (Potočnik et al., 2019; Potočnik et al., 2021), consistent with data reported in the current study (7-15%).

More specifically, findings from the current study support the application of strain *B. subtilis* Ch-13 at a concentration of 60 ml m^{-2} (1×10^8 CFU ml^{-1}) in three split doses to control cobweb disease and improve the yield of white button mushrooms.

CONCLUSION

More frequent application of the biofungicide *B. subtilis* Ch-13 enhanced its efficacy against natural infestation of *H. odoratus* in white button mushrooms. Used at a final concentration of 60 ml m^{-2} (1×10^8 CFU ml^{-1}), strain *B. subtilis* Ch-13 was substantially more effective in the large-scale production of white button mushrooms when applied in three split doses (42% efficacy), rather than two (30% efficacy). Compared to the low efficacy of the fungicide prochloraz (53%), optimal efficacy of *B. subtilis* Ch-13 was recorded in the suppression of *H. odoratus*. The greatest improvement in mushroom production, in comparison to the untreated control, was found when *B. subtilis* Ch-13 was applied in three split doses (15%), rather than two (7%). Therefore, current data support the application of the biofungicide *B. subtilis* Ch-13 in three split doses (30 + 15 + 15 ml m^{-2} on the second day after casing, two weeks after casing, and after the first fruiting flush, respectively) against the mycopathogen *H. odoratus* in white button mushrooms.

ACKNOWLEDGMENT

A large-scale study was conducted in the mushroom factory Delta Danube d.o.o., Kovin, Serbia, thanks to its CEO, Dr. Sunil Mamgain. Assistance in experiment implementation was offered by factory technologists: Marjan Paunov, agricultural engineer, Nebojša Slavković, mechanical engineer, and Tanja Lončarević, agricultural engineer. The study was financially supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, grant 451-03-136/2025-03/ 200214.

REFERENCES

Abbott, W.S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18*(2), 265-267. DOI: https://doi.org/10.1093/jee/18.2.265a

Australian Pesticides and Veterinary Medicines Authority (APVMA). (2025). Retrieved from https://portal.apvma.gov.au/es/permits

Back, C.-G., Kim, Y.-H., Jo, W.-S., Chung, H., & Jung, H.-Y. (2010). Cobweb disease on *Agaricus bisporus* caused by *Cladobotryum mycophilum* in Korea. *Journal of Plant Pathology*, *76*(3), 232-235. DOI: https://doi.org/10.1007/s10327-010-0236-3

Beyer, D.M., & Kremser, I.J. (2004). Evaluation of fungicide tolerance and control for three fungal diseases of mushrooms. In Romaine, C.P., Keil, C.B., Rinker, D.L., & Royse, D.J. (Eds.). *Mushroom Science XVI*, *Proceedings of the XVI International Congress on the Science and Cultivation of Edible and Medicinal Fungi*, *Miami, USA*, *March 14-17, 2004* (pp 521-529). Philadelphia, PA, USA: Pennsylvania State University Press.

Büchner, R., Vörös, M., Allaga, H., Varga, A., Bartal, A., Szekeres, A., Varga, S., Bajzát, J., Bakos-Barczi, N., Misz, A., Csutorás, C., Hatvani, L., Vágvölgyi, C., & 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. DOI: https://doi.org/10.3390/agronomy12020467

Carrasco, J., Navarro, M.J., Santos, M., Diánez, F., & Gea, F.J. (2016). Incidence, identification and pathogenicity of *Cladobotryum mycophilum*, causal agent of cobweb disease on *Agaricus bisporus* mushroom crops in Spain. *Annals of Applied Biology*, *168*(2), 214-224. DOI: https://doi: 10.1111/aab.12257

Carrasco, J., Navarro, M.J., & Gea, F.J. (2017a). Cobweb, a serious pathology in mushroom crops: A review. *Spanish Journal of Agricultural Research*, *15*(2), e10R01. DOI: https://doi.org/10.5424/sjar/2017152-10143

Carrasco, J., Navarro, M.J., Santos, M., & Gea, F.J. (2017b). Effect of five fungicides with different modes of action on mushroom cobweb disease (*Cladobotryum mycophilum*) and mushroom yield. *Annals of Applied Biology*, *171*(1), 62-69. DOI: https://doi.org/10.1111/aab.12352

Chakwiya, A., Van der Linde, E.J., & Korsten, L. (2015). *In vitro* sensitivity testing of *Cladobotryum mycophilum* to carbendazim and prochloraz manganese. *South African Journal of Science*, *111*(11/12), 1-7. DOI: https://doi.org/10.17159/sajs.2015/20140408

Chakwiya, A., Van der Linde, E.J., Chidamba, L., & Korsten, L. (2019). Diversity of *Cladobotryum mycophilum* isolates associated with cobweb disease of *Agaricus bisporus* in the South African mushroom industry. *European Journal of Plant Pathology*, *154*, 767-776. DOI: https://doi.org/10.1007/s10658-019-01700-7

Challen, M.P., & Elliott, T.J. (1985). The *in vitro* responses to a range of fungicides of two strains of the mushroom *Agaricus bisporus* and the pathogen *Verticillium fungicola*. *Mycopathologia*, *90*, 161-164. DOI: https://doi.org/10.1007/BF00436732

Chebotar, V.K., Makarova, N.M., Shaposhnikov, A.I., & Kravchenko, L.V. (2009). Antifungal and phytostimulating characteristics of *Bacillus subtilis* Ch-13 rhizospheric strain, producer of biopreparations. *Applied Biochemistry and Microbiology*, *45*(4), 465-469. DOI: https://doi.org/10.1016/j.micres.2006.04.001

Chrysayi-Tokousbalides, M., Kastanias, M.A., Philippoussis, A., & Diamantopoulou, P. (2007). Selective fungitoxicity of famoxadone, tebuconazole and trifloxystrobin between *Verticillium fungicola* and *Agaricus bisporus*. *Crop Protection*, *26*(4), 469-475. DOI: https://doi.org/10.1016/j.cropro.2006.02.016

Clarke, J., McGuinness, B., Fitzpatrick, D., Kavanagh, K., & Grogan, H. (2024). Response of the mushroom pathogen *Cladobotryum mycophilum* to the fungicides prochloraz and metrafenone and two *Bacillus*-based biological control agents in mushroom crop trials. *Crop Protection*, *177*, 106530. DOI: https://doi.org/10.1016/j.cropro.2023.106530

European and Mediterranean Plant Protection Organization (EPPO). (2010). Efficacy evaluation of fungicides: Fungal diseases on cultivated mushrooms of *Agaricus* spp. PP 1/270(1) in EPPO Standards. *OEPP/EPPO Bulletin*, *40*(3), 270-273. DOI: https://doi.org/10.1111/j.1365-2338.2010.02384.x

Fletcher, J.T. (2002). Cobweb disease, a new challenge. *Pest Control (Mushroom News)*, *50*, 20-23.

Food and Drug Administration (FDA). (1999). Code of Federal Regulations, Title 21: Food and drugs. In: *Chapter 1: Food and Drug Administration Department of Health and Human Services, Part 184: Direct food substances affirmed as Generally Recognized as Safe* (pp. 892-896). Washington, D.C., USA: US Government Printing Office.

Gea, F.J., Navarro, M.J., & Tello, J.C. (2005). Reduced sensitivity of the mushroom pathogen *Verticillium fungicola* to prochloraz-manganese *in vitro*. *Mycological Research*, *109*(Pt 6), 741-745. DOI: https://doi.org/10.1017/s095375620500242x

Gea, F.J., Tello, J.C., & Navarro, M.J. (2010). Efficacy and effect on yield of different fungicides for control of wet bubble disease of mushroom caused by the mycoparasite *Mycogone perniciosa*. *Crop Protection*, *29*(9), 1021-1025. DOI: https://doi.org/10.1016/j.cropro.2010.06.006

Gea, F.J., Navarro, M.J., Carrasco, J., González, A.J., & Suz, L.M. (2012). First report of cobweb on white button mushroom (*Agaricus bisporus*) in Spain caused by *Cladobotryum mycophilum*. *Plant Disease*, *96*(7), 1067. DOI: https://doi.org/10.1094/PDIS-02-12-0120-PDN

Grogan, H.M., Keeling, C., & Jukes, A.A. (2000). *In vitro* response of the mushroom pathogen *Verticillium fungicola* (dry bubble) to prochloraz-manganese. In *The BCPC 2000, Proceedings of the British Crop Protection Conference – Pests & Diseases, Brighton, UK, November 13-16, 2000* (pp 273-278). Farnham, UK: British Crop Protection Council.

Grogan, H.M. (2006). Fungicide control of mushroom cobweb disease caused by *Cladobotryum* strains with different benzimidazole resistance profiles. *Pest Management Science*, *62*(2), 153-161. DOI: https://doi.org/10.1002/ps.1133

Kayin, G.B., Öztüfekçi, S., Akin, H.F., Karaata, E.U., Katkat, A.V., & Turan, M.A. (2015). Effect of *Bacillus subtilis* Ch-13, nitrogen and phosphorus on yield, protein and gluten content of wheat (*Triticum aestivum* L.). *Journal of Agricultural Faculty of Uludag University*, *29*(1), 19-28. DOI: https://dergipark.org.tr/tr/download/article-file/154225

Kosanović, D., Potočnik, I., Duduk, B., Vukojević, J., Stajić, M., Rekanović, E., & Milijašević-Marčić, S. (2013). *Trichoderma* species on *Agaricus bisporus* farms in Serbia and their biocontrol. *Annals of Applied Biology*, *163*(2), 218-230. DOI: https://doi.org/10.1111/aab.12048

Liu, C., Sheng, J., Chen, L., Zhen, Y., Lee, D.Y.W., Yang, Y., Xu, M., & Shen, L. (2015). Biocontrol activity of *Bacillus subtilis* isolated from *Agaricus bisporus* mushroom compost against pathogenic fungi. *Journal of Agriculture and Food Chemistry*, *63*(26), 6009-6018. DOI: https://doi.org/10.1021/acs.jafc.5b02218

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. *Journal of Environmental Science and Health, Part B*, *56(*1), 54-63. DOI: https://doi.org/10.1080/03601234.2020.1838821

Marčić, D., Milijašević-Marčić, S., Drobnjaković, T., Luković, J., Šantrić, Lj., Grujić, N., & Potočnik, I. (2025). Bioprotection of the button mushroom from pests and diseases. *Agronomy*, *15*(6), 1323. DOI: https://doi.org/10.3390/agronomy15061323

McKay, G.J., Egan, D., Morris, E., Scott, C., & Brown, A.E. (1999). Genetic and morphological characterization of *Cladobotryum* species causing cobweb disease of mushrooms. *Applied and Environmental Microbiology, 65*(2), 606-610. DOI: https://doi.org/10.1128/aem.65.2.606-610.1999

Milijašević-Marčić, S., Stepanović, M., Todorović, B., Duduk, B., Stepanović, J., Rekanović, E., & Potočnik, I. (2017). Biological control of green mould on *Agaricus bisporus* by a native *Bacillus subtilis* strain from mushroom compost. *European Journal of Plant Pathology, 148(*3), 509-519. DOI: https://doi.org/10.1007/s10658-016-1107-3

Muhammad, I., Sossah, F.L., Yang, Y., Li, D., Li, S., Fu, Y., & Li, Y. (2019). Identification of resistance to cobweb disease caused by *Cladobotryum mycophilum* in wild and cultivated strains of *Agaricus bisporus* and screening for bioactive botanicals. *RSC Advances*, *9*(26), 14758-14765. DOI: https://doi.org/10.1039/c9ra00632j

Navarro, M.J., Santos, M., Dianez, F., & Gea, F.J. (2023). Chemical and biological control of wet bubble disease (*Hypomyces perniciosus*) in mushroom crops. *Agronomy*, *13*(7), 1672. DOI: https://doi.org/10.3390/agronomy13071672

Potočnik, I., Todorović, B., Rekanović, E., Luković, J., Paunović, D., & Milijašević-Marčić, S. (2018). Impact of *Bacillus subtilis* QST 713 mushroom grain spawn treatment on yield and green mould control. *Pesticides and Phytomedicine*, *33*(3-4), 205-211. DOI: https://doi.org./10.2298/PIF1804205P

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. *Pesticides and Phytomedicine*, *34*(1), 53-60. DOI: https://doi.org/10.2298/PIF1901053P

Potočnik, I., Todorović, B., Milijašević-Marčić, S., Luković, J., Kanižai Šarić, G., Majić, I., & Rekanović, E. (2021). A large-scale study on the effectiveness of a *Bacillus subtilis* Ch-13-based biofungicide against green mould

disease and mushroom yield improvement. *Pesticides and Phytomedicine*, *36*(2), 83-90. DOI: https://doi.org/10.2298/PIF2102083P

Regnier, T., & Combrinck, S. (2010). *In vitro* and *in vivo* screening of essential oils for the control of wet bubble disease of *Agaricus bisporus*. *South African Journal of Botany*, *76*(4), 681-685. DOI: https://doi.org/10.1016/j.sajb.2010.07.018

Shi, N., Ruan, H., Jie, Y., Chen, F., & Du, Y. (2020). Sensitivity and efficacy of fungicides against wet bubble disease of *Agaricus bisporus* caused by *Mycogone perniciosa*. *European Journal of Plant Pathology, 157*(4), 873-885. DOI: https://doi.org/10.1007/s10658-020-02047-0

Ślusarski, C., Uliński, Z., Szumigaj-Tarnowska, J., & Miszczak, A. (2012). Preliminary appraisal of the new preparations for protection of the white button mushroom against fungal diseases. *Progress in Plant Protection*, *52*(4), 1058-1063.

Sokal, R.R., & Rohlf, F.J. (1995). *Biometry: The principles and practice of statistics in biological research* (3rd edition). New York, NY, USA: W.H. Freeman and Company.

Stanojević, O., Berić, T., Potočnik, I., Rekanović, E., Stanković, S., & Milijašević-Marčić, S. (2019). Biological control of green mould and dry bubble diseases of cultivated mushroom (*Agaricus bisporus* L.) by *Bacillus* spp. *Crop Protection*, *126*(5), 104944. DOI: https://doi.org/10.1016/j.cropro.2019.104944

StatSoft Inc. (2004). STATISTICA (data analysis software system), version 7. Retrieved from https://www.statsoft.com

Tamm, H., & Põldmaa, K. (2013). Diversity, host associations, and phylogeography of temperate aurofusarin-producing *Hypomyces/Cladobotryum* including causal agents of cobweb disease of cultivated mushrooms. *Fungal Biology*, *117*(5), 348-367. DOI: https://doi.org/10.1016/j.funbio.2013.03.005

United States Environmental Protection Agency (US EPA). Grower resources. *Integrated Pest Management. Pesticides. Fungicides*. Retrieved from https://www.americanmushroom.org/integrated-pest-management/fungicides/

Efikasnost biofungicida *Bacillus subtilis* Ch-13 u suzbijanju prouzrokovača paučinaste plesni (*Hypomyces odoratus*) u uslovima industrijske proizvodnje šampinjona

REZIME

Učestalija primena biofungicida *Bacillus subtilis* Ch-13 povećala je njegovu efikasnost u suzbijanju prirodne zaraze *Hypomyces odoratus* (paučinate plesni) i pozitivan uticaj na prinos šampinjona (*Agaricus bisporus*). Soj *B. subtilis* Ch-13 je bio primenjen u ukupnoj koncentraciji od 60 ml m^{-2} (1×10^8 CFU ml^{-1}). Značajno veća efikasnost biofungicida u suzbijanju prouzrokovača paučinaste plesni je postignuta kada je primenjen u tri podeljene doze (42%), umesto u dve (30%), u uslovima industrijske proizvodnje šampinjona. Zadovoljavajuća efikasnost *B. subtilis* Ch-13 u suzbijanju *H. odoratus* je uočena u poređenju sa vrlo smanjenom efikasnošću fungicida prohloraza (53%). Najveće povećanje prinosa je zabeleženo kod primene *B. subtilis* Ch-13 u tri podeljene doze (15%), umesto u dve (7%), u poređenju sa netretiranom kontrolom. Može se preporučiti primena *B. subtilis* Ch-13 u tri podeljene doze: 30 + 15 +15 ml po m^2 (drugog dana, dve nedelje nakon pokrivanja i posle prve berbe) u suzbijanju *H. odoratus*.

Ključne reči: jestive gljive; agens biološke zaštite; zaštita od bolesti