JOURNAL OF AGRICULTURE AND PLANT SCIENCES, JAPS, Vol 22, No. 1, 2024

Manuscript received 11.05.2023
Accepted: 14.02.2024

In print: ISSN 2545-4447
On line: ISSN 2545-4455
doi: https://doi.org/10.46763/JAPS24221033p
Professional paper

BIOLOGICAL CONTROL OF GREEN MOULD DISEASE AND MUSHROOM FLY USING BIOFUNGICIDE *BACILLUS SUBTILIS* CH-13 AND BOTANICAL INSECTICIDE AZADIRACHTIN (TECHNICAL SOLUTION)

Ivana Potočnik[1*], Tanja Drobnjaković[1], Svetlana Milijašević-Marčić[1], Jelena Luković[1], Miloš Stepanović[1], Dejan Marčić[1], Emil Rekanović[1]

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

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

Abstract

The result of this study was accepted technical solution of disease/pest control of cultivated mushroom (*Agaricus bisporus* L.) based on biological pesticides (Biogenesis d.o.o., Serbia): microbial biofungicide *Bacillus subtilis* Ch-13 (Ekstrasol 1×10^8 CFU/cm^3) and botanical bioinsecticide azadirachtin (Ozoneem trishul 1 %). The efficiency of bio/pesticides in disease/pest control and impact on mushroom yield were evaluated in a large and small-scale experiments. The efficacy of biofungicide *Bacillus subtilis* Ch-13 to control of *Trichoderma aggressivum* Samuels & W. Gams (green mould disease) was evaluated in comparison with chemical fungicide prochloraz (2×1.5 mL/m^2). Biofungicide was applied in different procedures, in two (2×30 mL/m^2), three (30 + 2×15 mL/m^2), and six split doses (6×10 ml/m^2). The highest statistically significant effectiveness in pathogen control was shown when three (53.57-58.43%) or six doses (63.05%), were used. Biofungicide significantly improved yield in all different procedures, compared with untreated control in small-scale experiments 6.11-12.12% and in large-scale 5.07-8.41%. The impact of the bioinsecticide azadirachtin (4×0.5 ml/m^2) on the density of the mushroom fly *Lycoriella ingenua* Dufour (Diptera: Sciaridae) was compared to the effects of the chemical insecticide malathion (2×0.3 ml/m^2). The average number of the mushroom fly adults on yellow sticky traps per each mushroom row was significantly lower in the test chamber in comparison with two controlled chambers in commercial mushroom facility. The results of our study suggest that biofungicide *Bacillus subtilis* Ch-13 (30 + 2×15 mL/m^2 or 6×10 ml/m^2) and bioinsectide azadirachtin (4×0.5 ml/m^2) may provide a good alternative to conventional chemicals.

Keywords: *Agaricus bisporus, biopesticides, Lycoriella ingenua, Trichoderma aggressivum*

INTRODUCTION

The most important technological process in production of white button mushroom [*Agaricus bisporus* (Lange) Imbach] is the substrate preparation (Royse, 2010). Pesticide residues in straw and poultry manure obtained from the conventional agriculture production affect the quality of the substrates. Consequently, the genuine microbiome of the substrate is disturbed and no longer able to compete with harmful organisms. The major fungal disease of cultivated mushrooms is *Trichoderma aggressivum* Samuels & W. Gams causing green mould disease and crop losses exceeding 60% (Kosanović et al., 2013). The most significant pest in mushroom production is the mushroom fly, *Lycoriella ingenua* Dufour (Diptera: Sciaridae), which causes great economic losses and further dissemination of the conidia of the fungal infections (Mazin et al., 2019).

Disease and pest control in mushroom farms includes strict hygiene measures and application of disinfectants and pesticides. Considerable problems for mushroom growers are lack of effective pesticides and resistance development

of pests and pathogens to pesticides (Bartlett and Keil, 1997; Grogan, 2008). Pesticides may induce harmful effects on mushroom mycelia, causing loss of quality and yield, furthermore the residues present in the harvested mushrooms reduce the quality of the products (Navarro & Gea, 2006). In the recent years, a number of pesticides have been banned for use, by the European Commission. Currently, only fungicides such as prochloraz and metrafenone and some bioinsecticides have the approval to be used in mushroom cultivation by OEPPO (Luković et al., 2021).

Application of beneficial bacterium *Bacillus subtilis* (Ehrenberg) Cohn, which produces extra cellular metabolites for competition and antibiosis, is a good alternative to chemical fungicides (Milijašević-Marčiće et al., 2017). The bacterium is harmless to the environment and human health and is generally recognized as safe (GRAS) organism (FDA, U. S., 1999). Some metabolites and remains of *B. subtilis* could serve as a nutrient source for edible mushrooms and promote the yield (Carrasco and Preston, 2000). Pyrethrin-based products, which showed good efficacy in the mushroom fly suppression, have not been registered for edible mushrooms in Serbia, and the latest researches has revealed their high toxicity to non-target organisms (Drobnjaković et al., 2019). Besides, the use of entomopathogenic nematodes has shown instable efficacy against the mushroom flies. The triterpenoid azadirahtin from the class of limonoids is the primary active ingredient in plant extracts, oils and other derivatives obtained from seeds of the Indian neem tree [*Azadirachta indica* A. Juss. (Meliaceae)]. Neem based products have been shown to be active against more than 200 species of insects, including many dipterans and may act as repellents, feeding inhibitors, oviposition deterrents and insect growth regulators (Chaudhary et al., 2017). Neem-based products can be used together with other bioinsecticides based on living organisms, and in contrast to synthetic pesticides, the low persistence of azadirachtin products make them considerably safer for most beneficial arthropod species (Raguraman and Kannan, 2014). Due to the complex mode of action of azadirachtin, there is no risk of cross-resistance (Siegwart et al., 2015). Available literature data on azadirachtin activity against the mushroom flies are scarce and refere only to the mushroom phorid fly *Megaselia halterata* (Wood) (Diptera: Phoridae), and the first research on *L. ingenua* (Drobnjaković et al., 2019). In our study, biofungicide based on *B. subtilis* Ch-13 (Extrasol F) and bioinsecticide based on azadirachtin (Ozoneem trishul 1%) was tested in control of the green mould disease agent and the mushroom sciarid flies.

EXPERIMENTAL CONDITIONS

New strain *B. subtilis* Ch-13, recently available in Serbia, was compared with chemical fungicide prochloraz and biofungicide *Bacillus amyloliquefaciens* QST713 (formerly known as *B. subtilis*) (Priest et al., 1987) (Tab. 1). The impact on yield and efficacy in green mould disease control were evaluated on white button mushroom (*A. bisporus*) using artificial infection of *T. aggressivum* f. *europaeum* T77 ($10^6/m^2$) in the small-scale experiment and natural infection (*Trichodrma* spp.) in the commercial mushroom producing facility (large-scale). For preparing 0.7% spawned substrate of *A. bisporus* A15 (Sylvan, Hungária zRt) substrate was placed in: *a*) plastic boxes sized 0.340 x 0.215 x 0.130 m (*l* x *w* x *h*) contained 1.5 kg of compost, six replicates per treatment (small scale experiment), and *b*) plastic bags sized 0.6 × 0.4 × 0.2 m (*l* x *w* x *h*) contained 18 kg of compost, 224 replicates per treatment (large scale experiment). After 14 days of incubation, substrate was cased with casing soil Terahum (Treset d.o.o., Veliko Gradište, Serbia) in the hight of 3-5 cm. The harvested mushrooms were weighed and divided into two groups, with or without symptoms of green mould disease. The effect of fungicides on mushroom productivity was evaluated as biological efficiency (BE), calculated as the ratio of fresh weight of total fruiting body yield and the weight of dry spawned substrate, and expressed as %: BE = (fresh total fruiting body yield/dry spawned substrate mass) x 100. Fungicide efficacy in disease control was calculated: % efficacy = [(Ic -It)/Ic] x 100, where Ic - disease incidence in inoculated control; It - disease incidence in treated samples. Disease incidence was recorded as the percentage of fruiting bodies with symptoms compared to those without symptoms. Biofungicide *B. subtilis* Ch-13 was applied in different procedures, in

two (2×30 mL/m^2), three (30 + 2×15 mL/m^2), and six split doses (6×10 mL/m^2). Biofungicide *B. amyloliquefaciens* QST713 was applied 2×0.5 mL/m^2, and fungicide based on prochloraz 2×1.5 mL/m^2.

Furthermore, the impact of an azadirachtin-based bioinsecticide (applied 4×0.5 mL/m^2) on regulation of the abundance of sciarid mushroom fly adults, was investigated compared to a conventional insecticide based on malathion (2×0.3 mL/m^2) (Tab. 1). The density of mushroom flies was observed using yellow sticky traps. All traps were inspected under a binocular microscope to count mushroom flies caught.

Table 1. Pesticide products used in the study.

Trade name	Active ingredient	Concentration of active ingredient	Manufacturer
Ekstrasol F SC	*Bacillus subtilis* Ch-13	1 x 10^8 CFU/mL	BioGenesis d.o.o., Serbia
Serenade® ASO	*Bacillus amyloliquefaciens* QST713	5.13x10^9 CFU/g	Bayer CropScience, Serbia
Mirage® EC	Prochloraz	450 mL/L	ADAMA Agricultural Solutions UK Ltd., UK
Ozoneem trishul	Azadirachtin	1% (10 g/L)	BioGenesis d.o.o., Serbia
Etiol tečni	Malathion	600 g/L	Galenika-Fitofarmacija a.d., Serbia

Data were examined by using the one-way analysis of variance (ANOVA), including the comparison of means by *F* test. The test was used to compare the significance of differences among data on the average biological efficiency and efficacy in disease control of different biopesticide/pesticide treatments in the mushroom growing room. In all analyses, the level of significance was at least $P<0.05$. Statistical data analysis was performed by the software Statistica for Windows 6.0 (StatSoft Inc., 2004).

EFFICACY OF FUNGICIDES

The efficacy of biofungicides/fungicide in suppression of symptoms of green mould disease is shown in Fig. 1 and 2. Impact on yield is shown in Fig. 3. Preliminary small-scale experiment showed that *B. subtilis* Ch-13, applied at a concentration of 2 or 3×10^8 CFU/m^2, achieved respective better efficacy than strain QST713 (23%) formulated at higher concentration (5 × 10^9 CFU/m^2) (Potočnik et al., 2019). Despite the same final concentration of 60 mL/m^2, the efficacy of *B. subtilis* Ch-13 in green mould disease control was significantly higher when it was applied in three split doses (30 + 2×15 mL/m^2) than in two applications (2×30 mL/m^2) in the large-scale evaluation (Potočnik et al., 2021). Fungicide prochloraz showed the highest efficacy in disease control in all experiments. Efficacy of the prochloraz was 71% in the small-scale study after artificial infection (*T. aggressivum* 10^6 conidia/m^2), and 77% in the large-scale assay after natural infection (Potočnik et al., 2019; 2021). In the extended small-scale study, efficacy of the biofungicide was similar, 58.43%, when applied three times (30 + 2 × 15 mL m^{-2}), and 63.05% after six split doses (6×10 mL/m^2) applied to suppress green mould disease symptoms (Potočnik et al., 2022). No statistically significant difference was found in the efficacy in disease control of the biofungicide, applied in three (58.43%) or six (63.05%) split doses, and the fungicide prochloraz (71.08%) against *T. aggressivum* (Fig. 1).

IMPACT OF FUNGICIDES ON MUSHROOM YIELD

A statistically significant increase of mushroom yield was noted when the biofungicide *B. subtilis* Ch-13 was used in two and three split doses in comparison with the untreated control and prochloraz fungicide (Potočnik et al., 2021). The mushroom yield was enhanced in larger extent by *B. subtilis* Ch-13 when the biofungicide was used more frequently. Biofungicide *B. subtilis* Ch-13 considerably improved mushroom yield compared to uninoculated treatments: control plots (5-15%), fungicide prochloraz threated plots (7-12%),

and plots with biofungicide *B. amyloliquefaciens* QST713 (24-31%) (Potočnik et al., 2019; 2021). In extended small-scale experiment, the similar enhancement of mushroom yield by strain Ch-13 was recorded after three or six split doses (Potočnik et al., 2022). Some variation in yield promotion was noted depending of the mushroom compost quality and the seasonal changes.

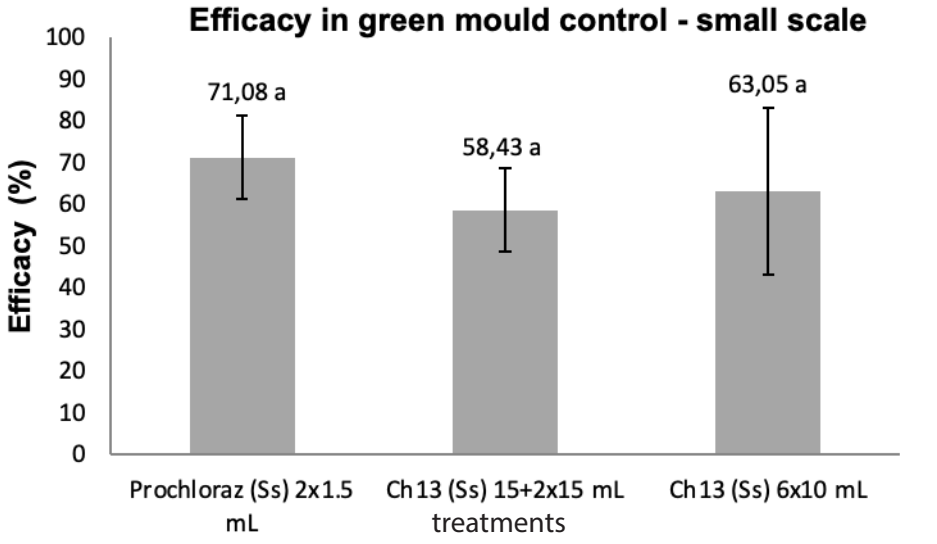

Figure 1. The efficacy of bio/fungicides against *Trichoderma aggressivum* T77 on white button mushroom (*Agaricus bisporus*). (Ss) – small scale experiment: SEDs, standard error of differences = 14.66; df, degree of freedom = 2; *F* = 0.26; *P*-value = 0.77. Values within series marked with the same letter are not significantly different according to *F* test (P<0.05).

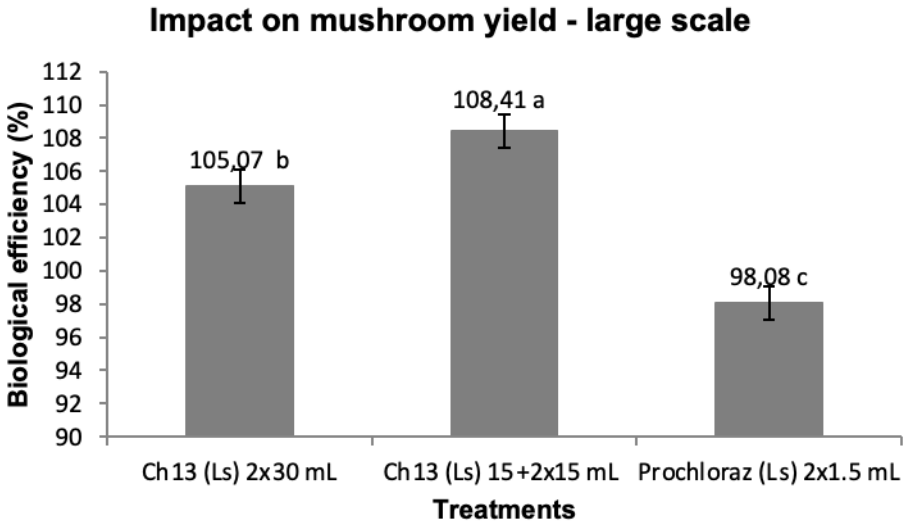

Figure 2. The efficacy of bio/fungicides against *Trichoderma aggressivum* T77 on white button mushroom (*Agaricus bisporus*). (Ls) – large scale experiment: SEDs, standard error of differences=9.41; df, degrees of freedom=3; *F*=70.22; *P*-value=0.001. Values with series marked with same letters are not significantly different according to *F* test (P<0.05). (Ss) – small scale experiment: SEDs, standard error of differences = 14.66; df, degree of freedom = 2; *F* = 0.26; *P*-value = 0.77. Values within series marked with the same letter are not significantly different according to *F* test (P<0.05).

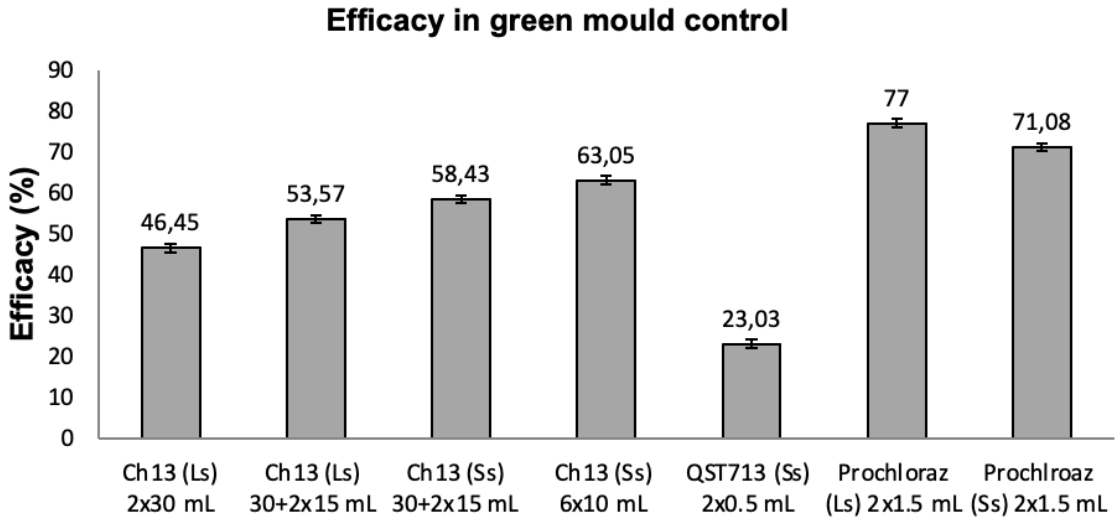

Figure 3. Impact on mushroom yield (*Agaricus bisporus*) of different treatments of bio/fungicides. (Ls) – large scale experiment: SEDs, standard error of differences=48; df, degrees of freedom=3; *F*=25; *P*-value=0.001. Values with series marked with the same letters are not significantly different according to *F* test (P<0.05).

Results regarding the management of the mushroom flies showed that, in comparison to the positive control chambers in the commercial facility (large scale), where the traditional malathion-based insecticide was used, the average number of sciarid fly adults over the entire test period was significantly lower in the test chambers (15, 22, 30 and 36 Days After Treatment - DAT), where the biorational azadirachtin-based product was tested (Fig. 4) (Drobnjaković et al., 2019).

The number of adult *L. ingenua* caught on yellow sticky traps was significantly lower in all three test chambers near the chamber entrance than in the chambers at the opposite end, suggesting that spatial isolation of fungus flies is a key element in preventing damage. The highest average number of adults of the mushroom fly was recorded at the third inspection (30 DAT), at the peak of mushroom production, corresponding to the time when the first harvest ended. In the fourth inspection period, 36 DAT, the average number of the mushroom fly adults started to decrease (Drobnjaković et al., 2019). No negative effect of azadirachtin on the growth of *A. bisporus* yield or quality was observed compared to the positive control. These are the first data on the efficacy of an azadirachtin-based product in reducing *L. ingenua* populations. Similarly to the results of our research, Erler et al. (2009) proposed that the neem-based products Neemazal (10 g/L azadirachtin EC) and Greeneem oil (3 g/L azadirachtin in the 100% pure natural cold-pressed neem oil) may suppress populations of the mushroom phorid fly *M. halterata* and may provide an alternative to the standard chemical chlorpyrifos-ethyl. The researchers discovered that phorid larvae treated with neem showed aberrant growth and remained at the immature stage of development before dying (Erler et al., 2009).

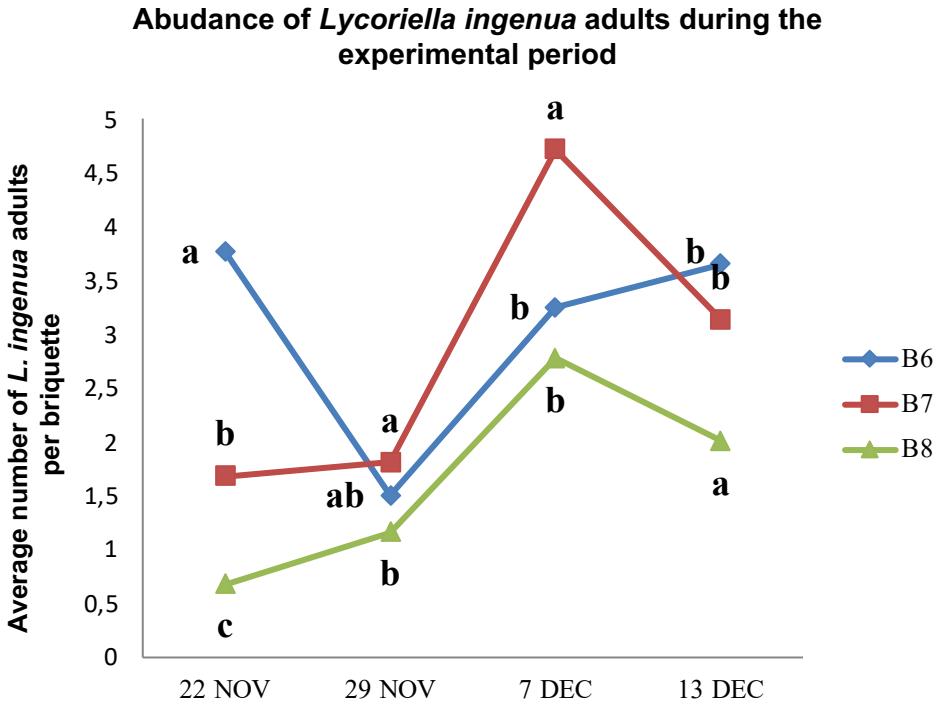

Figure 4. The average number of adults of *Lycoriella ingenua* (the mushroom fly) adults per briquette during the experimental period in the commercial facility (large scale) (B6, B7 – chambers treated with chemical insecticide malathion; B8 – chamber treated with bioinsecticide azadirachtin).

CONCLUDING REMARKS

The biofungicide based on *B. subtilis* Ch-13 showed better efficacy in green mould disease control and the highest positive impact on mushroom production when used in three or six split applications, rather than two in comparison with the chemical fungicide prochloraz. It suggests that the biofungicide should be applied three times (30 mL/m^2 on the second day after casing + 15 mL/m^2 two weeks after casing + 15 mL/m^2 after the first flush) or six times (10 mL/m^2 on the second day after casing + 5 × 10 mL/m^2 at seven-day intervals). Azadirachtin-based bioinsecticide applied four times at the rate of 4×0.5 mL/m^2 (starting with casing time and proceeding with successive treatments at seven-day intervals), significantly reduced the density of sciarid fly *L. ingenua*, compared with the conventional insecticide. Using those two biopesticides reduces the use of chemical pesticides in cultivated mushrooms, enabling the processing and export of the substrate and mushrooms according to the required standards for product safety and quality. It would further increase the competitiveness of domestic mushroom producers in a regional market.

ACKNOWLEDGEMENTS

The study was funded by grant 451-03-47/2023-1/200214 of the Ministry of Science, Technological Development and Innovation, Republic of Serbia.

REFERENCES

Bartlett, G. R., & Keil, C. B. (1997). Identification and Characterization of a Permethrin Resistance Mechanism in Populations of the Fungus Gnat *Lycoriella mali* (Fitch) (Diptera: Sciaridae). *Pesticide Biochemistry and Physiology*, *58*(3), 173-181.

Carrasco, J., & Preston, G., M. (2000). Growing edible mushrooms: a conversation between bacteria and fungi. *Environmental Microbiology*, *22*(3), 858-872. Doi: 10.1111/1462-2920.14765

Chaudhary, S., Kanwar, K. R., Sehgal, A., Cahill, D. M., Barrow, C. J., Shgal, R., & Kanwar, J. R. (2017). Progress on Azadirachta indica based biopesticides in replacing synthetic toxic pesticides. *Frontiers in Plant Science*, *8*(610), 1-13. doi: 10.3389/fpls.2017.00610

Drobnjaković, T., Marčić, D., Potočnik, I., Rekanović, E., Prijović, M., Milijašević-Marčić, S., & Stepanović, M. (2019). Control of mushroom sciarid fly *Lycoriella ingenua* (Dufour) with an azadirachtin-

based insecticide. *Pesticides and Phytomedicine/ Pesticidi i fitomedicina*, *34*(2), 111-121. Doi: https://doi.org/10.2298/PIF1902111D

Erler, F., Polat, E., Demir, H., Cetin, H., & Erdemir, T. (2009). Control of the mushroom phorid gnut, *Megaselia halterata* (Wood), with plant extracts. *Pest Management Science: formerly Pesticide Science, 65*(2), 144-149. Doi: https://doi.org/10.1002/ps.1658

FDA, U. S. (1999). Food and Drug Administration. Code of Federal Regulations. Title 21: Food and Drugs. Chapter 1: Food and Drug Administration Department of Health and Human Services. Part 184: Direct Food Substances Affirmed as Generally Recognized as Safe. Washington, DC: US Government Printing Office.

Grogan, H. M. (2008). Challenges facing mushroom disease control in the 21st century. In Proceeding of the Sixth International Conference on Mushroom Biology and Mushroom Products (pp. 120-127). Bonn, Germany: WSMBMP.

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

Luković, J., Milijašević-Marčić, S., Hatvani, L., Kredics, L., Szücs, A., Vàgvölgyi, 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: 10.1080/03601234.2020.1838821.

Navarro, M. J., & Gea, F. J. (2006). Estudio de la fitotoxicidad del insecticida diflubenzuron en el cultivo de champiñón. Determinación del nivel de residuos. *Boletín de la Asociación Española de Cultivadores de Champiñón*, *48*, 32-34.

Mazin, M., Harvey, R., Andreadis, S., Pecchia, J., Cloonan, K., Rajotte, E.. G. (2019). Mushroom sciarid fly, *Lycoriella ingenua* (Diptera: Sciaridae) adults and larvae vector Mushroom Green Mold (*Trichoderma aggressivum ft. aggressivum*) spores. *Applied Entomology and Zoology, 54*, 369–376. https://doi.org/10.1007/s13355-019-00632-2

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: 10.1007/s10658-016-1107-3

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/ Pesticidi i fitomedicina*, *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 of the effectiveness of a *Bacillus subtilis* Ch-13-based biofungicide against green mould disease and mushroom yield improvement. *Pesticides and Phytomedicine/ Pesticidi i fitomedicina*, *36*(2), 83-90. Doi: https://doi.org/10.2298/PIF2102083P

Potočnik, I., Luković, J., Todorović, B., Stepanović, M., Šantrić, Lj., Milijašević-Marčić, S., & Rekanović, E. (2022). Improvement of procedure for casing treatment with a *Bacillus subtilis* Ch-13-based biofungicide to control green mould disease of mushrooms. *Pesticides and Phytomedicine/Pesticidi i fitomedicina*, *37*(3), 94-102. Doi: https://doi.org/10.2298/PIF2203095P

Priest, F. G., Goodfellow, M., Shute, L. A., & Berkeley, R. C. W. (1987). *Bacillus amyloliquefaciens* sp. nov., nom. rev. *International Journal of Systematic and Evolutionary Microbiology*, *37*(1), 69-71. https://doi.org/10.1099/00207713-37-169

Raguraman, S., & Kannan, M. (2014). Non-target effects of botanicals on beneficial arthropods with special reference to *Azadirachta indica*. In *Advances in plant biopesticides*. New Delhi, India: Springer.

Royse, D. J. (2010). Effects of fragmentation, supplementation and the addition of phase II compost to 2nd break compost on mushroom (*Agaricus bisporus*) yield. *Bioresource Technology*, *101*, 188-192. Doi: 10.1016/j.biotech.2009.07.073.

Siegwart, M., Graillot, B., Blachere Lopez, C., Besse, S., Bardin, M., Nicot, P.C., & Lopez-Ferber, M. (2015). Resistance to bio-insecticides or how to enhance their sustainability: a review. *Frontiers in Plant Science*, *6*, 381. Doi: https://doi.org/10.3389/fpls.2015.00381

StatSoft Inc. (2004). STATISTICA (data analysis software system), version 7. Retrived from www.statsoft.com

БИОЛОШКА КОНТРОЛА НА БОЛЕСТА ЗЕЛЕНА МУВЛА И ГАБНА МУВА СО КОРИСТЕЊЕ НА БИОФУНГИЦИД *BACILLUS SUBTILIS* Ч-13 И БОТАНИЧКИ ИНСЕКТИЦИД АЗАДИРАХТИН (ТЕХНИЧКО РЕШЕНИЕ)

Ивана Поточник[1], Тања Дробњаковић[1], Светлана Милијашевић-Марчић[1], Јелена Луковић[1], Милош Степановић[1], Дејан Марчић[1], Емил Рекановић[1]*
[1]Институт за пестициди и заштита на животната средина, Банатска 31б, Поштенски фах 163, 11080 Белград-Земун, Србија

**Контакт автор: ivana.potocnik@pesting.org.rs*

Резиме

Резултатот од ова истражување беше прифатено техничко решение за контрола на болести/штетници на култивирана печурка (*Agaricus bisporus* L.) базирано на биолошки пестициди (Биогенесис Д.О.О., Србија): микробен биофунгицид *Bacillus subtilis* Ch-13 (Ekstrasol 1×108 CFU/cm^3) и ботанички биоинсектицид азадирахтин (Ozoneem trishul 1 %). Ефикасноста на био/пестицидите во контролата на болести/штетници и влијанието врз приносот на печурките беа оценети во експерименти од големи и мали размери. Ефикасноста на *биофунгицидот Bacillus subtilis* Ч-13 за контрола на *Trichoderma aggressivum* Samuels & W. Gams (болеста зелена мувла) беше оценета во споредба со хемискиот фунгицид прохлораз (2×1,5 mL/m^2). Биофунгицидот беше применет во различни постапки, во две (2×30 mL/m^2), три (30 + 2×15 mL/m2) и шест поделени дози (6×10 ml/m^2). Статистички највисока значајна ефективност во контролата на патогенот беше достигната кога беа користени три (53,57-58,43 %) или шест дози (63,05 %). Биофунгицидот значително го подобри приносот во сите различни процедури, во споредба со нетретираната контрола во експериментите од мал 6,11-12,12 % и во експериментите од голем размер 5,07-8,41 %. Влијанието на биоинсектицидот азадирахтин (4×0,5 ml/m^2) врз густината на габната мува *Lycoriella ingenua* Dufour (Diptera: Sciaridae) беше споредено со ефектите на хемискиот инсектицид малатион (2×0,3 ml/m^2). Просечниот број на возрасни габни муви на жолти лепливи стапици на секој ред од печурки беше значително помал во комората за тестирање во споредба со две контролирани комори во комерцијалните капацитети за печурки. Резултатите од нашата студија сугерираат дека биофунгицидот *Bacillus subtilis* Ch-13 (30 + 2×15 mL/m^2 или 6×10 ml/m^2) и биоинсектидот азадирахтин (4×0,5 ml/m^2) можат да обезбедат добра алтернатива на конвенционалните хемикалии.

Клучни зборови: *Agaricus bisporus*, *биопестициди*, *Lycoriella ingenua, Trichoderma aggressivum*.