

PRODUCTIVITY, BIOLOGICAL EFFICIENCY, AND NUMBER OF *Agaricus blazei* MUSHROOMS GROWN IN COMPOST IN THE PRESENCE OF *Trichoderma* sp. AND *Chaetomium olivacearum* CONTAMINANTS

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ABSTRACT

This experiment was carried out to evaluate the effect of the fungi *Trichoderma* sp. and *Chaetomium olivacearum* on the productivity, biological efficiency and number of *Agaricus blazei* mushrooms grown in compost (mixture of crushed sugarcane, coast-cross grass trash, soybean meal, gypsum, and calcitic limestone). The experiment consisted of 3 treatments (*Trichoderma* sp., *C. olivacearum*, and a control) with 8 replications each (box containing 12kg of compost colonized by *A. blazei*). Later, 150g of inoculum of each contaminant fungus (*Trichoderma* sp. and *C. olivacearum*) were distributed on the surface of the compost previously colonized by *A. blazei*. The experiment was conducted in a greenhouse with a plastic roof, under relative humidity of about 60-90% and temperature between 20-34°C. Productivity was determined from the relation between fresh weight of the mushroom and fresh weight of the compost. Biological efficiency was determined from the relation between fresh weight of the mushroom and dry weight of the compost at the end of the harvesting period. Based on results obtained, the contaminant fungi did not affect the productivity, biological efficiency, and number of *A. blazei* mushrooms grown in compost when introduced into previously colonized composts.

Keywords: *Agaricus blazei*, *Chaetomium olivacearum*, *Trichoderma* sp., contaminants

INTRODUCTION

The compost used to cultivate mushrooms of the order Agaricales is subject to the action of contaminant fungi, and their presence has been pointed out as one of the most important factors that frequently affect yield. During this process, competition occurs for space and nutrients, as well as chemical alterations in the substrate, which will hinder or prevent mushroom development. Among the most important contaminant fungi found in *Agaricus blazei* cultivations are *Dactylium dendroides* (cobweb disease), *Trichoderma* sp. (green and white mould), *Chaetomium olivacearum* (olive green mould), *Papulospora* sp., *Peziza* sp., and *Diehliomyces microsporus* (false truffle) (14).

The negative influence of contaminant fungi on the cultivation of several edible mushrooms is already known and has been studied for some years now (1-3,6,8,10,11,17). Nevertheless, for *A. blazei*, most articles report on the occurrence and morphological characteristics of these fungi (7,14), and only a few refer to their effect on mushroom production (15,16). In this respect, Nascimento (14) verified that the presence of *Diehliomyces microsporus* in the compost or in the casing soil can reduce *A. blazei* productivity by up to 90%.

Regarding *C. olivacearum*, compost or casing soil areas containing mycelium of this fungus are exclusory to *A. blazei*. The incidence of this contaminant agent frequently results from inadequate compost formulation, containing high nitrogen as

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well as high moisture contents. These characteristics are further compounded by inappropriate management during composting phase II, characterized by deficient aeration and early filling of the compost in the tunnels. All these procedural errors are easily revealed by the presence of high ammonia contents in the compost, at the end of phase II. The fungus has a green olive color, but may also show gray to brown colors (9,15,20).

Trichoderma sp., in turn, is very common during *A. blazei* colonization and production, especially in environments with deficient fresh air intake and circulation, in addition to high moisture, either in the compost, casing soil, or the air. At the initial stage, it appears in the form of white spots that generally become green due to sporulation of the fungus. In the compost, contaminants may occur in all *A. blazei* cultivation phases (13,15). Like *C. olivacearum*, areas occupied by mycelium of this fungus become unfavorable to *A. blazei* mycelial growth.

There are no papers in the scientific literature evaluating the effect of *Trichoderma* sp. and *C. olivacearum* on *A. blazei* productivity. Consequently, the results presented in this experiment serve as a reference for future researches involving contaminant fungi.

The aim of this work was to evaluate the effect of the fungi *Trichoderma* sp. and *C. olivacearum* on *A. blazei* productivity, biological efficiency, and number of mushrooms.

MATERIAL AND METHODS

A. blazei, *Trichoderma* sp., and *C. olivacearum* inocula

Pure fungal cultures were used for the production of *A. blazei*, *Trichoderma* sp., and *C. olivacearum* inocula, maintained as part of the Culture Collection of the Mushroom Research Center (Módulo de Cogumelos) of College of Agronomic Sciences/São Paulo State University (FCA/UNESP – Brazil).

A. blazei strain ABL 04/49 was used, originating in Porto Alegre/RS, reisolated from a basidioma from Mogi das Cruzes/SP. The fungi *Trichoderma* sp. and *C. olivacearum* were isolated from contaminated composts and identified at Instituto Biológico, in São Paulo/SP.

Substrate preparation, inoculation, and colonization

The substrates used to produce the spawn were triticale grains (*Triticum secale*), gypsum, and limestone (13,14). The grains were initially cooked in water for 40 minutes after the water started boiling. After draining the excess water, 20g kg⁻¹ of calcitic limestone and 160g kg⁻¹ of gypsum were added in relation to grain dry fresh weight. After homogenization, the substrate was placed in clear glass flasks, with a metal lid sealed with Tyvek® film. The flasks were autoclaved for 3 hours at 121°C and 1 atm. Upon cooling, *A. blazei*, *Trichoderma* sp., and *C. olivacearum* were inoculated separately. Incubation was carried out in a BOD chamber at 28 ± 1°C for 10 days, in the dark.

Compost and Composting

Composting phase I was performed in a covered hut with concrete floor, open sides, and natural ventilation. The ingredients used to formulate the compost consisted of 720kg crushed sugarcane, 315kg coast-cross grass trash, 117kg soybean meal, 20kg gypsum and 20kg calcitic limestone, totaling 1,192kg dry matter, as adapted from Kopytowski Filho (12). Prior to forming the windrow, the coast-cross grass straw was pre-moistened and turned every 2 days, for a period of 8 days.

The windrow was formed by depositing layers of crushed sugarcane and coast-cross (20 cm in height), followed by a layer of crushed sugarcane (20 cm) and so on until the windrow reached approximately 1.8 m in height by 1.8 m in width. A mixture was prepared separately, consisting of one half limestone and soybean meal (fresh weight), which was equitably added between the windrow layers. Irrigation was performed manually, with a hose, to maintain moisture around 70%.

The compost was turned every three days. The second half of the mixture consisting of limestone and soybean meal (fresh weight) was added during the first turning. Gypsum was added in the subsequent turnings, and water was added whenever needed to maintain moisture at 70-75%. Four turnings were performed, totaling 12 days in Phase I.

In phase II, the compost was transferred to polypropylene boxes, measuring 56.5 × 46.5 × 28.5 cm (length, width, and height). The boxes were randomly arranged inside a Dalsem container for pasteurization and conditioning. Pasteurization was performed for 6 hours at 61 ± 1°C and conditioning was carried out for 13 days at 48 ± 1°C.

At the end of composting phases I and II, three compost samples were collected, dehydrated at 65°C for 48 hours and then ground in a knife mill with a 30 mesh sieve. According to FCA/UNESP Fertilizers and Correctives Laboratory, carbon and nitrogen contents, C/N ratio, and pH in the samples at the end of composting phase I were 46.13%, 1.57%, 29/1, and 6.64, respectively. At the end of Phase II, these values were 41.37%, 2.28%, 18/1, and 5.07, respectively.

Inoculation and spawn run

Inoculation was made by hand; 15g *A. blazei* spawn were added per kg fresh compost. To accomplish this, the compost was apportioned and transferred to other smaller polyethylene boxes internally lined with transparent polyethylene plastic film containing holes in the bottom. The *A. blazei* spawn was added simultaneously and homogenized until reaching 12-12.5 kg fresh weight. After inoculation, the boxes were randomly arranged in a container and incubated in the dark for 15 days, at a compost temperature of 28 ± 1°C.

After that, 150g of *Trichoderma* sp. or *C. olivacearum* inoculum were added to the compost, according to the treatments, and the casing soil was then added.

A loamy oxisol-alfisol from Fazenda Lageado was used as

casing soil (FCA/UNESP). Initially, the pH was corrected to 7.0 by adding limestone (30 kg per m³ soil). The soil was moistened to 60% and left to rest for 20 days. Then, 30% wood charcoal (1-2 cm) were added and pasteurization was carried out at 62°C for 8 hours, in a Dalsem container. The casing soil was sown over the surface of the compost by hand. Each box received about 15 kg casing soil, resulting in a thickness of approximately 4 cm. The compost was covered with plastic and incubated for 15 days in a Dalsem chamber at 28±1°C.

Induction

After the casing soil was colonized, the boxes were arranged at random in shelves inside a greenhouse with semi-controlled environment, constructed with an iron frame measuring 6m × 1.8m × 2.1m (length, width, height, respectively) and covered with 150 micron AUV (Anti-Ultraviolet) plastic coating. Micro-sprinklers with a flow of 7 L h⁻¹ were employed to control the relative humidity (60-90%). The temperature ranged from 20 to 34°C.

Variables analyzed

Mushroom number and fresh weight

The number and fresh weight of mushrooms (FWM) were determined daily during the harvesting phase.

Productivity and biological efficiency

Productivity (P, %) was determined from the relation between mushroom fresh weight (FWM) and compost fresh weight (FWC) at the end of phase II, according to the equation: $P = \text{FWM}/\text{FWC} \times 100$.

Biological efficiency (BE, %) was determined at the end of the harvesting period, according to the equation: $\text{BE} = \text{FWM}/\text{DWC} \times 100$, where DWC corresponds to compost dry weight.

Experimental Design and Statistical Analysis

A completely randomized design was adopted, with three treatments and eight replicates. Each replicate corresponded to one box containing 12-12.5 kg wet compost. The data were submitted to analysis of variance and the means were compared by Tukey test (5%) (17).

RESULTS AND DISCUSSION

Harvest started at 40 days for the control and *Trichoderma* sp. treatments (Figs. 1 and 2) and at 41 days for the *C. olivacearum* treatment (Fig. 3), after casing. This behavior is in agreement with results obtained by Braga (4), who verified the first flush on the 41st day. Kopytowski Filho (12,13), in turn, started at 26 days after casing when producing in a controlled environment (14).

The inoculation of *Trichoderma* sp. and *C. olivacearum* into the compost already colonized by *A. blazei* did not affect

productivity, biological efficiency, and number of mushrooms (Table 1). Even though results were not significantly different, the control group presented a tendency of better productivity (10.19%) and biological efficiency (33.63%), up to 22.03% and 22.06% higher as compared to the compost inoculated with *C. olivacearum*. Braga (4) tested cultivation rooms, compost weight, and casing soil thickness, and obtained productivities ranging from 9.36% - 15.76%, while Kopytowski Filho (14)

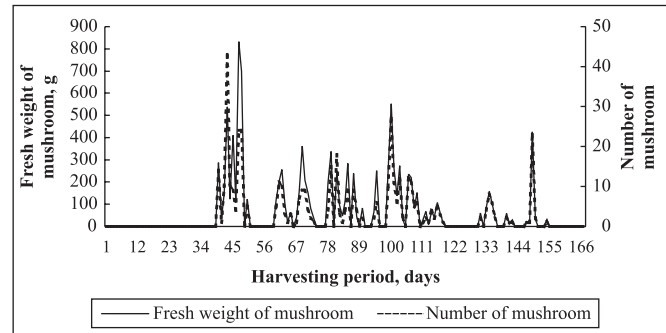


Figure 1. Fresh weight and number of mushrooms, based on a harvesting period of 167 days in the control treatment.

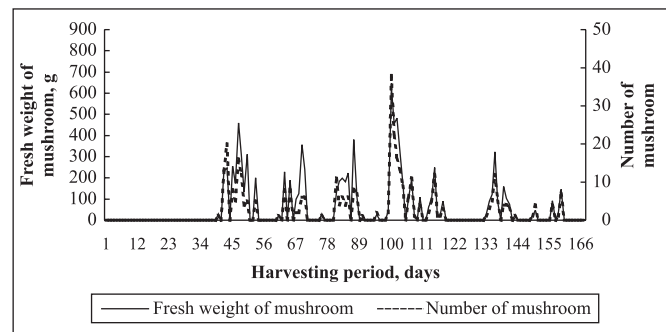


Figure 2. Fresh weight and number of mushrooms, based on a harvesting period of 167 days in the *Trichoderma* sp. treatment.

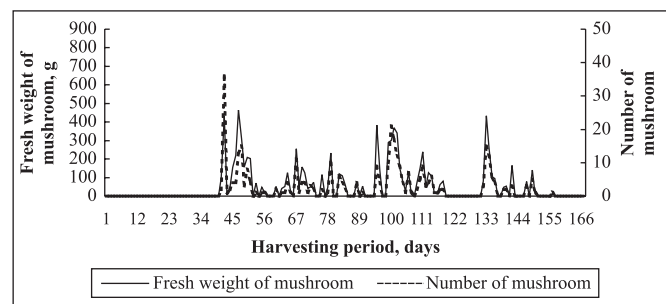


Figure 3. Fresh weight and number of mushrooms, based on a harvesting period of 167 days in the *Chaetomium olivacearum* treatment.

obtained productivities between 5.61 and 9.65% when testing compost supplementation.

Although a higher number of mushrooms was obtained in the control (34.88% higher than the *C. olivacearum* treatment) it does not mean good profits for mushroom farms, since prices are usually based on the color, shape, and size of dehydrated mushrooms (14). Therefore, small mushrooms are associated with light-weight mushrooms. In our experiment, mean fresh mushroom weights were 23.62, 24.71 and 26.5g for the control group, *Trichoderma* sp., and *C. olivacearum*, respectively.

In the control group (Fig. 1), the three greatest flushes occurred at 47, 100, and 149 days after casing, and corresponded to 832.45g, 551.4, and 425.5g in fresh mushroom weight, respectively. In the *Trichoderma* sp. treatment (Fig. 2), these peaks occurred at 47, 100, and 102 days after the casing soil was added, and corresponded to fresh mushroom weights of 459.3, 625.2, and 481g, respectively. In the *C. olivacearum* treatment (Fig. 3), however, these peaks occurred at 42, 47, and 133 days after adding the casing soil, and corresponded to fresh mushroom weights of 432.3, 465, and 433.7g, respectively.

The first flush occurred at the same time in the three treatments; however, fresh mushroom production obeyed an order of 46, 29, and 32% in relation to the total fresh weights produced in the control, *Trichoderma* sp., and *C. olivacearum* treatments, respectively (Figs. 1, 2, and 3). For mushroom farmers, shorter cultivation cycles mean faster investment return and reduced infestations of flies (13) and contaminants. In this experiment, all treatments showed several flushes, not defined in terms of duration and days after casing. This behavior is probably due to partial control of the environment, with both high and low temperatures in the same day, in addition to many changes in relative humidity. Nevertheless, *A. blazei* cultivation under environmental control can be carried out in 4-5 flushes lasting 3-5 days, with a cycle of up to 100 days after casing (14).

Damage resulting from the presence of contaminant fungi

Table 1. Total mean productivity values (mushroom fresh weight/compost fresh weight), biological efficiency (mushroom fresh weight/compost dry weight), and number of *Agaricus blazei* mushrooms in the control, *Trichoderma* sp., and *C. olivacearum* treatments.

Treatments	Productivity (%)	Biological efficiency (%)	Number of mushrooms
Control	10.19 A ¹	33.63 A	54.63 A
<i>Trichoderma</i> sp.	8.70 A	28.70 A	44.50 A
<i>C. olivacearum</i>	8.35 A	27.55 A	40.50 A

¹Means followed by common letters in the columns do not differ by Tukey test (5%). P: CV= 30.93%, LSD= 3.54; BE: CV= 30.95%, LSD= 11.69 NB: CV= 40.54%, LSD= 23.78.

may become significant in situations in which they colonize the compost prior to *A. blazei*. Andrade (2) verified this behavior in a *L. edodes* cultivation. *Eucalyptus* logs submitted to high inoculum potentials of *Trichoderma* sp., *Hypoxylon* sp., *Poria* sp., and *Stemonitis* sp., prior to colonization by *L. edodes*, were unproductive. Jandaik et al. (10) evaluated the effect of *C. dendroides* on *A. bisporus* yield and reported reductions in the productivity and mean weight of mushrooms, as a result of an increase in *C. dendroides* inoculum density of 1-5g kg⁻¹ compost.

CONCLUSION

The contaminants *C. olivacearum* and *Trichoderma* sp. did not affect the productivity, biological efficiency, and number of *A. blazei* mushrooms grown in compost when introduced into previously colonized composts.

RESUMO

Produtividade, eficiência biológica e número de cogumelos de *A. blazei* em composto na presença dos contaminantes *Trichoderma* sp. e *C. olivacearum*

Foi avaliado o efeito dos fungos contaminantes *Trichoderma* sp. e *C. olivacearum* na produtividade, eficiência biológica e número de cogumelos da produção do *A. blazei* em composto (mistura de cana-de-açúcar, palha de capim coast-cross, farelo de soja, gesso e calcário calcítico). O delineamento foi inteiramente casualizado com três tratamentos (*Trichoderma* sp., *C. olivacearum* e testemunha) e oito repetições (caixa com 12 kg de composto colonizado com *A. blazei*). Após a colonização do composto pelo *A. blazei*, adicionou-se 150g de inóculo à base de triticales de cada um dos fungos contaminantes na superfície do composto seguido da camada de cobertura. O experimento foi conduzido em estufa com cobertura plástica, umidade relativa entre 60-90% e temperatura de 20-34°C. A produtividade foi determinada pela relação entre a massa fresca de basidiomas e a massa úmida do composto. A eficiência biológica foi determinada pela relação entre a massa fresca de basidiomas e a massa seca do composto ao final do período de colheita. De acordo com os resultados obtidos, os fungos contaminantes *C. olivacearum* e *Trichoderma* sp. não afetaram a produtividade, eficiência biológica e número de cogumelos da produção do *A. blazei* em compostos previamente colonizados.

Palavras-chave: *Agaricus blazei*, *Chaetomium olivacearum*, *Trichoderma* sp., contaminantes.

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