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Biofungicide *Bacillus subtilis* Ch-13 in the control of *Hypomyces perniciosus* (wet bubble disease) in industrial-scale mushroom cultivation

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SUMMARY

The current study aimed to evaluate the efficacy of the biofungicide *Bacillus subtilis* Ch-13 in the suppression of natural infection of white button mushrooms (*Agaricus bisporus*) with *Hypomyces perniciosus* (causal agent of wet bubble disease), as well as its impact on mushroom yield in industrial-scale cultivation. The biofungicide *B. subtilis* Ch-13 was applied at a total concentration of 60 ml per m^2 of casing layer in two different procedures – using either three (30 + 2 × 15 ml m^{-2}) or two split doses (2 × 30 ml m^{-2}) – and then its effects were compared to those of the fungicide prochloraz applied at the standard application rate. The efficacy of the biofungicide was significantly higher when applied in three split doses (29.7%), than in two (15.7%). Though the efficacy of *B. subtilis* Ch-13 (≈30%) against *H. perniciosus* was low in comparison to that of prochloraz (≈68%), *B. subtilis* Ch-13 slightly reduced wet bubble symptoms. Furthermore, the highest increase in mushroom yield was achieved when *B. subtilis* Ch-13 was applied in three split doses (14%), rather than two (2%), compared to the untreated control. In comparison to prochloraz, three and two split applications of *B. subtilis* Ch-13 enhanced mushroom yield by up to 17% and 4%, respectively. Regarding its efficacy in wet bubble disease control and augmentation of mushroom yield, *B. subtilis* Ch-13 was much more effective when applied in three split doses, than in two. Therefore, this study supports the application *B. subtilis* Ch-13 in three split doses (30 + 2 × 15 ml m^{-2}, on the second day and two weeks after casing, and after the first fruiting flush, respectively) to suppress *H. perniciosus* and increase mushroom yield.

Keywords: edible mushrooms; biocontrol agent; disease control

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INTRODUCTION

The causal agent of wet bubble disease, *Hypomyces perniciosus* Magnus [formerly *Mycogone perniciosa* (Magnus) Delacroix], is a significant fungal pathogen of white button mushrooms [*Agaricus bisporus* (Lange) Imbach] worldwide (Umar & Van Griensven, 1999; Meyer & Korsten, 2008; Glamočlija et al., 2008; Siwulski et al., 2011; Shi et al., 2020). As a soil-borne fungus, *H. perniciosus* spreads mainly by black peat used for casing soil, which serves as a cover layer for the mushroom compost. Mostly appearing in mixed infestations with other significant mycopathogens – such as *Zarea fungicola* (Preuss) Khons., Thanakitp. and Luangsa-ard. [formerly *Lecanicillium fungicola* (Preuss) Zare & W. Gams], *Cladobotryum mycophilum* (Oudem.) W. Gams & Hoozem., and *Trichoderma aggressivum* Samuels & W. Gams – *H. perniciosus* has occasionally caused yield losses of 15-30% (Gea et al., 2010; Potočnik et al., 2010; Shi et al., 2020). Symptoms of wet bubble disease manifest as large undifferentiated tumorous fruiting bodies with amber-coloured droplets on the surface (Umar & Van Griensven, 1999). After *Z. fungicola* (Gea et al., 2005) and *C. mycophilum* (Grogan, 2006) developed resistance to prochloraz, the fungicide was withdrawn from the EU market in June 2023 (Clarke et al., 2024). However, prochloraz is still used in China (Shi et al., 2020) and Australia (Australian Pesticides and Veterinary Medicines Authority [APVMA], 2025). Furthermore, strains of *Cladobotryum* tolerant to metrafenone, the only chemical fungicide approved in the cultivation of mushrooms in the EU (Carrasco et al., 2017), have already been detected (Clarke et al., 2024). However, no evidence of *H. perniciosus* resistance to fungicides has been recorded (Gea et al., 2010; Shi et al., 2020). Resistance of pathogens to fungicides, in addition to the harmful effects of fungicides on human health and the environment, has redirected mushroom disease control to the application of biocontrol agents, predominantly antagonistic microorganisms. Mushrooms are highly connected with the microbiota in their substrate, which may be beneficial or harmful for their growth. Supplementing the substrate with favourable microbial species helps the mushrooms to cope with pathogen infection (Marčić et al., 2025). Biofungicides based on various *Bacillus* species compete with pathogens for space and nutrients by producing antibiotics, enzymes, iron chelators, and various volatile compounds (Pandin et al., 2018). Moreover, *B. subtilis* (Ehrenberg) Cohn is regarded as harmless to the environment and humans, and has been classified as a safe organism (i.e. generally recognized as safe [GRAS]) (Food and Drug Administration [FDA], 1999). The biocontrol agent *B. subtilis* also promotes the growth of cultivated plants and mushrooms (Liu et al., 2015). A commercial biofungicide based on the strain *Bacillus velezensis* (formerly *B. subtilis*) QST 713 (Ehrenberg) Cohn has been registered for use against many fungal pathogens of cultivated plants and mushrooms (Védie & Rousseau, 2008; Pandin et al., 2018; Marčić et al., 2025), and was recently approved for use in Serbia. However, the efficacy of *B. velezensis* QST 713 against different mushroom pathogens varies, especially in the suppression of *H. perniciosus* (Navarro et al., 2023) and *C. mycophilum* (Clarke et al., 2024). As a commercial strain with antifungal and phytostimulating properties, *B. subtilis* Ch-13 was approved for use as a microbiological fertilizer, fungicide, and wheat seed disinfectant in Serbia and several other countries (Chebotar et al., 2009; Kayin et al., 2015; Potočnik et al., 2019). Strain *B. subtilis* Ch-13 was tested against *T. aggressivum* f. *europaeum* and compared to strain *B. velezensis* QST 713 and the fungicide prochloraz (Potočnik et al., 2019; Potočnik et al., 2021). Few industrial-scale studies of disease control in cultivated mushrooms are available, especially those concerning the biological control of *H. perniciosus*. Only one such study was performed by Regnier and Combrinck (2010), who determined the application rate (40 µl l^{-1}) of non-formulated plant essential oils (lemon verbena, thyme, lemongrass, and their main components nerol and thymol) in the suppression of *H. perniciosus* on naturally infected mushrooms.

The goal of the current study was to compare the biofungicide *B. subtilis* Ch-13 to the fungicide prochloraz in relation to their efficacy in wet bubble disease control in natural infection conditions, as well as their impact on mushroom yield. For this purpose, two different application protocols for *B. subtilis* Ch-13 (two *vs.* three split doses) on naturally infected mushrooms were evaluated.

MATERIAL AND METHODS

Antifungal agents

The efficacy of *B. subtilis* Ch-13 (Ekstrasol F SC, BioGenesis d.o.o., Belgrade, Serbia; content of active ingredient [a.i.] 1×10^8 CFU ml^{-1}) against the

mycopathogen *H. perniciosus* (wet bubble disease agent) was tested on naturally infested white button mushrooms (*A. bisporus*). The trial was conducted in a growing room (A2) of mushroom producer Delta Danube d.o.o., Kovin, Serbia. Efficacy in disease control and impact on yield (biological efficiency) of the biofungicide were evaluated and compared to those of the commercial fungicide prochloraz (Mirage® EC, ADAMA Agricultural Solutions UK Ltd., UK; content of a.i. 450 ml l^{-1}).

Tests in the mushroom growing room

Mushroom compost (fermented and pasteurized straw, supplemented with chicken manure) for phase III was produced by the compost factory Champicomp d.o.o., Pločica, Kovin, Serbia. The compost was packed in plastic briquettes sized 0.6 × 0.4 × 0.25 m (*l* x *w* x *h*). Each briquette contained 18 kg of compost inoculated with 0.7% of grain spawn of *A. bisporus* F56 (Italspawn, Onigo di Pederobba, Italy). Compost in each briquette was covered with a 50 mm layer of casing soil (7 kg) made of black peat soil (Pešter peat soil, Dallas Company d.o.o., Tutin, Serbia), and disinfected with 90 ml of 0.02% peracetic acid (Peral-S 15%, Vetprom, Belgrade, Serbia) per m^2 of casing layer. To calculate the doses for fungicide application, the surface area of the casing layer of five briquettes was estimated at 1 m^2. After the substrate was incubated at 25°C for 16 days, the air temperature was gradually reduced for 8 days to 17°C for the case-run. The day of casing time was regarded as day one. The trial was prepared according to standard PP 1/270 (1) methodology (European and Mediterranean Plant Protection Organization [EPPO], 2010). The fungicide prochloraz and biofungicide *B. subtilis* Ch-13 were applied onto the casing soil as a drench application. Prochloraz was applied at the standard product application rate – divided into two split applications (2 × 1.5 ml in 1 l of water per 1 m^2 of casing surface, four days after casing time and after the first fruiting flush). The biofungicide *B. subtilis* Ch-13 was applied in the total amount of 60 ml per m^2 of casing surface using two different application procedures: (*1*) three split applications (30 + 2 × 15 ml in 1 l of water per m^2 of casing surface, two days after casing, two weeks after casing, and after the first fruiting flush, respectively); and (*2*) two split applications (2 × 30 ml in 1 l of water per m^2 of casing surface, two days after casing, and after the first fruiting flush). The fungicide and biofungicide were applied using an automatic "fir" sprayer with 10 full cone nozzles, in the amount of 1 l per m^2 of casing surface. Control (untreated) briquettes were sprayed with tap water.

Each treatment was repeated in a randomized block design, on 43 m^2 of casing area per block. Each block consisted of 214 briquettes (replicates) with mushroom substrate. The fruiting bodies were hand-picked during the first production flush (14 to 21 days after casing) and the second flush (24 to 34 days after casing). The harvested mushrooms were classified into two groups, healthy and diseased, and then weighed. The impact of the biofungicide and fungicide on mushroom yield was evaluated via biological efficiency (BE) according to Chrysayi-Tokousbalides et al. (2007). Biofungicide and fungicide efficacy (E) were estimated using Abbott's formula (Abbott, 1925), i.e. the calculation was based on disease incidence (proportion of healthy and diseased mushrooms) in treated mushrooms (Gea et al., 2010).

Statistical analyses

Data obtained for efficacy in disease control and biological efficiency (impact on mushroom yield) were analyzed using the one-way analysis of variance (ANOVA); means were compared using the *F*-test. The *F*-test was used to compare mean values for efficacy obtained from repeated treatments of mushrooms with the fungicide, biofungicide, and water against the *H. perniciosus* mycopathogen *in vivo*, and to detect any significant difference among the treatments. The level of significance was determined at $p<0.05$ (Sokal & Rohlf, 1995). Statistical data analysis was performed using the software Statistica for Windows 7.0 (StatSoft Inc., 2004).

RESULTS

The efficacy of the biofungicide *B. subtilis* Ch-13, applied at the total concentration of 60 ml m^{-2} using two different application protocols – two (2 × 30 ml m^{-2}) or three (30 + 2 × 15 ml m^{-2}) split doses – was compared to that of the fungicide prochloraz in industrial-scale conditions of white button mushroom cultivation and their natural infestation with *H. perniciosus*. The impact of prochloraz and *B. subtilis* Ch-13 on mushroom yield was also evaluated. Wet bubble disease symptoms developed and were detected 18 to 34 days after casing time.

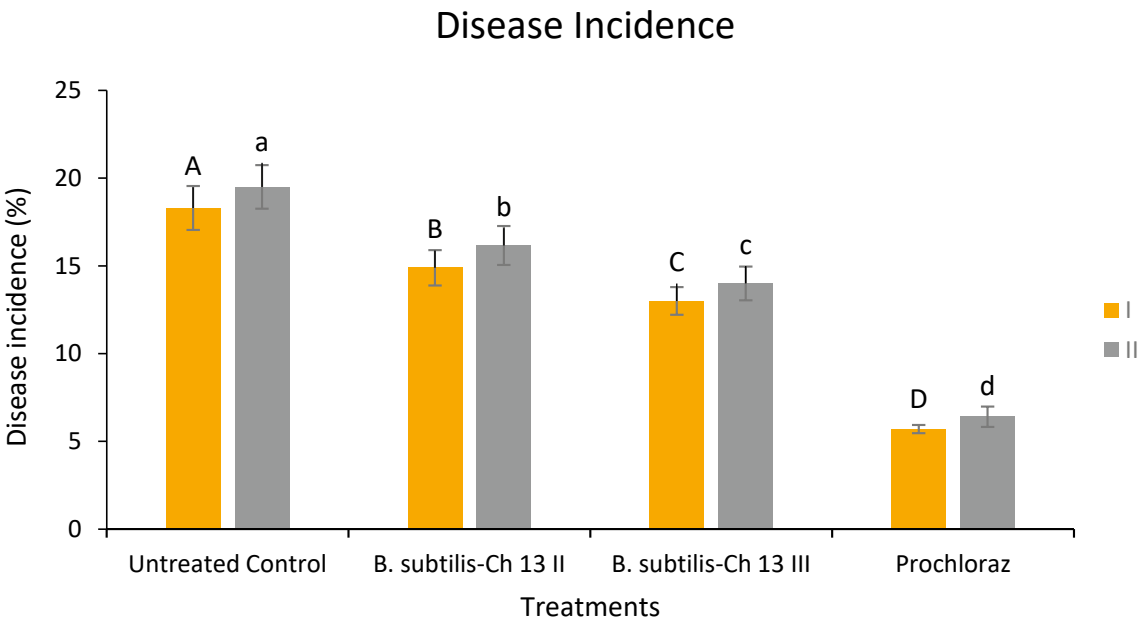

Figure 1. Incidence of wet bubble disease caused by *Hypomyces perniciosus* after treatment of *Agaricus bisporus* with bio/fungicides (*Bacillus subtilis* Ch-13 II/III – used in two/three split doses); data represent means of repetitions I and II (214 experimental briquettes per repetition) ± SE, standard error of the mean; SEDs, standard error of difference = 8.5 (I); 9.7 (II); df, degrees of freedom = 3; $F = 65.22$ (I); 78.32 (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 ($p<0.05$).

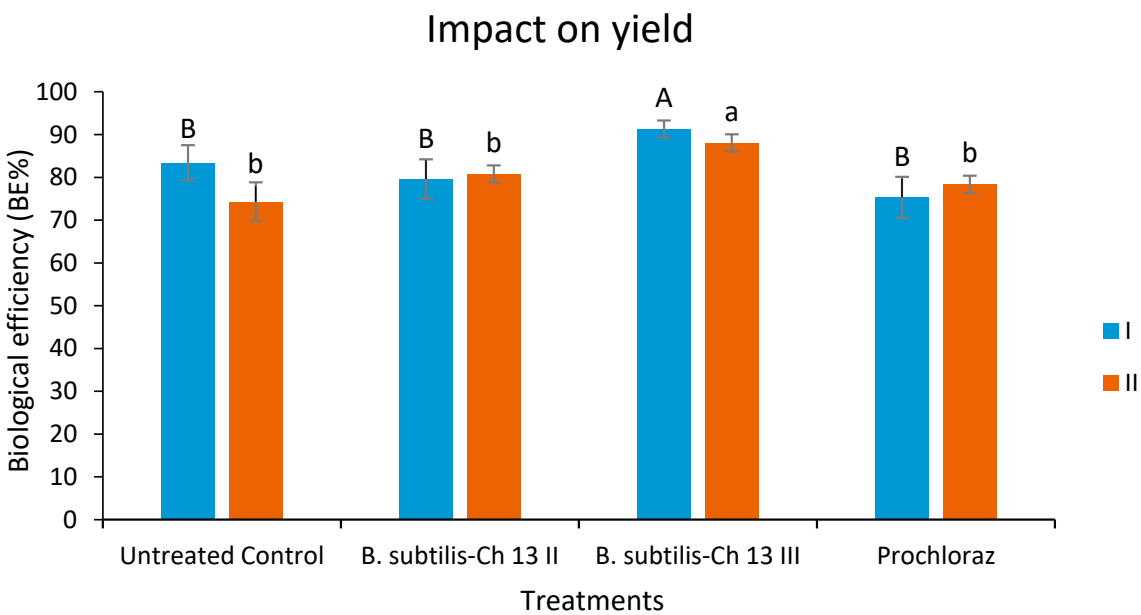

Figure 2. Impact of bio/fungicides on yield (biological efficiency – BE%) of *Agaricus bisporus*, naturally infested with *Hypomyces perniciosus* (*Bacillus subtilis* Ch-13 II/III – used in two/three split doses). Data represent means of repetitions I and II (214 experimental briquettes per repetition) ± SE, standard error of the mean; SEDs, standard error of difference = 37.7 (I); 35.8 (II); df, degrees of freedom = 3; $F = 28.4$ (I); 32.6 (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 ($p<0.05$).

Effects of bio/fungicides on wet bubble disease incidence in white button mushrooms are shown in Figure 1. The highest disease incidence was found in the untreated mushroom control (18.3-19.5%). Disease incidence significantly decreased by applying biofungicide *B. subtilis* Ch-13 in two (14.9-16.2%) or three (13-14%) split doses. The lowest disease incidence was found in plots treated with prochloraz (5.7-6.4%). Efficacy in disease control was calculated in two ways: in relation to the fungicide prochloraz as a standard (E_{st}), which was set to 100% efficacy, or to the untreated control (E_c) (Table 1). Statistically significant differences in efficacy against the fungal pathogen were recorded for all treatments, with respect to either the fungicide standard or the untreated control. In relation to the untreated control (E_c), prochloraz exhibited the highest efficacy in the suppression of wet bubble disease (67.9%), followed by *B. subtilis* Ch-13 applied in three split doses (28.7%) (Table 1). The biofungicide *B. subtilis* Ch-13 used in two split doses showed the lowest efficacy in the suppression of *H. perniciosus* (15.7%), even though the same final concentration of *B. subtilis* Ch-13 was used in both protocols (with two or three split applications). Relative to efficacy of prochloraz set to 100% (E_{st}), *B. subtilis* Ch-13 used in three split doses demonstrated 55% higher efficacy in disease control (42.3%), than when used in two split doses (23.1%) (Table 1). Therefore, *B. subtilis* Ch-13 used three times significantly decreased the incidence of wet bubble disease of white button mushrooms, by up to 29% compared to the untreated control. In addition, efficacy of *B. subtilis* Ch-13 against *H. perniciosus* was significantly higher when the biofungicide was applied in three split doses, instead of two, regardless of the same total concentration of *B. subtilis* Ch-13 used in both protocols. Although efficacy of *B. subtilis* Ch-13 (≈30%) against *H. perniciosus* was low in the current study, treatment of mushrooms with *B. subtilis* Ch-13 slightly diminished wet bubble symptoms.

The impact of prochloraz and *B. subtilis* Ch-13 on mushroom yield (Table 2; Figure 2) was calculated

Table 1. Efficacy of bio/fungicides in the suppression of *Hypomyces perniciosus* on naturally infested *Agaricus bisporus,* in relation to the fungicide prochloraz (standard) or untreated control

Treatments	Fungicide application rate	[2]E_{st} (%)	[2]E_c (%)	SE
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1} II[1]	2 × 30 ml m^{-2}	23.10 c[4]	15.70 c	0.36
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1} III	30 + 2 × 15 ml m^{-2}	42.30 b	28.74 b	0.48
Prochloraz 450 ml l^{-1}	2 × 1.5 ml m^{-2}	100.00	67.94 a	0.22
Untreated Control	–	8.60 a	–	0.57

[1]*Bacillus subtilis* Ch-13 II/III – used in two/three split doses. The data represent means of two repetitions (214 experimental units per repetition) ± SE, standard error of the mean; efficacy in relation to the fungicide prochloraz (standard) set to 100% [2](E_{st}), or the untreated control [3](E_c); SEDs, standard error of difference = 36.75; df, degrees of freedom = 3; $F = 71.77$; p-value = 0.001. [4]Values marked with the same letter within each repetition series 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 fungicide prochloraz (standard) treatment of *Agaricus bisporus* naturally infested with *Hypomyces perniciosus*

Treatments	Fungicide application rate	[2]BE_{st} (%)	[3]BE_c (%)	SE
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1} II[1]	2 × 30 ml m^{-2}	104.35 b[4]	101.67 b	0.86
Bacillus subtilis Ch-13 1 × 10^8 CFU ml^{-1} III	30 + 2 × 15 ml m^{-2}	116.71 a	113.72 a	2.28
Prochloraz 450 ml l^{-1}	2 × 1.5 ml m^{-2}	100.00	97.44 b	2.14
Untreated Control	–	102.63 b	100.00	6.43

[1]*Bacillus subtilis* Ch-13 II/III – used in two/three split doses. The data represent means of two repetitions (214 briquettes per repetition) ± SE, standard error of the mean; fungicide prochloraz (standard) impact [2](BE_{st}) or untreated control impact [3](BE_c) is set to 100 %; SEDs, standard error of difference = 36; df, degrees of freedom = 3; $F = 30.5$; p-value = 0.001. [4]Values marked with the same letter within each repetition series are not significantly different according to the F-test ($p<0.05$).

by using the formula for biological efficiency (BE) according to Chrysayi-Tokousbalides et al. (2007). A statistically significant increase in mushroom yield relative to the untreated control was found when *B. subtilis* Ch-13 was applied in three split doses (88.1-91.3%), while no significant difference in yield was recorded between the untreated control (74.3-83.4%) and treatments with either prochloraz (75.4-78.4%) or *B. subtilis* Ch-13 applied in two split doses (80-81%) (Figure 2). Biological efficiency was calculated in two ways: relative to prochloraz (standard) (BE_{st}) or to the untreated control (BE_c), each set to 100% biological efficiency (Table 2). Relative to the untreated control, *B. subtilis* Ch-13 applied in three split doses had the highest and statistically most significant impact on mushroom yield (113.7%) (Table 2). In contrast, treatment of mushrooms with *B. subtilis* Ch-13 applied in two split doses, as well as with prochloraz, resulted in respective yield values of 101.7% and 97.4%, which did not significantly differ from that of the untreated control (Table 2). Relative to prochloraz set to 100% biological efficiency (BE_{st}), *B. subtilis* Ch-13 used in three split doses supported a mushroom yield (116.7%) that was 12.4% higher than the yield it supported when applied in two split doses (104.3%) (Table 2). Concerning its impact on yield, *B. subtilis* Ch-13 used in two split doses did not significantly differ from the untreated control in relation to prochloraz (BE_{st}) set to 100%, and did not significantly differ from prochloraz in relation to the untreated control (BE_c) set to 100%. The application of *B. subtilis* Ch-13 in three split doses significantly increased mushroom yield in contrast to its application in two split doses, the untreated control, or prochloraz. The biofungicide *B. subtilis* Ch-13, when applied in three split doses, considerably improved mushroom yield: by up to 14% and 17% relative to the untreated control and the fungicide prochloraz, respectively.

DISCUSSION

Large tumorous fruiting bodies with extracellular fluid on the surface of diseased mushrooms, detected in all experimental briquettes, were consistent with symptoms of wet bubble disease caused by *H. perniciosus* and described by Umar and Van Griensven (1999).

Rather low efficacy of *B. subtilis* Ch-13 applied in three (≈29%) or two split doses (≈16%) was recorded against *H. perniciosus,* in comparison to that of the fungicide prochloraz (≈68%). However, wet bubble symptoms were slightly reduced after *B. subtilis* Ch-13 treatment relative to the untreated control. Having found that two commercial strains, *B. velezensis* QST 713 (5.13×10^{10} CFU g^{-1}) and *B. amyloliquefaciens* subsp. *plantarum* D747 (5×10^{13} CFU g^{-1}) – both applied in two doses, at a concentration of 3 g m^{-2} on the first and fourth day after casing time – were not effective against *H. perniciosus*, Navarro et al. (2023) stated that *Bacillus* strains were not applicable against *H. perniciosus* on white button mushrooms. Nevertheless, when Navarro et al. (2023) evaluated strains *B. velezensis* QST 713 and *B. amyloliquefaciens* subsp. *plantarum* D747 at a concentration of 1×10^5 CFU m^{-2} against *H. perniciosus* in artificially infested mushrooms, they recorded efficacy ranges of 14.8-19% and 17-20.7%, respectively, which correspond to disease incidence values found after natural infestation of mushrooms with the pathogen in the present study. Efficacy of strain *B. subtilis* Ch-13 applied in the current study in two split doses (≈16%) was consistent with the previously reported findings of Navarro et al. (2023). However, strain *B. subtilis* Ch-13 applied in three split doses in the present study achieved higher efficacy (28.7%) than strains *B. velezensis* QST 713 and *B. amyloliquefaciens* subsp. *plantarum* D747 applied at much higher concentrations by Navarro et al. (2023). Strain *B. subtilis* Ch-13 exhibited higher efficacy against compost pathogen *T. aggressivum* f. *europaeum* and also increased mushroom yield more than *B. velezensis* QST 713, when both were applied at a concentration (10^7 CFU m^{-2}) lower than their standard application rates (Potočnik et al., 2019). This finding suggests that the timing of *Bacillus* spp. application is of great importance for its effect on mushroom production.

The biofungicide *B. subtilis* Ch-13 showed higher efficacy against *Trichoderma aggressivum* when applied either in three (53.6%) or two split doses (46.4%) in a large-scale experiment (Potočnik et al., 2021), than when used at the same concentration against *H. perniciosus* in the current study. The fungicide prochloraz exhibited efficacy in the control of *T. aggressivum* (71%) (Potočnik et al., 2021) similar to that against *H. perniciosus* in the present study (68%).

In several studies, the biofungicide widely used for plant and mushroom protection, *B. velezensis* QST 713 (Védie & Rousseau, 2008), displayed different efficacies against green mould disease agents *Trichoderma* spp. on *A. bisporus*: against *T. harzianum,* 44% (Kosanović et al., 2013; Milijašević-Marčić

et al., 2017), and against *T. aggressivum,* 48% (Milijašević-Marčić et al., 2017), 53% (spawn treatment) (Potočnik et al., 2018), 55% (Potočnik et al., 2019), and 54-58% (Stanojević et al., 2019). Stanojević et al. (2019) recorded an efficacy of 45-62% of strain *B. velezensis* QST 713 in the suppression of *Z. fungicola* (dry bubble disease). Clarke et al. (2024) noted that *B. velezensis* QST 713 prevents disease symptoms caused by *C. mycophilum* with an efficacy of 30-40%. Variation in biofungicide efficacy may be based either on differences in compost quality or among pathogen strains. Recently, strain *B. amyloliquefaciens* subsp. *plantarum* D747 was approved for use against *T. aggressivum,* while *B. amyloliquefaciens* MBI 600 was approved for suppression of *T. harzianum* and *T. aggressivum* on white button mushrooms (Marčić et al., 2025).

Milijašević-Marčić et al. (2017) recorded efficacies of 50% and 36% of the indigenous strain *B. subtilis* B-38 in the control of green mould agents *T. harzianum* and *T. aggressivum*, respectively. Stanojević et al. (2019) found that the native strain *B. amyloliquefaciens* B-241 suppressed *Z. fungicola* and *T. aggressivum* f. *europaeum* with efficacies of 46-58% and 53-68%, respectively. Clarke et al. (2024) found that the native strain *B. velezensis* Kos reduced *C. mycophilum* symptoms by up to 30-40%. Carrasco and Preston (2020) noted that native *Bacillus* spp. may diminish wet bubble disease symptoms only if the concentration of disease inoculum is low, whilst having a significant role in increasing mushroom yield.

Liu et al. (2015) confirmed that the indigenous strain *B. subtilis* B154 significantly increased the yield of *A. bisporus* infected with red bread mould *Neurospora sitophila* in comparison to the untreated control. Büchner et al. (2022) recorded that native strains of *B. velezensis,* used against *Trichoderma* spp. and *Z. fungicola,* increased mushroom yield by up to 18-26%. Milijašević-Marčić et al. (2017) found that *B. velezensis* QST 713 improved mushroom production by up to 15% in plots inoculated with *T. aggressivum* f. *europaeum,* while in uninoculated treatments, no increase in yield was found. Navarro et al. (2023) found that strains *B. velezensis* QST 713 and *B. amyloliquefaciens* subsp. *plantarum* D747 increased mushroom yield significantly in comparison to the untreated control in one out of six trials. In a small-scale trial, Potočnik et al. (2019) recorded that strain *B. subtilis* Ch-13 applied in two split doses (two days after casing and after the first flush) at a final concentration of 60 ml per m^2 (1×10^8 CFU ml^{-1}) enhanced mushroom yield by up to 12% in pathogen-free briquettes. Though applied at the same total concentration (60 ml per m^2, 1×10^8 CFU ml^{-1}) in the current study, *B. subtilis* Ch-13 increased mushroom yield much more when applied more frequently, in three split doses (14% and 17%, relative to the untreated control and prochloraz, respectively), than when applied in two split doses (2% and 4%, relative to the untreated control and prochloraz, respectively). Therefore, the results of this study support the application of strain *B. subtilis* Ch-13 at a concentration of 60 ml per m^2 (1×10^8 CFU ml^{-1}) in three split doses to suppress *H. perniciosus* and improve *A. bisporus* yield.

CONCLUSION

In the industrial-scale cultivation of *A. bisporus,* the efficacy of *B. subtilis* Ch-13, used at a total concentration of 60 ml per m^2 (1×10^8 CFU ml^{-1}) against *H. perniciosus* on naturally infected white button mushrooms, was significantly higher when applied in three split doses (28.7%), than in two (15.7%). Although the efficacy of *B. subtilis* Ch-13 (≈29%) against *H. perniciosus* was low in comparison to that of the fungicide prochloraz (≈68%), wet bubble symptoms in mushrooms were slightly reduced. Additionally, the greatest increase in mushroom yield was achieved when *B. subtilis* Ch-13 was applied in three split doses (14%), rather than in two (2%), compared to the untreated control. Therefore, the results of this study support that *B. subtilis* Ch-13 should be applied in three split doses ($30 + 2 \times 15$ ml m^{-2} on the second day after casing, two weeks after casing, and after the first fruiting flush, respectively) to suppress *H. perniciosus*.

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Biofungicid *Bacillus subtilis* Ch-13 u suzbijanju *Hypomyces perniciosus* (mokra trulež) u uslovima industrijske proizvodnje šampinjona

REZIME

Cilj rada je bio da se ispita efikasnost biofungicida *Bacillus subtilis* Ch-13 u suzbijanju prirodne zaraze *Hypomyces perniciosus* (prouzrokovača bolesti mokre truleži) i njegovog uticaja na prinos u uslovima industrijske proizvodnje šampinjona (*Agaricus bisporus*). Biofungicid *B. subtilis* Ch-13 je bio primenjen u ukupnoj koncentraciji od 60 ml m^{-2} na dva različita načina: u tri ($30 + 2 \times 15$ ml m^{-2}) ili dve podeljene doze (2×30 ml m^{-2}) u poređenju sa standardnom dozom primene fungicida prohloraza. Efikasnost biofungicida je bila značajno veća kada je primenjen u tri podeljene doze (28.7%), nego u dve (15.7%). Iako je uočena niska efikasnost *B. subtilis* Ch-13 (≈29%) u suzbijanju *H. perniciosus* u poređenju sa fungicidom prohlorazom (≈68%), simptomi mokre truleži su smanjeni u određenoj meri. Takođe, najveće povećanje prinosa šampinjona je postignuto kada je *B. subtilis* Ch-13 primenjen u tri podeljene doze (14%), umesto u dve (2%), u poređenju sa netretiranom kontrolom. Tri podeljene doze biofungicida *B. subtilis* Ch-13 su povećale prinos šampinjona 17%, a dve podeljene doze 4%, u poređenju sa fungicidom prohlorazom. Efikasnost biofungicida *B. subtilis* Ch-13 u suzbijanju prouzrokovača mokre truleži, kao i u povećanju prinosa šampinjona, bila je veća kada je primenjen u tri podeljene doze, umesto u dve. Dakle, preporučuje se primena biofungicida u tri podeljene doze ($30 + 2 \times 15$ ml m^{-2}, drugog dana i dve nedelje nakon pokrivanja i posle prve berbe) radi efikasnog suzbijanja *H. perniciosus* i povećanja proizvodnje šampinjona.

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