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Original scientific paper

Impact of neem cake amendment in the casing soil on control of *Trichoderma aggressivum* Samuels & W. Gams and *Lycoriella ingenua* (Dufour) and mushroom yield

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SUMMARY

The study was focused on improvement of the integrated management strategy against green mould disease agent *Trichoderma aggressivum* Samuels & Gams and mushroom fly *Lycoriella ingenua* (Dufour) as pests of the white button mushroom *Agaricus bisporus* (Lange) Imbach. The impact of neem cake amendment in casing soil on regulation of the abundance of mushroom sciarid fly adults, efficacy in controlling the green mould disease agent, and mushroom yield was evaluated. Casing soil was supplemented with different concentrations of neem cake: 1, 2.5, 5, 10 and 15%. Neem cake added as a supplement to casing soil at a rate of 2.5% reduced the number of mushroom fly adults by 83.93% and green mould disease incidence by 59.6% in comparison to the control. No adverse effect on mushroom formation, yield and quality of fruiting bodies was observed at that concentration. Amendment of 2.5% neem cake in the casing soil could be recommended for application in mushroom production to control *L. ingenua* and symptoms of green mould disease without negative impact on mushroom yield.

Keywords: *Agaricus bisporus*, *Azadirachta indica*, green mould disease, mushroom fly

INTRODUCTION

Substrate preparation is the most important process in the production of white button mushroom [*Agaricus bisporus* (Lange) Imbach]. Mushroom substrate is made from fermented and pasteurized mixture of plant material and animal manure. Application of pesticides during wheat production and the use of antibiotics in poultry farming results in reduced compost quality. Chemical residues disrupt the genuine microbiome of substrate in favour of harmful organisms. After completing spawn-run of mushroom mycelia, compost is cased with a 3-5 cm thick layer of casing soil. Casing soil is made mainly of black peat, neutralized with limestone and sterilized by various disinfectants. Casing layer is essential for mushroom development and fructification, enabling suitable microbiome, humidity and aeration (Jarial et al., 2005). Preventive disinfection and treatment of casing soil with chemical pesticides is a common practice in disease and pest control.

The prevailing mycosis of cultivated mushrooms in Serbia and worldwide is green mould disease caused by *Trichoderma aggressivum* Samuels & W. Gams, which results in crop losses exceeding 60% (Kosanović et al., 2013). The most significant mushroom pest in Serbia and globally is the mushroom fly (fungus gnat), *Lycoriella ingenua* (Dufour) (Sciaridae: Diptera), which causes great economic losses in commercial mushroom production (Rinker, 2017; Drobnjaković et al., 2019). Fletcher and Ganney (1968) recorded that mushroom pests and diseases were mostly transmitted by casing soil, and suggested formaldehyde as a control method. Other widely used casing soil disinfectants are also: sodium hypochlorite, potassium permanganate, sulphur, calcium chloride and chlorinated compounds (Sharma & Guleria, 1999). Recently, casing soil disinfection based on the eco-friendly substance peracetic acid was implemented (Potočnik et al., 2014).

Chemicals induce harmful effects on mushroom mycelia, causing losses in quality and yield (Shamshad, 2010), and resulting in residues presence in harvested mushrooms (Gea et al., 2021). Lately, many pesticides have been withdrawn from the market. Only two chemical fungicides (prochloraz and metrafenone) and bioinsecticides based on pyrethrin and entomopathogenic nematodes have been officialy recommended for use in mushroom cultivation by the OEPPO (Carrasco et al., 2017; Navarro et al., 2021). Pyrethrin-based bioinsecticides, which are very effective in the control of mushroom flies, have appeared to be highly toxic to non-target organisms. On the other side, the use of bioinsectides based on entomopathogenic nematodes has shown variable success in the control of mushroom flies (Navarro et al., 2021; Ruchika et al., 2021). However, none of the mentioned formulated bioinsecticides is registered in Serbia.

Natural products of the Indian neem tree [*Azadirachta indica* A. Juss. (Meliaceae)] have several uses in agriculture, industry, medicine, and the environment (Adusei & Azupio, 2022). The limonoid-based azadirahtin, the primary and most important active ingredient in all neem tree derivates (plant extracts, essential oils, neem cake, etc.) (Gupta et al., 2019), accounts for over 90% of the pest-control actions. It acts as a natural fertilizer with pesticidal properties (Schmutterer, 1995).

In agriculture, neem products are used as pesticides, pest fumigants, fertilizers, manures, compost, urea coating agents, and soil conditioners. Neem-based pesticides are effective against many plant pests and disease agents, such as insects, nematodes, fungi and bacteria (Lokanadhan et al., 2012). Neem cake is the by-product obtained in the process of cold pressing of whole neem tree fruits, depulped seed/kernels, and either by expeller or solvent extraction process.

Azadirachtin acts as antifeedant, repellent, feeding inhibitor, oviposition deterrent, growth regulator in insects (Isman, 1993), and growth-inhibitor in fungal pathogens (Adusei & Azupio, 2022). Due to the complex mode of action of azadirachtin, there is no risk of cross-resistance (Siegwart et al., 2015). Neem components affect the insect endocrine system, rather than neurological or digestive system like chemical insecticides [Mordue (Luntz) & Nisbet, 2000]. It has demonstrated typical insect growth regulating actions in the larval stages of insects (Koul, 2007). More than 105 insect pests from 10 orders are controlled with neem kernels, among which are numerous pests in the order Diptera (Roychoudhury, 2016). Besides, neem cake organic manure protects plant roots not only from soil insects but from nematodes as well (Alam, 1993; Abbasi et al., 2005). We are not aware of any earlier study conducted to determine the impact of neem cake on control of *L. ingenua* populations.

Neem and its constituents are effective in inhibiting the growth of a wide range of microorganisms, including viruses, bacteria, and fungi. Extracts and aerosols of seeds and leaves of neem have been found to suppress the growth of many plant pathogenic fungi: *Alternaria alternata* (Fr.) Keissl. (Chaudhary et al., 2003), *Fusarium solani* (mart.) Sacc. and *Rhizoctonia solani* J.G. Kühn (Darwish & Shaker, 2005), *Verticillium dahliae* Kleb. and *Pythium aphanidermatum* (Edson) Fitzp. (Abbasi et al., 2005), *Alternaria solani* Sorauer (Moslem & El-Kholie, 2009; Jabeen et al., 2013), *Cercospora canesens* Ellis. & Martin (Trivedi et al., 2014). Neem powder inhibited the growth of fungal species *Fusarium oxysporum* Schlecht (Hadian et al., 2011). Neem seed extracts were efficient against the bacteria *Pseudomonas syringae* pv. *syringae* Van Hall, *Xanthomonas arboricola* pv. *corylina* Dye, and *Agrobacterium tumefaciens* Smith & Townsend (Goel et al., 2016).

Neem as a biopesticide has no adverse effects on plants or soil (Mukhopadhyay et al., 1992). Neem products serve as nitrification inhibitors as they block soil bacteria from converting nitrogenous compounds into nitrogen gas, and prolong the availability of nitrogen to both short and long duration crops (Puri, 2004). They reduce alkalinity in soil as they produce organic acids upon decomposition. Their application is compatible with soil microbes and rhizosphere microflora, and ensures soil fertility. They improve the organic matter content in soil, soil texture and its aeration, as well as water holding capacity (Puri, 2004).

In view of a large knowledge gap regarding azadirachtin and neem activity against harmful mushroom pests and diseases, neem oil has already been evaluated in control of the mushroom sciarid fly, *L. ingenua* (Drobnjaković et al., 2019). The aim of this study was to determine the impact

of neem cake amendment in casing soil on regulation of the abundance of *L. ingenua* adults. Moreover, its effect on the control of *T. aggressivum*, the green mould disease agent, and impact on yield of white button mushroom (*A. bisporus*) were also evaluated. The study was focused on improving mushroom fly and green mould disease integrated management strategies.

MATERIAL AND METHODS

Tests in mushroom growing room

Mushroom substrate was provided by the compost producer Uča, Vranovo, Serbia. Plastic boxes sized 0.340 x 0.215 x 0.130 m (*l* x *w* x *h*) were filled with 1.5 kg of compost mixed with 15 g of grain spawn of *A.bisporus* A15 (Sylvan, Hungária zRt) to prepare 1% spawned substrate. The boxes were incubated at 25°C (spawn-run) for 18 days. Compost was cased with 1.2 kg of black peat casing soil Wokas Casing Soil - Typ S (Wokas S.A., Łosice, Poland), and disinfected with peracetic acid 0.02% (Peral-S 15%, Vetprom, Belgrade, Serbia), equaling 90 ml per m^2 of casing soil. Neem cake – »Azadiroko« (BioGenesis d.o.o., Serbia) was tested as a potential casing soil amendment in the range of: 1, 2.5, 5, 10, and 15% of casing soil, and placed on mushroom compost. Casing soil was cased in a 50 mm layer and incubated at 22°C for 8 days (case-run). The day of casing was regarded as day one. Over the following seven days air temperature was reduced in stages to 17°C. The trial consisted of two groups, uninoculated plots and inoculated with *T. aggressivum* f. *europaeum* T77. Control plots within both groups were sprayed with tap water. Efficacy of the neem cake was evaluated against the green mould disease agent *T. aggressivum* f. *europaeum* T77 (artificial infection) and the mushroom fly *L. ingenua* (natural infection). The pathogenic fungus *T. aggressivum* f. *europaeum* T77, isolated in 2010 from a mushroom farm in Lisovići, Barajevo, was identified previously based on morpho-physiological characteristics and ITS1/ITS4 sequence analyses (Kosanović et al., 2013). Inoculation with *T. aggressivum* f. *europaeum* T77 was arranged from a culture grown on PDA at 25°C for three days. Mycelia of the pathogenic fungi were scraped from the surface of PDA plates, mixed with water and Tween 20 (v/v 0.01%) (REANAL Finomvegyszergyár Rt., Hungary, No. 805383) and filtered through sterile gauze. Spore concentration was determined by counting on a hemocytometer and the suspension was diluted to achieve the final concentration of 10^6 conidia ml^{-1}. Inoculation of *T. aggressivum* f. *europaeum* T77 was performed two days after the spawned compost was placed into boxes, by pipetting 1 ml of spore suspension and 9 ml of tap water (10^6 conidia ml^{-1} per m^2) down the inner walls of each box. The plots were arranged in a completely random design with six replicates per treatment.

Efficacy in disease/pest control and impact on yield (biological efficiency) were evaluated by comparison with the uninoculated and inoculated control, respectively. The fruiting bodies were hand-picked in two successive production flushes. The harvested mushrooms were weighed and divided into two groups based on visual observation, i.e. with and without symptoms of green mould disease. The effect of fungicides on mushroom productivity was evaluated by biological efficacy (BE), calculated as the ratio of fresh weight of total fruiting body yield and weight of dry spawned substrate, expressed as %: BE = (fresh total fruiting body yield/dry spawned substrate mass) × 100 (Chrysayi-Tokousbalides et al., 2007). Fungicide effectiveness was calculated by Abbott's formula (Abbott, 1925): % effectiveness = [(Ic -It)/Ic] × 100, where Ic - disease incidence in inoculated control; It - disease incidence in treated samples. Disease incidence was recorded as a percentage of fruiting bodies with symptoms compared with those without symptoms.

All experimental boxes were placed inside the insect rearing cages (one box per cage). The density of *L. ingenua* was observed using yellow sticky traps inside each insect rearing cage to enable early observation of fly adults, and their abundance during the first mushroom flush. The yellow sticky traps were collected at five inspection periods (5, 8, 14, 18 and 22 days after treatment - DAT), and replaced with new ones. After each inspection period the collected traps were inspected under a binocular microscope to establish mushroom fly presence and density. The flies were identified to the species level based on the identification key given by Menzel & Mohrig (2000). The observed treatment parameter was the number of adult flies on yellow sticky traps per insect cage containing mushroom substrate. The trial was performed in six replicates.

Statistical analyses

Data were examined using the one-way analysis of variance (ANOVA), including comparison of means by Duncan's test. The test was used to compare the significance of differences in data based on the average efficacy in disease/pest control and biological efficiency (impact on yield) of different neem cake amendment concentrations in casing soil against *T. aggressivum* f. *europaeum* T77 and *L. ingenua* in experimental mushroom growing room. In all analyses, the level of significance was at least $p<0.05$ (Sokal & Rohlf, 2013). Statistical data analysis was performed using the software Statistica for Windows 6.0 (StatSoft Inc., 2004).

RESULTS

The highest statistically significant disease incidence was recorded in the control (2.97%), followed by the plot amended with 5% neem cake (2.94%) (Figure 1). Experimental plots amended with 1 or 2.5% neem cake showed the lowest number of green mould disease symptoms with respective disease incidence of 1.23 and 1.2%, without statistically significant differences. Those concentrations of neem cake reduced disease symptoms by 60% in comparison to control. Hence, the highest efficacy in control of *T. aggressivum* f. *europaeum* was found in these plots treated with neem cake 1 % (58.58%) or 2.5% (59.59%) (Figure 2). The lowest efficacy in reducing symptoms of green mould disease was noted in plots supplemented with 5% neem cake (1.01%).

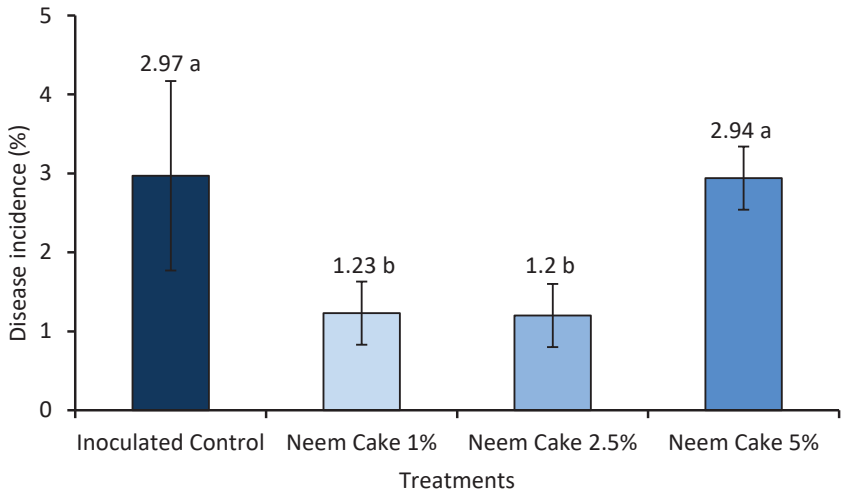

Figure 1. Green mould disease incidence on *Agaricus bisporus* after artificial inoculation of *Trichoderma aggressivum* f. *europaeum* T77; disease incidence average ± SE; SEDs, standard error of differences=11.61; SS=16.9; df, degrees of freedom=3; *F*=0.48; *P*-value=0.0001 (One-way ANOVA; *Means marked by different letters are significantly different; Duncan's test, p<0.05).

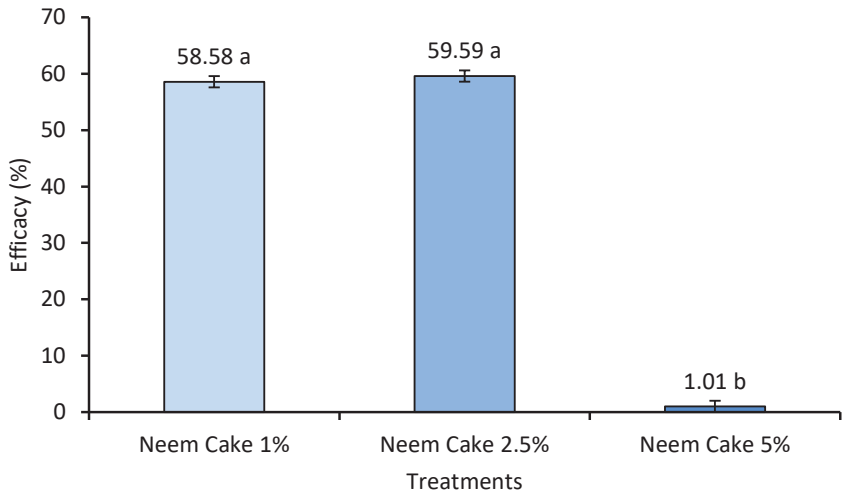

Figure 2. Efficacy of neem cake in control of *Trichoderma aggressivum* f. *europaeum* T77 after artificial inoculation on *Agaricus bisporus*; efficacy average SE; SEDs, standard error of differences=0.57; SS=16.9; df, degrees of freedom=3; *F*=0.48; *P*-value=0.0001 (One-way ANOVA; *Means marked by different letters are significantly different; Duncan's test, p<0.05).

Regarding the impact on yield, statistically significant differences were not found between the control, 1 or 2.5 % neem cake enhancement, with respective values of biological efficiency 82.1, 69.3 and 71.47% (Figure 3). Statistically significant negative impact on yield was noted in plots amended with higher concentrations of neem cake than previous (5, 10 and 15%). The lowest yield was recorded in plots supplemented with 5% neem cake (23.23%), while no fructification was noted when casing soil was supplemented with 10 or 15% neem cake. Neem cake at concentrations 1, 2.5 and 5% did not reduce the time taken for pinhead formation, compared to control.

Examination of the yellow sticky traps under a binocular microscope revealed only one species of mushroom fly pest, the mushroom sciarid fly *L. ingenua*, during the entire experimental period. The highest number of *L. ingenua* was found in plots covered with 10 and 15% neem cake (Figure 4). No statistically significant differences in the number of *L. ingenua* were found between the control plots and plots supplemented with 5 and 15% neem cake (Figure 4). The lowest number of mushroom fly adults was recorded in plots with 2.5% neem cake, followed by 1% neem cake at $p<0.05$ significance.

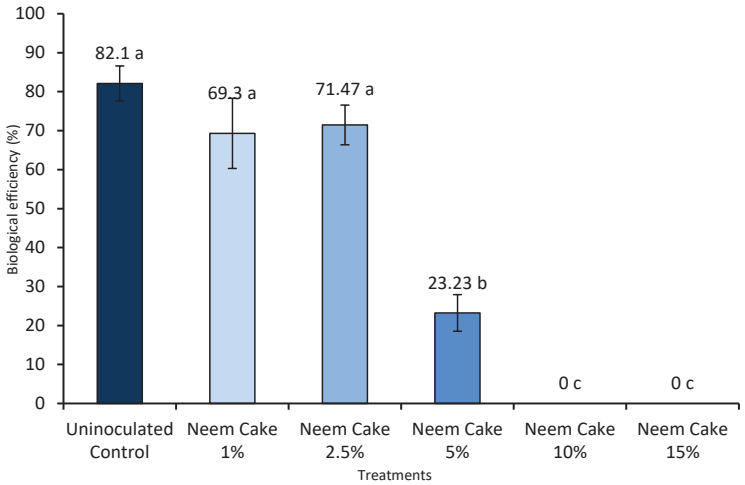

Figure 3. Impact of treatment on yield of *Agaricus bisporus* shown through biological efficiency of neem cake; biological efficiency average ± SE; SEDs, standard error of differences=4.99; SS=277980; df, degrees of freedom=3; *F*=6.16; *P*-value< 0.004 (One-way ANOVA; *Means marked by different letters are significantly different; Duncan's test, $p<0.05$).

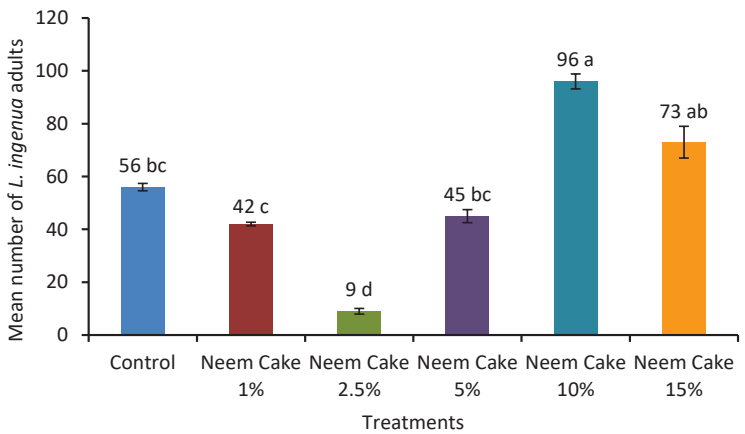

Figure 4. Abundance of *Lycoriella ingenua* adults per insect cage depending of concentrations of neem cake throughout the experimental period (average±SE). SEDs, standard error of differences=11.61; SS=2188.75; df, degrees of freedom=5; *F*=12.19; *P*-value<0.004 (One-way ANOVA; *Means marked by different letters are significantly different; Duncan's test, $p<0.05$).

During the experimental period, the highest statistically significant number of mushroom fly adults was recorded on day 14 after treatment (DAT) (Figures 5 and 6). Lower numbers of *L. ingenua* were noted in all other inspection periods (5, 8, 18 and 22 DAT) with no significant differences among them at $p<0.05$ level of significance.

DISCUSSION

The neem cake biopesticide was successfully applied at the concentration of 2.5% in casing soil, both as an insecticide against mushroom fly *L. ingenua* adults and as a fungicide reducing *T. aggressivum* incidence. The abundance of *L. ingenua* adults decreased by 83.93%

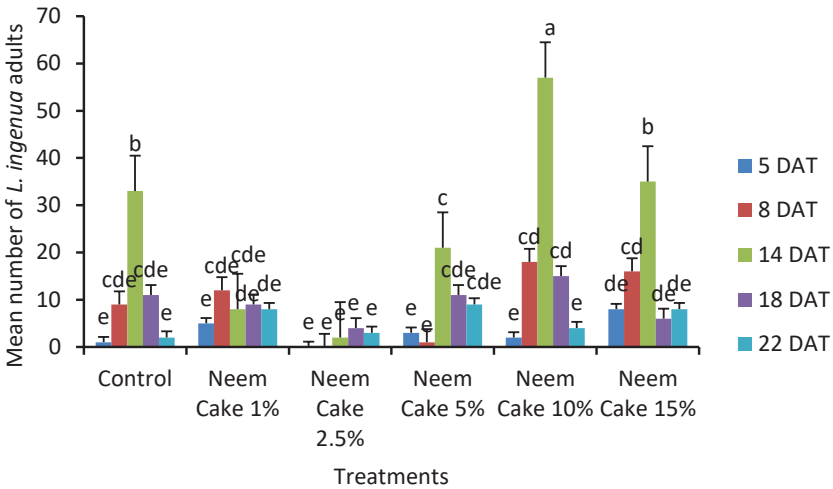

Figure 5. Abundance of *Lycoriella ingenua* adults depending of concentration of neem cake 5, 8, 14, 18 and 22 days after treatment (DAT) (average±SE). SEDs, standard error of differences=9.05; SS=1593.33; df, degrees of freedom=29; *F*=7.93; *P*-value<0.0001 (One-way ANOVA; *Means marked by different letters are significantly different; Duncan's test, $p<0.05$).

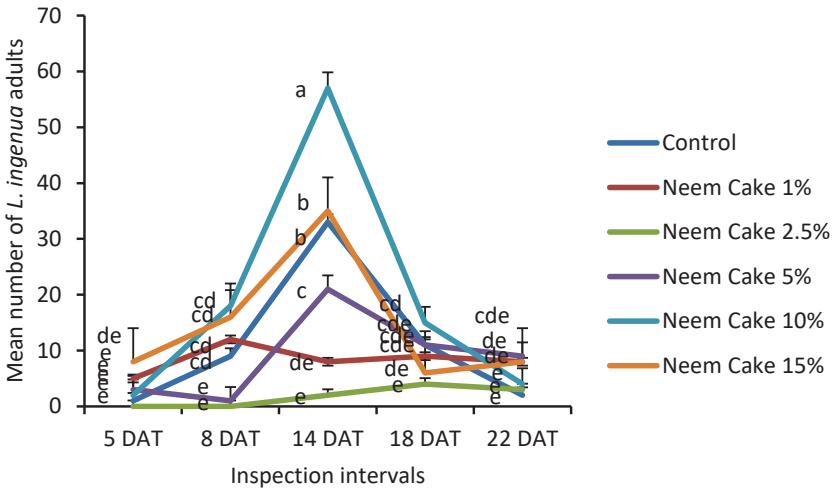

Figure 6. Abundance of *Lycoriella ingenua* adults depending of neem cake concentration during the experimental period (average±SE). SEDs, standard error of differences=9.05; SS=1593.33; df, degrees of freedom=29; *F*=7.93; *P*-value<0.0001 (One-way ANOVA; *Means marked by different letters are significantly different; Duncan's test, $p<0.05$).

and *T. aggressivum* incidence by 59.6% in comparison with control plots. After neem cake application, adverse effects on mushroom formation, yield and quality of fruiting bodies were not observed. The 5% concentration of neem cake drastically decreased mushroom yield (71.7%), while 10 or 15% neem cake amendement completely prevented fructification, which is in accordance with numerous other studies. In agreement with the results of the present study, Inam-Ul-Haq et al. (2010) found that concentrations of neem cake higher than 4% decreased mushroom yield. Previous studies have also shown that all neem tree derivatives, such as essential oils, neem cake and plant extracts, were efficient in pest/disease control in various mushroom crops. Shah et al. (2011) revealed that neem oil inhibited *Trichoderma harzianum* Rifai isolated from the oyster mushroom [*Pleurotus sajor-caju* (Fr.) Singh] by 34.1% in *in vitro* experiments. Furthermore, the authors reported that 1, 2, and 3% neem cake amendments increased oyster mushroom yields by 29, 34.3 and 35.3% (32.8% mean value) and reduced disease incidence by 33.3, 27.7 and 22.2 (mean value 27.7%), respectively, in comparison with control in *in vivo* tests. Besides, Shah et al. (2011) found that neem cake slightly reduced the time taken for pinhead formation as compared to control (7.6 days). Regarding the use of neem cake against disease agents, Sharma and Jarial (2000) reported that this neem three derivative inhibited the growth of *Diehliomyces microsporus* (Diehl & E.B. Lamb.) Gilkey, the false truffle disease agent of *Agaricus* spp. *in vitro.* The authors also discovered that neem cake incorporated in compost, drastically reduced disease incidence and increased the yield of button mushroom in *in vivo* experiments. Additionally, Sharma and Rajesh (2005) noted that 10% neem leaf extract inhibited the growth of *Sepedonium chrysospermum* (Bull.: Fr.) Link, causing yellow mould in button mushroom. Mishra (2009) reported that several neem three derivatives, i.e. neem leaf extract, neem cake solution and neem sawdust, inhibited *Trichoderma viride* Pers. on *Agaricus* spp. Grewal and Grewal (1988) noticed that incorporation of dried leaves of neem tree into mushroom compost eliminated pathogenic fungi belonging to *Fusarium* and *Sependonium* species. Moreover, Sharma and Jandiak (1994) reported that neem tree leaves incorporated in compost inoculated with various weed fungi, increased mushroom yield. Concerning the impact of neem cake on mushroom yield, Khade et al. (2019) reported that 2% neem cake per 3 kg substrate resulted in a significantly higher number of fruiting bodies of elm oyster mushroom [*Hypsizygus ulmarius* (Bull. Ex Fr.) Redhead] (89.56%). The authors also found that neem cake supplement achieved maximum mushroom yield (841.11 g per kg substrate), compared with different other organic and inorganic supplements. Also, the impovement of yield of oyster mushrooms *Pleurotus florida* Cetto and *P. sajor-caju* was detected after 2 and 4% neem cake supplementation (Sharma & Kumar, 2009). Furthermore, Inam-Ul-Haq et al. (2010) discovered that 2% neem cake amendment was the most promising in improving the yield of oyster mushroom [*Pleurotus ostreatus* (Jacq. Ex Fr.) Kumer] as it increased it up to 4%. Besides, Kumar et al. (2012) found that neem cake as a casing supplement of milky mushroom (*Calocybe indica* Purkay & A. Chandra) significantly inhanced its yield and pinhead initiation in comparison with control mushrooms, by 40-45%. It is noteworthy to highlight that neem tree based products have extremely low mammalian toxicity (Kleeberg, 1992), and they are relatively safe to non-target organisms (Schmutterer, 1995).

As in the case with *T. aggressivum*, we are not aware of any earlier research conducted to determine the impact of a neem cake on regulation of *L. ingenua* populations. Drobnjaković et al. (2019) found that a bioinsecticide based on azadirachtin (Ozoneem trishul 1%), the component of neem oil, succesfully controlled *L. ingenua*, compared to the malathion-based chemical insecticide Etiol tečni. Ozoneem trishul was applied at 2 ml m^{-2}, split in four applications, during casing time and later at seven-day intervals. The azadirachtin-based bioinsecticide supressed populations of the mushroom fly significantly better than malathion applied in the control chambers. The highest average number of mushroom flies was recorded 30 days after treatment (25 days after casing). There have been only a few reports with some other agricultural pests demonstrating that neem-based products may suppress pest populations and provide good alternative to conventional insecticide control. Depressed development and mortality were also recorded for neem-treated phorid larvae (Erler et al., 2009) and some dipteran pests (Stark et al., 1990; Okumu et al., 2007).

Pest and disease control in mushroom crops in Serbia is mainly based on using chemical pesticides, toxic to non-target organisms, humans and the environment. The European Commission Regulation 1107/2009 stimulates the application of low-risk active ingredients and use of sustainable alternatives

to chemical pesticides (Villaverde et al., 2014). Beside the European and national policies, the United Nations (UN) Sustainable Development Goals also urgently promote environmentally-friendly disease/pest control in mushroom production, which will improve the productivity and income of smallholder farming families (as part of a vulnerable population) (UNDP, 2023). Neem cake 2.5% amendment in casing soil could be recommended for application in mushroom production to control the mushroom sciarid fly and the causal agent of green mould disease without any negative impact on mushroom yield. The use of neem cake will reduce the use of chemical pesticides in mushroom industry and allow the processing and export of mushroom substrate and fresh mushrooms in accordance with the required standards for product safety and quality. It will further promote competitiveness among local farmers in regional markets. Furthermore, sustainable mushroom production will improve human health and safekeep the environment, strengthen the ecosystem's capacity to adapt to climate change, and improve land and soil quality.

CONCLUSION

Neem cake added as a supplement to casing soil for mushroom growing, applied at the concentration of 2.5%, reduced the number of *L. ingenua* adults by 83.93% and *T. aggressivum* green mould disease incidence by 59.6%, compared to control. After the application of that neem cake concentration, no adverse effects on mushroom formation, yield or quality of fruiting bodies was observed. Neem cake applied as a 2.5% amendment in casing soil could be recommended for application in white button mushroom production to control the mushroom sciarid fly and symptoms of green mould disease without negative impact on mushroom yield. The use of neem cake at the recommended application rate is in accordance with the basic postulates of the European Commission Regulation 1107/2009 and the UN Sustainable Development Goals.

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Uticaj dodavanja neem cake-a pokrivci za gajenje šampinjona na suzbijanje *Trichoderma aggressivum* Samuels & W. Gams i *Lycoriella ingenua* (Dufour) i prinos šampinjona

REZIME

Cilj rada je unapređenje integralne zaštite šampinjona *Agaricus bisporus* (Lange) Imbach od prouzrokovača bolesti zelene plesni *Trichoderma aggressivum* Samuels & W. Gams i šampinjonske mušice *Lycoriella ingenua* (Dufour). Ispitivan je uticaj *neem cake*-a dodatog pokrivci u smanjenju broja odraslih jedinki šampinjonske mušice, pojave simptoma zelene plesni i uticaj na prinos šampinjona. Pokrivka za gajenje šampinjona je obogaćena različitim koncentracijama *neem cake*-a: 1; 2,5; 5; 10 i 15%. Dodavanje *neem cake*-a pokrivci u udelu od 2,5% smanjilo je broj šampinjonskih mušica 83,93% i pojavu simptoma bolesti zelene plesni 59,6% u poređenju sa kontrolom. Nisu uočeni nepovoljni uticaji na obrazovanje, prinos i kvalitet plodnosnih tela šampinjona pri primeni navedene koncentracije. Dodatak 2,5% *neem cake*-a pokrivci se može preporučiti za primenu u proizvodnji šampinjona za suzbijanje šampinjonske mušice i simptoma bolesti zelene plesni, bez negativnog uticaja na prinos.

Ključne reči: *Agaricus bisporus*, *Azadirachta indica*, bolest zelene plesni, šampinjonska mušica