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ADAPTATION OF A PLANT GROWTH CHAMBER FOR THE EXPERIMENTAL CULTIVATION OF CHAMPIGNONS (*AGARICUS BISPORUS*)

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Abstract

The aim of the present study was to adapt a 1200 liter volume, Weiss Gallenkamp SGC120 standard plant growth chamber by optimizing the environmental parameters (temperature, humidity) for the cultivation of champignons on mushroom compost covered with casing material in commercially available compost blocks wrapped in polyethylene and mushroom growing boxes. The III. phase compost in the products consisted of wheat straw, chicken manure, horse manure, gypsum and white hybrid *Agaricus* spawn, while the casing material was black peat. A cultivation experiment of 36 days was designed with the temperature and humidity values set up in the ranges of 17-21 ℃ and 85-100%, respectively, by adapting the conditions of mushroom growing houses to the volume of the plant growth chamber.

Mushroom compost blocks yielded 142% higher mushroom crop (kg/m^2) than the growing boxes in the first harvesting period (days 20 to 22). Compost colonization and fruiting body formation proved to be appropriate under the controlled conditions. However, the mushroom compost blocks became affected by fungal infections by the end of the second harvesting period (days 34 to 36) and sciarid mushroom flies also appeared.

Our findings suggest that optimal parameters for champignon cultivation can be provided in the 1200 liter volume Weiss Gallenkamp SGC120 standard plant growth chamber, however, to reduce the risk of fungal contamination and crop losses, sterilization of the black peat by autoclaving is recommended before casing.

Key Words: champignon, pathogenic moulds, mushroom flies, plant growth chamber

Introduction

World mushroom cultivation is realized partly in a traditional way, partly within the frames of intensive cultivation. The traditional way of cultivation is performed without sterilization and it is primarily based on handwork, while the intensive cultivation strategies involve sterilization of the compost and they are mechanized. Industrial mushroom growing takes place in bags, blocks or on shelving systems. The phases of cultivation are composting, compost sterilization (in intensive cultivation), spawning, sprouting, casing and harvest (van Griensven, 1988; Visscher, 1988; Zied et al., 2011).

Cultivated mushrooms can be affected by numerous diseases caused by insects (including mushroom flies), mites, nematodes, viruses, bacteria as well as moulds resulting in serious crop losses worldwide. The most important mushroom pathogenic ascomycetous moulds include several members of the Hypocreaceae family, like the green moulds from the genus *Trichoderma* (Hatvani et al., 2008; Kredics et al., 2010), wet bubble disease caused by *Mycogone perniciosa* (Umar et al., 2000) or *Cladobotryum* species causing cobweb disease (Carrasco et al., 2015), as well as *Lecanicillium fungicola* from Cordycipitaceae causing dry bubble disease (Berendsen et al., 2010).

There are different types of control mechanisms for the protection of cultivated mushrooms against pathogenic moulds. Among them, chemical control is the most widely applied strategy worldwide. Imidazole demethylation inhibitors (DMI's) like prochloraz-manganese proved to be efficient against all fungal pathogens of mushroom cultivation by the inhibition of the demethylation step in ergosterol biosynthesis (van Zaayen and van Adrichem, 1982; Chrysayi-Tokousbalides et al., 2007; Potočnik et al., 2015). In European countries, prochloraz is used for the control of dry bubble

and green mould diseases (Potočnik et al., 2015). However, the number of chemicals permitted for application in mushroom production is already limited and decreasing, therefore, the development of various biocontrol strategies is of increasing importance. An example is a biofungicide product based on *Bacillus subtilis*, which is successfully used in France (Védie and Rousseau, 2008). Such novel, biology-based tools against mould infections also have the potential to be integrated into complex prevention and control strategies, the application of which may reduce the amount of the chemicals used.
The examination of different pests and pathogens of cultivated champignon (*Agaricus bisporus*) and the development of potential biological or integrated tools of disease control require *in vivo* experiments modelling mushroom cultivation under controlled conditions. For this purpose it is important to accurately set up and maintain environmental parameters – primarily temperature and humidity – in order to simulate the conditions of mushroom growing houses, enabling the development of fruiting bodies and also allowing the realization of infection and biocontrol experiments.

Materials and Methods

Compost and casing soil

Blocks of 70x45x15 cm wrapped in polyethylene (approx. 12 kg) and 30×20×15 cm mushroom growing boxes (approx. 4.5 kg) along with casing material were purchased from two different Hungarian commercial suppliers. The III. phase compost in the products consisted of wheat straw, chicken manure, horse manure, gypsum and white hybrid *Agaricus* spawn. The appearance of the compost in both products was brown, with slight manure smell and fine fibery consistence, while the casing layer was black peat in both products with soil smell and fine grainy consistence. The prescribed quality conditions for *Agaricus* compost and casing material are summarized in Table 1 and Table 2, respectively, while Table 3 shows the threshold limit values of heavy metals for both the compost and the casing material.

Two compost blocks and three mushroom growing boxes (with a compost height of 15 cm for both) were covered with approx. 5 cm of the respective casing material supplied by the producers. Compost blocks were placed in a 70x45x30 cm plastic box. Both cultivation systems were placed into a 1200 liter volume, Weiss Gallenkamp SGC120 standard plant growth chamber. Table 4 shows the chamber temperature and air humidity values set up during the 36-day cultivation period, along with the recorded compost temperature values. The casing layer was irrigated before the development of the fruiting bodies.

Table 1. Prescribed physico-chemical parameters of the compost

Volume mass	kg/dm^3	max. 0.9
Dry matter	m/m%	min. 50.0
Organic matter	m/m%	min. 70.0
pH (in 10% water suspension)	-	7.9 (±0.5)* 6.5 (±0.5)**
Total water soluble salt	-	max. 4.0
Particle size composition < 25 mm	m/m%	min. 100.0
N	m/m%	min. 1.0
P_2O_5	m/m%	min. 0.5
K_2O	m/m%	min. 0.5
Ca	m/m%	min. 1.2
Mg	m/m%	min. 0.5

*: compost block; **: mushroom growing box

Table 2. Prescribed physico-chemical parameters of the casing material

Volume mass	kg/dm^3	max. 0.9
Dry matter	m/m%	min. 50.0
Organic matter	m/m%	min. 50.0
pH (10% H_2O)	-	7.9(± 0.5)
Total water soluble salt	-	max. 4.0
Particle size < 25 mm	m/m%	min. 100.0
N	m/m%	min. 1.0
P_2O_5	m/m%	min. 0.5
K_2O	m/m%	min. 0.5
Ca	m/m%	min. 1.2
Mg	m/m%	min. 0.5

Table 3. Prescribed maximum threshold limit values (mg/kg) of heavy metals in the compost and the casing material

As	10.0
Cd	2.0
Co	50.0
Cr	100.0
Cu	100.0
Hg	1.0
Ni	50.0
Pb	100.0
Se	5.0

Table 4. Growth conditions in plant growth chamber

Days	Air temperature (°C)	Compost temperature (°C)	Air humidity (%)	Treatment	Observations
1	19	20	95	Compost loading, casing	
2	17	20	95	Irrigation: 2.4 L/m^2	
3	17	20	98	Irrigation: 2.9 L/m^2	
4	18	20	98		
5	19	20	98	Irrigation: 0.8 L/m^2	
6	20	20	99	Irrigation: 0.5 L/m^2	
7	21	20	100		
8	22	20	100		
9	21	21	95		casing layer colonized by *Agaricus* in compost block
10	21	21	95		
11	22	21	95		
12	21	22	95		initiation of primordia in compost block
13	20	21	95		
14	19.5	20	94		
15	19	19	93		casing layer colonized by *Agaricus* in growing box
16	18.7	18	92		
17	18.5	18	91		initiation of primordia in growing box
18	18.5	19	90		
19	18.5	19	89		

20	18.5	19	88	I. harvest from compost blocks	
21	17.5	19	88	I. harvest from compost blocks	
22	19	18	90	I. harvest from compost blocks	
23	17	18	95	I. harvest from growing boxes	
24	17	16	95		
25	18	17	95		
26	19	18	95		
27	20	19	95	I. harvest from growing boxes	
28	21	19	95		
29	22	21	95		infection emerging in compost blocks
30	21	22	95		
31	20	21	95	II. harvest from growing boxes	
32	19.5	20	94	II. harvest from growing boxes	
33	19	18	93		
34	18.7	18	92	II. harvest from compost blocks	
35	18.5	18	91	II. harvest from compost blocks	
36	18.5	18	90	end of II. harvest	

Isolation of fungi

A total of 12 fungal isolates were recovered from the infected compost blocks on potato dextrose agar (PDA) medium supplemented with 100 µg/mL streptomycin-sulfate and chloramphenicol. The sampling was performed from the casing layer and the surface of fruiting bodies. Five isolates were selected for species identification on the basis of their colony morphology characteristics.

DNA isolation and species-level identification of moulds and mushroom flies

DNA was extracted from fungal isolates and mushroom flies with the E.Z.N.A. DNA Mini Kit (OMEGA Bio-tek) and the Quick DNA Miniprep Plus kit (Zymo Research), respectively. The identification of fungi was based on the sequence analysis of the internal transcribed spacer (ITS) region of the ribosomal RNA gene cluster using primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al. 1990). The reactions were carried out in a final volume of 20 µl consisting of 2 µl 10× DreamTaq Buffer with 20 mM $MgCl_2$, 2 µl of 2 mM dNTP mix and 0.1 µl of 5 U/µl Dream Taq DNA Polymerase (Thermo Scientific), 4 µl of each primer (1 µM), 6.9 µl bidistilled water and 1 µl template DNA. The temperature profile was set to an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 48 °C for 40 s and 72 °C for 40 s, with a final elongation at 72 °C for 2 min in a VWR Doppio Gradient Thermocycler. The collected flies were identified to the species level by the sequence analysis of a fragment of the cytochrome oxidase I (COI) gene by primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Chen, 2013; Shin et al. 2013). The 20 µl volume reactions contained 2 µl 10× DreamTaq Buffer with 20 mM $MgCl_2$, 2 µl of 2 mM dNTP mix and 0.1 µl of 5 U/µl DreamTaq DNA polymerase (Thermo Scientific), 4 µl of each primer (1 µM), 6.9 µl bidistilled water and 1 µl template DNA. The PCR was carried out in a VWR Doppio Gradient Thermocycler with a program set to an initial denaturation at 94 °C for 2 min, followed by

35 cycles of 94 °C for 1 min, 51 °C for 1 min and 72 °C for 1 min 30 s, with a final elongation at 72 °C for 5 min. The obtained fragments were sequenced by an external service (BRC, Szeged). Sequences were analyzed using the NCBI BLAST online platform (https://blast.ncbi.nlm.nih.gov).

Results and discussion

Compost colonization and fruiting body formation proved to be appropriate under the controlled conditions. *Agaricus* colonization became visible in the casing material on days 9 and 15 in compost blocks (Figure 1) and growing boxes, while the formation of primordia started on days 12 (Figure 2) and 17, respectively.

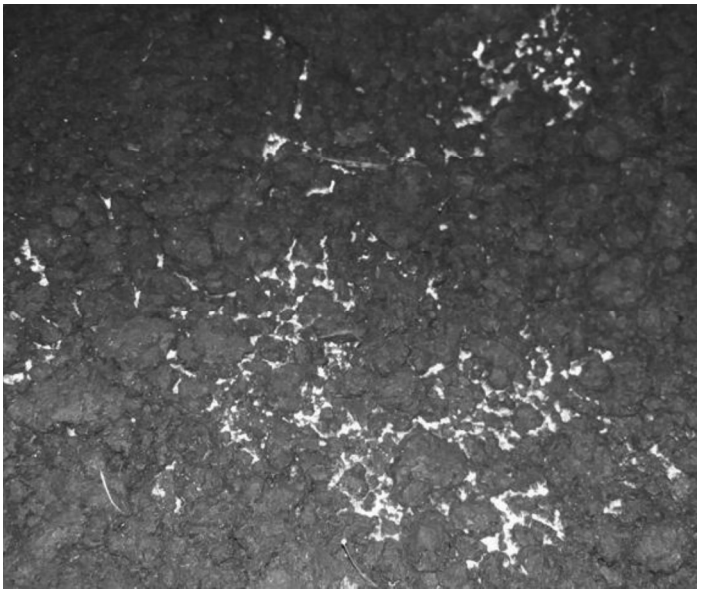

Figure 1. Colonization of the casing material on a compost block by Agaricus mycelia on day 9 of cultivation in a Weiss Gallenkamp SGC120 plant growth chamber

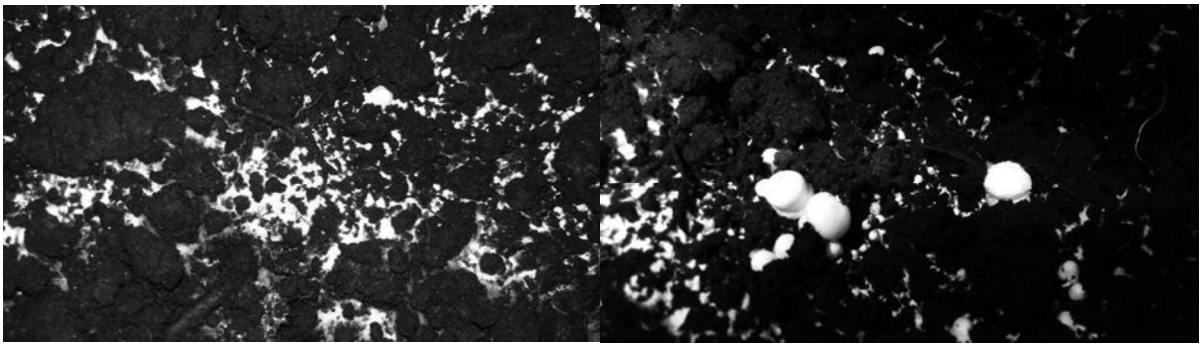

Figure 2. Initiation of primordia formation in a compost block on day 12 of cultivation in a Weiss Gallenkamp SGC120 plant growth chamber

Mushroom compost blocks yielded 11.72 kg mushroom crop (kg/m^2), while the growing boxes yielded 4.84 kg/m^2 during the first harvesting period (days 20 to 22). The mushroom compost blocks became affected by fungal infections by the end of the second harvesting period (days 34 to 36, Figure 3) and yielded 85% less crop than in the first harvesting period. Mushroom flies also appeared, which are also known to serve as vectors of mould infections. At the same time, the mushroom growing boxes remained symptomless, suggesting that the casing material supplied with the mushroom compost blocks may have been the source of fungal contamination.

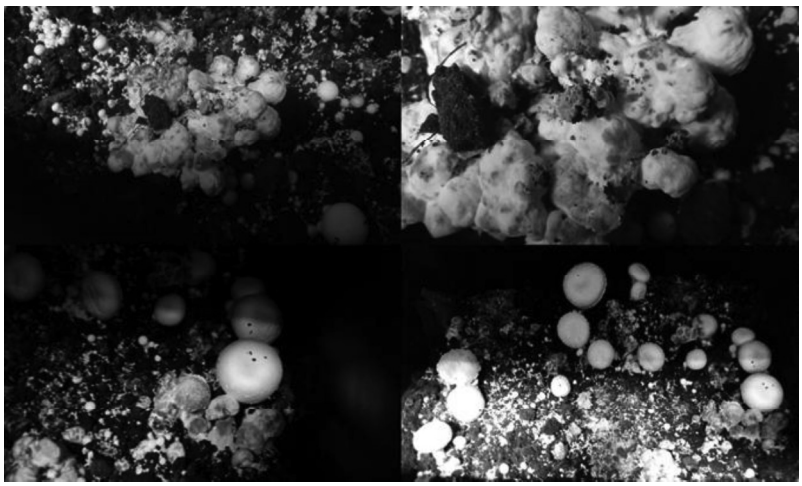

Figure 3. Symptoms of fungal infection on the casing layer of a compost block on day 34 of cultivation in a Weiss Gallenkamp SGC120 plant growth chamber.

Out of 5 fungal isolates, 3 were identified as *Mycogone perniciosa*, the causal agent of „wet bubble" (Umar et al., 2000; Bora and Özaktan, 2000; Sharma and Singh, 2003), while 2 strains proved to be *Acremonium* cf. *camptosporum* (Figure 4). *A. camptosporum* (Clavicipitaceae) is a species known for its polyketide production, which was described as an endophyte from the leaves of *Bursera simaruba* (Burseraceae) (Meléndez-González et al., 2015). A compost fungal community characterisation research showed the appearence of *Acremonium* species (*A. charticola*, *A. chrysogenum, A. humicola, A. persicinum, A. fusidioides,* and *A. sclerotigenum*) in commercial composts (Anastasi et al., 2005). *Acremonium* species are identified mainly based on the large and small subunit nuclear rRNA gene sequences (nucLSU and nucSSU), as the ITS region is not always accurate within the genus (Summerbell et al., 2011, de Hoog et al., 2000). The identification of the 2 fungal isolates as *A. camptosporum* in this study needs therefore further confirmation. *Lecanicillium fungicola* – the species known as the causal agent of „dry bubble" disease (Potočnik et al., 2008; Berendsen et al., 2010) – was not identified. The collected mushroom flies were classified to the Sciaridae family on the basis of their microscopic characteristics, and the DNA sequence-based species identification revealed their identity as *Lycoriella ingenua* (Figure 5). Sciarid flies (*Lycoriella* spp.) represent one of the three fly groups most commonly encountered in mushroom cultivation (Erler et al., 2011). *Lycoriella ingenua* can infect the compost during the composting process. Sciarids can survive the inadequate pasteurisation method or infect the compost after pasteurisation (Hussey and Gurney, 1968; Jess et al., 2007). Mushroom flies cause huge crop losses, as their larvae can feed on mycelia or mushrooms and indirectly cause compost damage which inhibits mycelial growth, furthermore, their adults may become as vectors of microbial mushroom pathogens (Clift 1979, Clancy 1981; Kim and Hwang 1996).

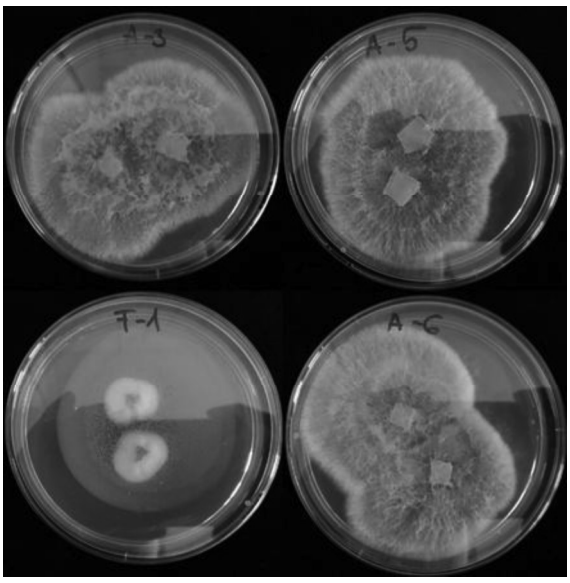

Figure 4. Strains Mycogone perniciosa A-3, A-5, A-6 and Acremonium cf. camptosporum F-1 isolated from an infected compost block. Colony morphology on potato dextrose agar medium after 7 days of incubation at 25 ℃

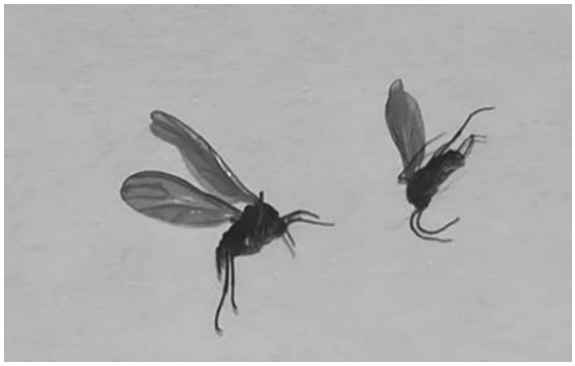

Figure 5. Lycoriella ingenua mushroom flies collected from the Weiss Gallenkamp SGC120 plant growing chamber during the second harvesting period

Conclusions

Our findings suggest that optimal parameters for champignon cultivation can be provided in the 1200 liter volume Weiss Gallenkamp SGC120 standard plant growth chamber, however, to reduce the risk of fungal contamination and crop losses, sterilization of the casing material by autoclaving is recommended before casing. Further studies will be carried out in different cultivation volumes, using sterilized casing material and CACing (compost addition to casing) to evaluate the possibilities of the establishment of experimental microcompost systems, which would allow parallel cultivation in several replicates. Such microcompost systems could be powerful tools for studying the interactions of mushroom pathogenic microorganisms, cultivated mushrooms and potential biocontrol agents.

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