RESEARCH ON THE TRANSFER MATERIAL TO OBTAIN MYCELIUM ON GRANULAR SUPPORT AT THE AGARICUS BLAZEI MURRILL MUSHROOMS

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Abstract

Agaricus blazei mushrooms have nutritional and therapeutic values which differentiates it from other fungi: the highest protein level (46,2 % in comparison to the average 43%), the highest level of non-fibrous carbohydrates (38%), contains more glutamic acid than the other *Agaricus* fungi available on the market. It is used for treating tumors located in various places, low immunity, viral diseases, hypercholesterolemia, atherosclerosis and viral hepatitis. The beta glucan they contain stops the evolution of malignant cells.
Being relatively new introduced crop, the *Agaricus blazei* Murill mycelium production technology is not exactly known. In the study of the transfer material to obtain the granular support mycelium inoculum was intended to achieve the production of mycelium. We have tested many materials to produce mycelium. As granular support, 4 experimental variants were studied: wheat kernels, kernels millet, mustard seed and mixed seeds. Was studied at each experimental variant the effect of amendments on *Agaricus blazei* Murrill mushroom mycelial growth. The best mycelial increase, of 1.85 mm/day was accomplished in mixture of seeds (wheat kernels and kernels of millet), with the calcium sulphate amendment.

Key words: mycelia, spawn, mushrooms, transfer material, *Agaricus blazei* Murrill

Using sustainable natural resources is one of the great challenges of our times. This challenge is directly linked to local livelihood and economic viability, without which the use of natural resources (nature conservation) cannot be managed (Heinemann P., 1993).

Along with medicinal plants, fungi had been appreciated for thousands of years for their culinary value and therapeutic properties. The cultivation of the so called "medicinal fungi" has become a large-scale economic activity, being a source of income for producers, tradesmen and manufacturers, while the medical researches of the last decades prove their extraordinary antitumoral properties. (Kawagishi H. *et al,* 1988, 1989; Sorimachi T *et al,* 2001; Kawakami S. *et al*, 2002).

For the successful cultivation of any mushroom on a small scale or commercial scale, one of the most important requirements is the seed of that species or variety. The spawn, a pure culture of the mycelium grown on a special medium, is the mushroom seed, comparable to the vegetative seed in crop plants. The production of spawn is done in the laboratory under controlled conditions of temperature, light and humidity. (Stamets P., 2005).

The success of mushroom cultivation and its yield depend to a large extent on the purity and quality of the spawn used. Mushrooms for spawn production can be grown on sterilized cereal grain (wheat, millet, mustard, rye, sorghum), but usually grain colonized with mycelium (grain-spawn) is used as an inoculum for composts. Grain is preferred as a substrate for mushroom spawn because grain gives a large number of inoculation sites, each with a high inoculum potential derived from the nutrient base the outgrowing fungi can utilize. This helps to ensure that the compost is rapidly permeated, which is important for the exclusion of competitors as well as for the rapid production of fruit bodies. The media used for maintenance, multiplication and preservation of the *Agaricus blazei* Murrill mushroom culture are PDA (potato dextrose agar) and CEA (compost extract agar) medium. (Stamets P., 2010).

MATERIAL AND METHOD

Materials used in the experience: pure culture of *Agaricus blazei* Murrill, cereal grains

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(wheat, millet, mustard and mix of these cereal grains), bottles, cotton, paper squares 7x7 cm, calcium sulphate (gypsum), calcium carbonate (chalk), glucose bottles/milk bottles, alkathene sheets, autoclave, laminar flow cabinet, incubator/storage room, wire gauge balance, bunsen burner and water.

Method:

1. Substrate preparation:

a. the cereal grains were soaked for one night; 2L of water per 1 kg of grain, the cereal grains were washed and strained to remove all water;

b. the cereal seeds were steamed for 30-45 minutes to soften grains, and the water was drained, and the cereal grains spreaded to cool down and decrease moisture;

c. the three-fourth of the bottle was filled with cereal grains, carefully was prepare cotton plug, and tightly plugged in the mouth of bottle with cotton, and leave out for ventilation.

d. the grain was allowed to surface dry by spreading over alkathene sheets, in shade, for a few hours.

e. the grain was mixed thoroughly with chemicals (calcium sulphate and calcium carbonate at 2% and 0.5%, respectively, on dry weight basis of the grain), to adjust pH of the grain at 7-7.8. the grain must not be coagulated at this stage;

f. the grain-chemical mixture was filled in 500 mL glucose/milk bottles (300-350 g boiled grains/container). However, the first generation spawn (master spawn or transfer material) must be prepared in glass bottles due to their convenience in handling for further subculturing;

g. the bottles/containers were plugged with nonabsorbant cotton; (*figure 1*)

h. the substrate was sterilized by autoclaving at 121ºC (15 psi) for 30 minutes;

i. the process of sterilization was repeated after 24h of first autoclaving

j. the substrate container was allowed to come to room temperature for making the substrate ready for inoculation.

Figure 1 **Bottles filled with grain**

2. Inoculation of substrate:

a. the substrate was inoculated (grain in containers) with the mycelium of the mushroom grown on a specific medium by transferring mycelium in agar on the grain under aseptic (sterile) condition. (*figure 2*)

b. the containers were agitated, after plugging, to distribute the fragments of the mycelium.

Figure 2 **Inoculation of substrate**

3. Incubation:

a. the inoculated containers were stored (incubated) at 25-27ºC in darkness for 3 weeks (*figure 3*).

b. the containers were agitated for an even distribution of mycelium, after a few days of incubation or as soon as mycelium was visible on grain.

Figure 3 **Incubation of substrate**

Mycelial characteristics: Longitudinally striate mycelium (*figure 4*), with radiating rhizomorphs overlaying a cottony mycelial undergrowth. Rhizomorphic mycelia in culture produces hyphal aggregates and pseudo-primordia after one month of incubation on 2% CEA, which fail to enlarge to maturity. Becoming loosely aerial in age, mycelia often exude a yellowish, almond-smelling metabolite.

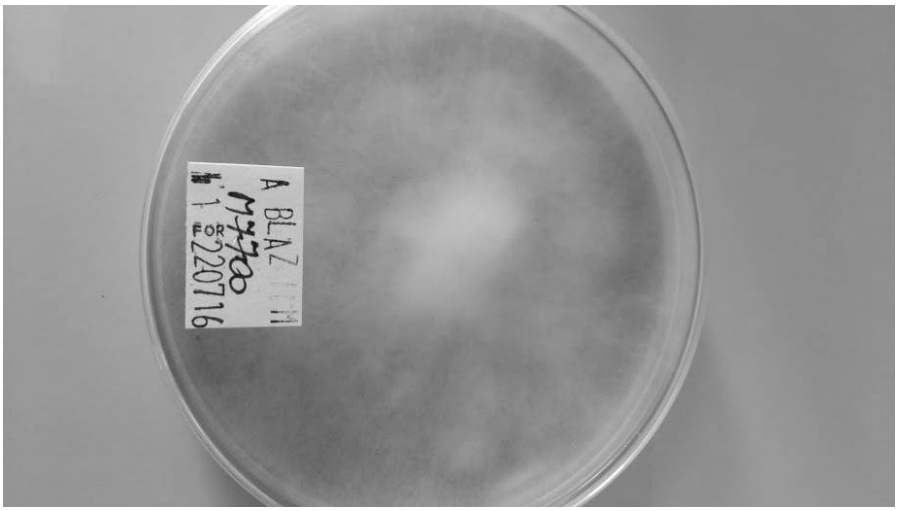

Figure 4 ***Agaricus blazei*** **Murrill mycelial characteristics**

The experimental factors and their graduation are shown below:

A - biological material with the following graduations:

a1 – wheat grains
a2 – millet grains
a3 – mustard grains
a4 – mix of grains (33.3% wheat, 33.3% millet and 33.4% mustard

B – amendments with the following graduations:

b1 – calcium sulphate
b2 – calcium carbonate
b3 – no amendments used

RESULTS AND DISCUSSIONS

The combination of experimental factors resulted in 12 variants shown in Table no. 1, each version having 3 repetition.The average of repetitions is presented in Table 1 and figure 5.

Table 1

The combination of experimental factors and the average of mycelial growth

Variant	Biological material	Amendments	Mycelial growth mm/day
V1 a1b1	Wheat grains	calcium sulphate	1.67
V2 a1b2	Wheat grains	calcium carbonate	1.45
V3 a1b3	Wheat grains	no amendments used	1.38
V4 a2b1	Millet grains	calcium sulphate	1.50
V5 a2b2	Millet grains	calcium carbonate	1.28
V6 a2b3	Millet grains	no amendments used	1.17
V7 a3b1	Mustard grains	calcium sulphate	1.38
V8 a3b2	Mustard grains	calcium carbonate	1.25
V9 a3b3	Mustard grains	no amendments used	1.07
V10 a4b1	Mix of grains	calcium sulphate	1.85
V11 a4b2	Mix of grains	calcium carbonate	1.72
V12 a4b3	Mix of grains	no amendments used	1.32

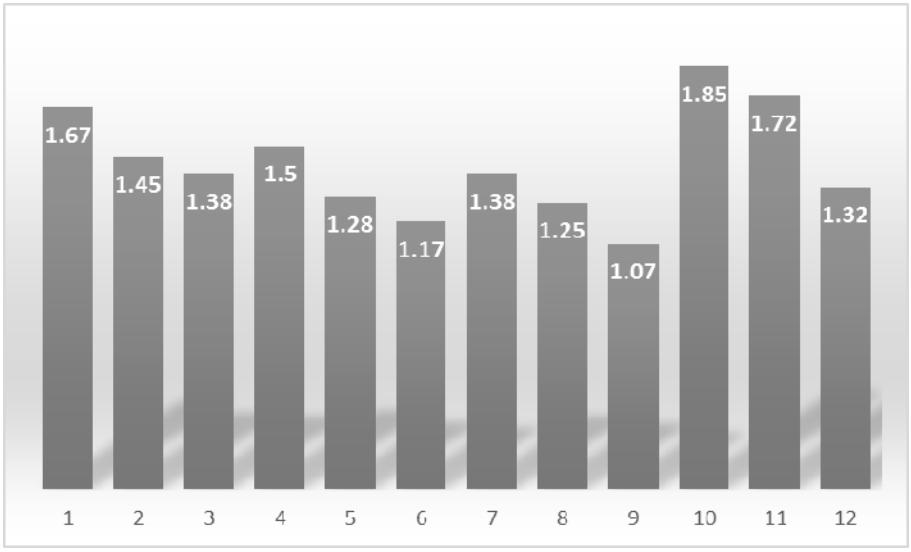

Figure 5 **The average of mycelial growth in mm/day**

Taking into account the unilateral influence of biological material on the *Agarius blazei* Murrill mycelial growth, we can be seen as it recorded a difference of 1.63 mm/day being very significant positive, to the average taken as controls (*table 2*) which registered 1.42 mm/day.

Table 2

Unilateral biological material influence on mycelial growth *Agaricus blazei* Murrill mushrooms

Biological material	Growth mm/day		Difference ±D mm/day	Significationof difference
	Value	%		
	1.42	100	0.00	Mt
Wheat grains	1.50	105.6	0.08	*
Millet grains	1.32	92.7	-0.10	0
Mustard grains	1.23	86.9	-0.19	00
Mix of grains	1.63	114.8	0.21	***
DL (p 5%)			0.08	
DL (p 1%)			0.12	
DL (p 0.1%)			0.19	

Summary comparisons by Duncan test, the influence of biological material on the *Agarius blazei* Murrill mycelial growth, is presented in *Table 3*, the highest value of growth was recorded for seed mix with 1.63 mm / day in last place was located mustard seed with a rise of 1.23 mm / day.

Table 3

Summary comparisons by Duncan test, the influence of biological material on the *Agarius blazei* Murrill mycelial growth

Biological material	Growth mm/day	Significance*
Mix of grains	1.63	A
Wheat grains	1.50	B
Millet grains	1.32	C
Mustard grains	1.23	D

DS values 0,08-0,08

* Values marked with different letters are significant

Taking into account the unilateral influence of amendments on the *Agarius blazei* Murrill mycelial growth, we can be seen as it recorded a difference of 1.60 mm/day being very significant positive, to the average taken as controls (*table 4*) which registered 1.42 mm/day.

Table 4

Unilateral amendments influence on mycelial growth *Agaricus blazei* Murrill mushrooms

Amendments	Growth mm/day		Difference ±D mm/day	Signification of difference
	Value	%		
	1.42	100.0	0.00	Mt
$CaSO_4$	1.60	112.7	0.18	***
$CaCO_3$	1.43	100.4	0.00	-
No amendments	1.24	87.0	-0.19	000
DL (p 5%)			0.04	
DL (p 1%)			0.05	
DL (p 0.1%)			0.07	

In combining experimental factors, amendments and biological material, on mycelial growth we recorded significant values (*table 5*).

The influence of combined factors, amendments and biological material, on the mycelial growth, were recorded the highest values, 1.85 mm/day with $CaSO_4$ amendments, followed by the wheat grain with $CaSO_4$ amendments 1.67 mm/day. On the last place was located the mustard grain with no amendments. It can be concluded that the mycelial growth is more intensive with $CaSO_4$ amendments.

Table 5

Combining experimental factors, amendments and biological material, on mycelial growth of *Agaricus blazei* Murill mushroom

Amendment / Biological material	Mycelium growth		Difference ±D mm/day	Signification of difference
	mm/day	%		
	1.50	100.0	0.00	Mt.
$CaSO_4$ / Wheat grain	1.67	111.1	0.17	***
$CaCO_3$ / Wheat grain	1.45	96.7	-0.05	-
No amendments / Wheat grain	1.38	92.2	-0.12	00
	1.32	100.0	0.00	Mt.
$CaSO_4$ / Millet grain	1.50	113.9	0.18	***
$CaCO_3$ / Millet grain	1.28	97.5	-0.03	-
No amendments / Millet grain	1.17	88.6	-0.15	000
	1.23	100.0	0.00	Mt.
$CaSO_4$ / Mustard grain	1.38	112.2	0.15	***
$CaCO_3$ / Mustard grain	1.25	101.4	0.02	-
No amendments / Mustard grain	1.07	86.5	-0.17	000
	1.63	100.0	0.00	Mt.
$CaSO_4$ / Mix of grain	1.85	113.5	0.22	***
$CaCO_3$ / Mix of grain	1.72	105.3	0.09	*
No amendments / Mix of grain	1.32	81.2	-0.31	000
DL (p 5%)			0.08	
DL (p 1%)			0.11	
DL (p 0,1%)			0.15	

In combining experimental factors, biological material and amendments, on mycelial growth we recorded significant values (table no. 6).

The influence of combined factors, biological material and amendments, on the mycelial growth, were recorded the highest values, 1.85 mm/day on mix of grain with $CaSO_4$ amendments, followed by the wheat grain with $CaSO_4$ amendments 1.67 mm/day. On the last place was located the mustard grain with no amendments. It can be concluded that the mycelial growth is more intensive on mix of grain with amendments.

Table 6

Combining experimental factors, biological material and amendments, on mycelial growth of *Agaricus blazei* Murill mushroom

Biological material / Amendment	Mycelium growth		Difference ±D mm/day	Signification of difference
	mm/day	%		
	1.60	100.0	0.00	Mt.
Wheat grain / $CaSO_4$	1.67	104.2	0.07	-
Millet grain / $CaSO_4$	1.50	93.8	-0.10	-
Mustard grain / $CaSO_4$	1.38	86.5	-0.22	00
Mix of grain / $CaSO_4$	1.85	115.6	0.25	***
	1.43	100.0	0.00	Mt.
Wheat grain / $CaCO_3$	1.45	101.8	0.02	-
Millet grain / $CaCO_3$	1.28	90.1	-0.14	0
Mustard grain / $CaCO_3$	1.25	87.7	-0.18	00
Mix of grain / $CaCO_3$	1.72	120.5	0.29	***
	1.24	100.0	0.00	Mt.
Wheat grain / No amendments	1.38	112.0	0.15	**
Millet grain / No amendments	1.17	94.5	-0.07	-
Mustard grain / No amendments	1.07	86.4	-0.17	00
Mix of grain / No amendments	1.32	107.2	0.09	-
DL (p 5%)			0.10	
DL (p 1%)			0.15	
DL (p 0,1%)			0.22	

CONCLUSIONS

All the recipes presented in this paperwork may be used for *Agaricus blazei* Murrill mushroom mycelium production, all of them with result retrieved in foreign scientific literature, with 1.42 mm/day mean value.

The mycelial growth is more intensive on mix of grain with amendments (1.72-1.85 mm/day), followed by wheat grain without amendments (1.38 mm/day).

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