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FRUITING YIELD OF SOME *PLEUROTUS* STRAINS

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Abstract
Oyster mushrooms are highly popular and farmed extensively given their culinary and therapeutic properties alongside their development versatility on a broad assortment of substrate materials. Many biologically active compounds produced by this macromycetes have been found to be important in forestall and therapy of some medical conditions. Structural polysaccharides like glucans, phenolic compounds, tocopherols, triterpenes are noteworthy biocompounds produced by these mushrooms that have numerous health benefits acting as immunomodulator, antitumoral, antioxidant, hypocholesterolemic, antiviral and antibacterial factors. The current study attempted to explore the production yields of some Pleurotus spp. strains in relation to various technological methods and different nutritive supports that consisted of several industrial left overs. Two granular spawn variations were obtained from solid (SISS) and liquid (LISS) inoculum, while the third thechnological spawn was constituted by liquid spawn (LS) derived from liquid inoculum. Vegetal resources including winery pomace, brewery spent grains, corn cobs, coffee grinds and fruits scraps have been examined.

Keywords: fruiting, mycelium, Pleurotus, spawn, substrates.

1. INTRODUCTION

Mushrooms belonging to the genus *Pleurotus* are among the most popular and cultivated species due to their gastronomic properties, adaptability to a wide range of culture substrates, in addition to their therapeutic qualities, which along with other species, give them the status of functional foods (nutraceuticals). Each of the organic components evaluated in this research is commonly available and generated in substantial quantities annually in Romania and most other countries. These biomasses represent an excellent nutritional source for the cultivation of *Pleurotus* mushrooms apart from their huge biotechnological potential in different applications like biofuels, cosmetics, enzymes etc. An impressive evolution of the technologies involved in the production of the spawn (commercial mycelium) has been implicated, along with its economic value, in the spectacular development of the *Pleurotus* culture. These xylophagous macromycetes exists in nature on woody debris and represents efficient agents for the biological recycling of a very large number of by-products from food, textile, paper and other industries (Mandeel et al., 2005, Akcay et al., 2023). The submerged *in vitro* mycelia cultivation technique have numerous advantages including an increased colonisation rates on grain caryopses, dispersion of metabolic products throughout the media and with more uniform characteristics of the colonies. Increasing the availability of nutrients

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in the culture media and enriching them with organic carbon, nitrogen and mineral sources may stimulate mycelial growth. As a result, this increases the mycelia's nutritional content, which has a positive impact on fruiting, crop quantity and quality (Stajić et al., 2006, Hoa and Wang, 2015, Dedousi et al., 2023). Mushrooms business includes two major phases: the generation of spawn and the mushrooms harvest. The spawn production represents the final step of *in vitro* multiplication of the mycelia and is incorporated into the growing substrate for its occupancy and the mushrooms crop (Narh et al., 2011, Higgins et al., 2017).

2. MATERIALS AND METHODS

The biological material used in this paper belongs to the macromycetes collection of RDIVFG Vidra, where all practical laboratory and fruiting experiments were carried out. Three *Pleurotus* strains were examined: *Pleurotus ostreatus* PoM-77, *Pleurotus columbinus* PcM-98 and *Pleurotus eryngii* PeM-41.

Each of the six agarized media that were examined had been supplemented with natural carbon sources collected from agricultural and industrial enterprises. In order to produce the crude extracts, equivalent parts (m/m) of every vegetal material were thoroughly blended with distilled water (50–60°C). Following decantation, separation and filtration, a 50% Malt Extract (VWR Chemicals) volume was homogenized with 50% extract from each lignocellulosic category, leading to a final concentration of 25% and labelled as: V0 (control, malt extract), V1 (white grape pomace), V2 (red grape pomace, V3 (brewery spent grains), V4 (corn cobs), V5 (coffee grinds) and V6 (fruit scraps). The pH values were rectified to 6.5, subsequently being added 20 g/l of agar per flask and sterilized at 121°C for 25'. After a slightly cooling, the media were dispensed into Petri dishes, 18-20ml per plate. For inoculation, Ø5mm mycelial fragments has been placed in the center of each plate, with 4 repetitions per variant. The incubation lasted 10 days at 25–26°C.

Six liquid media were produced in identical way as above excepting the agar, in order to generate the liquid inoculum represented by the ensuing immersed mycelial tissue. After preparation, 200ml of every sample were divided equally among 100ml glass containers and autoclaved at 121°C for 25'. Each flask was inoculated with five Ø5mm mycelial pieces. The incubation was carried out for 2 weeks at 25–26°C, without illumination and with periodic agitations.

Partially boiled wheat grains were mixed with 1% $CaCO_3$ and 3% $CaSO_4$ in order to prepare the granular support. It was then disposed into glass vessels each containing 300g of nutritive support and autoclaved at 123°C for 1.5h. Both solid media (five mycelial fragments per replicate) and liquid media (15ml per replicate) were used to source the mycelial inoculum. A new batch of liquid cultures was created in order to acquire the third mycelium variation , liquid spawn. Each flask held 300ml of media and were injected with 15ml of liquid inoculum per replicate. Incubation was conducted for up to 21 days at 26°C, with regular agitations for the liquid spawn.

The first step of culture substrates preparation consisted of processing the vegetal components in a motorized grinder and submerged for a full day in water. After the excess moisture was removed, the components were mixed with 2% $CaCO_3$, 6% $CaSO_4$ plus 3% sunflower millfeed, maize and wheat millbran. The control sample consisted of 100% wheat straws with the nutrient supplements, while the experimental variants included a base of 75% wheat straws and 25% from each by-products category and nutrient supplements. The samples were sterillized at 121°C for 1.5h in PP bags (0.5kg/ replicate). Spread across 189 containers, the culture support comprised 94.5kg in total (3 species x 3 spawn variants × 3 replications x 7 substrate types). A 5% spawn rate, specifically

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25g of granulated spawn and 25ml of liquid spawn per variant, was utilized in the inoculation process and incubated after at 24-26°C for up to 3 weeks.
Following the closure of the incubation period and the complete colonization of the substrates by the spawn variants, the bags were transferred into the fruiting area, with a prior negative thermic shock.
The onset of fruiting is marked by the emergence of primordia while the microclimate conditions established a range temperature of 16-18°C, RH of at least 92-95% at the emergence of primordia that reduces over time to values of 80-85% as the mushrooms develop, daily 8h illumination, CO_2 levels of 500-1200ppm and adequate ventilation to ensure air recirculation. Carpophores were collected within 6-10 days of fruiting pins appearance.
After harvesting, the yields were quantified and statistically examined using Anova two-factor analysis.

3. RESULTS AND DISCUSSIONS

Colonies of all *Pleurotus* strains displayed multiple nuances of white, tightly compacted mycelia, with an average horizontal growth, several circular formations and cultural traits specific for every variety. Two replicates of each sample are displayed in Figure 1 at 7 days following inoculation. The liquid cultures (Figure 2) produced clustered spherical biomasses, hyphal edges at the colony's margins, totally immersed, minor stroma at the upper region of media across certain samples and a high mycelial biomass volume.

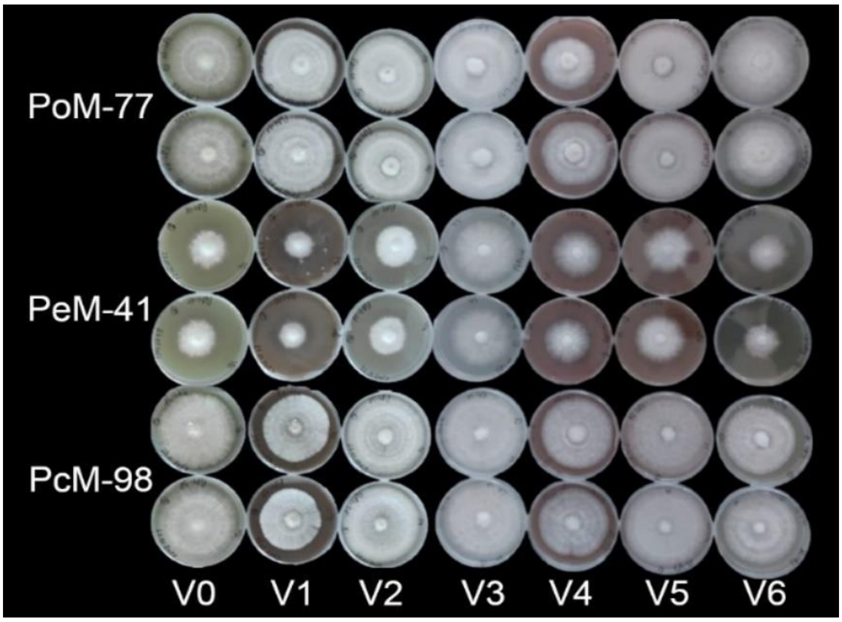

Figure 1. Pleurotus mycelia on solid media

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Figure 2. Pleurotus mycelia on liquid media
(PoM-77 front row, PcM-98 centre row, PeM-41 back row)

The most common type of spawn is cultivated on a grain-based substrate made from cereal kernels, especially rye, wheat and millet, less commonly used barley or sorghum (Sánchez, 2010, Challa et al., 2019). The liquid inoculum is prolific in colonizing the wheat grains caryopses due to the multitude of inoculation points resulting in a faster colonization compared to the solid inoculum (Cohen et al., 2002, Gregori et al., 2007).
The liquid spawn had the same appearance as for the liquid inoculum, the difference being only the size of the mycelial biomass being grown in larger vessels. Figure 3 shows all spawn variants obtained in 63 Erlenmeyer flasks (3 strains x 3 spawn var. x 7 media var.), 14 days after inoculation.

Figure 3. Spawn variants
(PoM-77 left, PeM-41 centre, PcM-98 right)

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For each spawn variation, all three *Pleurotus* strains exhibited viability and typical specie specific cultural traits. They colonized the lignocellulosic substrate at varying rates in the early post-inoculation period, but overall, these macromycetes developed in similar manners. The next three following Figures, 4, 5, and 6, displays the onset of fruiting for all strains examined.

Figure 4. Pleurotus ostreatus fruiting debut

Figure 5. Pleurotus columbinus fruiting primordia

Figure 6. Pleurotus eryngii primordia onset

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Pleurotus ostreatus formed bouquets of mushrooms with a curved pileus, a sooty cuticle that deepened near the margins. The mushroom's stipes were not large, radially inserted and of a pale white in color while the trama was moderatly firm.
Pleurotus columbinus generated groups of mushrooms with a dark blue cuticle that eventually faded to gray as the mushrooms developed. The stipes had a pearly hue, short and outwardly inserted, while the trama was quite firm.
Both strains are displayed in a randomized system in Figure 7.

Figure 7. Pleurotus ostreatus and Pleurotus columbinus mushrooms

Pleurotus eryngii (Figure 8) yielded robust, meaty mushrooms featuring a brun cuticle and distinct squamosus formations. The stipes was short and with a wider center and a shorter base.

Figure 8. Pleurotus eryngii mushrooms

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The following 3 tables displays the average mushrooms yield of each strain, based on the average of three replicates.

Table 1. Average mushrooms yield of Pleurotus ostreatus

Strain/ variant	Spawn type	Mean value (g)	Martor raport (%)	Martor difference (g)	Significance of difference
PoM-77/V0	SISS	167	100	0	Mt
	LISS	167.17	110.10	0.17	
	LS	156.67	93.81	-10.33	
PoM-77/V1	SISS	152.67	91.42	-14.33	
	LISS	154	92.22	-13	
	LS	127.83	76.55	-39.13	0
PoM-77/V2	SISS	136.67	81.84	-30.33	
	LISS	177.83	106.49	10.83	
	LS	137	82.04	-30	
PoM-77/V3	SISS	177.33	106.19	10.33	
	LISS	201	120.36	34	*
	LS	182	108.98	15	
PoM-77/V4	SISS	164.83	98.70	-2.16	
	LISS	168.83	101.10	1.83	
	LS	157.83	94.51	-9.16	
PoM-77/V5	SISS	148.33	88.82	-18.66	
	LISS	162.17	97.11	-4.83	
	LS	142.33	85.23	-24.66	
PoM-77/V6	SISS	154.67	92.61	-12.33	
	LISS	164.33	98.40	-2.66	
	LS	157.83	94.51	-9.16	

DL 5%	32.19
DL 1%	43.04
DL 0.1%	56.56

Tabel 2. Average mushrooms yield of Pleurotus columbinus

Strain/ variant	Spawn type	Mean value (g)	Martor raport (%)	Martor difference (g)	Significance of difference
PcM-98/V0	SISS	222.97	100	0	Mt
	LISS	186.40	83.60	-36.56	0
	LS	130.33	58.45	-92.63	000
PcM-98/V1	SISS	183.07	82.10	-39.89	0
	LISS	172.57	77.40	-50.39	00
	LS	139	62.34	-83.96	000
PcM-98/V2	SISS	200.10	89.74	-22.86	
	LISS	235.37	105.56	12.40	
	LS	154	69.07	-68.96	000

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PcM-98/V3	SISS	188.17	84.39	-34.80	0
	LISS	285.07	127.85	62.10	***
	LS	206.33	92.54	-16.63	
PcM-98/V4	SS	209.13	93.80	-13.83	
	LISS	190.33	85.36	-32.63	0
	LS	169.67	77.10	-53.30	00
PcM-98/V5	SISS	217.17	97.40	-5.80	
	LISS	192.33	86.26	-30.63	
	LS	124.50	55.84	-98.46	000
PcM-98/V6	SISS	183.33	82.22	-39.63	0
	LISS	164.67	73.85	-58.30	000
	LS	141.50	63.46	-81.46	000

DL 5% 30.93
DL 1% 41.39
DL 0.1% 54.36

Tabel 3. Average mushrooms yield of Pleurotus eryngii strain

Strain/ variant	Spawn type	Mean value (g)	Martor raport (%)	Martor difference (g)	Significance of difference
PeM-41/V0	SISS	214.83	100	0	Mt
	LISS	185.67	86.42	-29.16	
	LS	193.17	89.91	-21.66	
PeM-41/V1	SISS	255.33	118.85	40.50	
	LISS	186.50	86.81	-28.33	
	LS	178.83	83.24	-36	
PeM-41/V2	SISS	229.33	106.75	14.50	
	LISS	223.83	104.19	9	
	LS	209.67	97.60	-5.16	
PeM-41/V3	SISS	364.17	169.51	149.33	***
	LISS	272	125.61	57.17	
	LS	214.50	99.84	-0.33	
PeM-41/V4	SISS	237.83	110.71	23	
	LISS	252.50	117.53	37.67	
	LS	204.93	95.39	-9.90	
PeM-41/V5	SISS	203.20	94.58	-11.63	
	LISS	195.33	90.92	-19.50	
	LS	192.33	89.53	-22.50	
PeM-41/V6	SISS	160.83	74.86	-54	
	LISS	211.30	98.36	-3.53	
	LS	124.33	57.87	-90.50	00

DL 5% 60.89
DL 1% 81.47
DL 0.1% 107

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The following table illustrates the total mushrooms production for each strain according to the spawn types and substrate variants based on the sum of three replicates and two fruiting flushes.

Table 4. Total strains productivity according to spawn variations and organic sources

Strain/ variant	Spawn type	Total harvest (g)	Yield (%)	Biological efficacy (%)
PoM-77/V0	SSIS	501	33.40	125.25
	LISS	501.51	33.43	125.38
	LS	470.01	31.33	117.50
PoM-77/V1	SISS	458.01	30.53	114.50
	LISS	462	30.80	115.50
	LS	383.49	25.57	95.87
PoM-77/V2	SISS	410.01	27.33	102.50
	LISS	533.49	35.57	133.37
	LS	411	27.40	102.75
PoM-77/V3	SISS	531.99	35.47	133.00
	LISS	603	40.20	150.75
	LS	546	36.40	136.50
PoM-77/V4	SISS	494.49	32.97	123.62
	LISS	506.49	33.77	126.62
	LS	473.49	31.57	118.37
PoM-77/V5	SISS	444.99	29.67	111.25
	LISS	486.51	32.43	121.63
	LS	426.99	28.47	106.75
PoM-77/V6	SISS	464.01	30.93	116.00
	LISS	492.99	32.87	123.25
	LS	473.49	31.57	118.37
PcM-98/V0	SISS	668.91	44.59	167.23
	LISS	559.2	37.28	139.80
	LS	390.99	26.07	97.75
PcM-98/V1	SISS	549.21	36.61	137.30
	LISS	517.71	34.51	129.43
	LS	417	27.80	104.25
PcM-98/V2	SISS	600.3	40.02	150.08
	LISS	706.11	47.07	176.53
	LS	462	30.80	115.50
PcM-98/V3	SISS	564.51	37.63	141.13
	LISS	855.21	57.01	213.80
	LS	618.99	41.27	154.75
PcM-98/V4	SISS	627.39	41.83	156.85
	LISS	570.99	38.07	142.75

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	LS	509.01	33.93	127.25
PcM-98/V5	SISS	651.51	43.43	162.88
	LISS	576.99	38.47	144.25
	LS	373.5	24.90	93.38
PcM-98/V6	SISS	549.99	36.67	137.50
	LISS	494.01	32.93	123.50
	LS	424.5	28.30	106.13
PeM-41/V0	SISS	644.49	42.97	161.12
	LISS	557.01	37.13	139.25
	LS	579.51	38.63	144.88
PeM-41/V1	SISS	765.99	51.07	191.50
	LISS	559.5	37.30	139.88
	LS	536.49	35.77	134.12
PeM-41/V2	SISS	687.99	45.87	172.00
	LISS	671.49	44.77	167.87
	LS	629.01	41.93	157.25
PeM-41/V3	SISS	1092.51	72.83	273.13
	LISS	816	54.40	204.00
	LS	643.5	42.90	160.88
PeM-41/V4	SISS	713.49	47.57	178.37
	LISS	757.5	50.50	189.38
	LS	614.79	40.99	153.70
PeM-41/V5	SISS	609.6	40.64	152.40
	LISS	585.99	39.07	146.50
	LS	576.99	38.47	144.25
PeM-41/V6	SISS	482.49	32.17	120.62
	LISS	633.9	42.26	158.48
	LS	372.99	24.87	93.25

4. CONCLUSIONS

For all *Pleurotus* strains, the best yields were obtained on the substrate variant enriched with brewery residues (V3). This corresponds primarily to the sugars found in the brewery spent grains (Liguori et al., 2013, Nyhan et al., 2023), affecting also the hyphal density considerably, with a greater value in contrast to other subtrate materials. Regarding spawn variations, the most fruitful mycelia for *Pleurotus ostreatus* and *Pleurotus columbinus* strains were produced from liquid inoculum on granular support (LISS), while *Pleurotus eryngii* obtained better crops from mycelia propagated with solid inoculum on granular support (SISS).

The winemaking process results in the generation of by-products that can be used in various applications. Despite the fact that these components contain substantial tannins and molecules that may prevent mycelial development, the winemaking by-products can still be incorporated into different substrates for *Pleurotus* mushroom cultivation to guarantee moderate results (Papadaki et al., 2019, Koutrotsios et al., 2022).

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Corn cobs are one of the classic materials for growing oyster mushrooms, with high nutrient intake but low digestibility due to high lignification levels, leading to longer incubation times. In some cases, when they are finely chopped, they can conduce to lower growth rates and yields due to compaction of the substrate and hindering the advancement of the mycelial front. Nevertheless, corn cobs remain an excellent supplement for enrichment of *Pleurotus* spp. culture substrates, an organic material available in most countries (Singleton, 2009, Stanley et al., 2011, Sufyan et al., 2022).

Even though coffee grinds delivered lower yields than the control sample, it remain a cheap material providing acceptable yields for the production of *Pleurotus* mushrooms. Other researchers have also found positive outcomes and the literature on the subject is abundant on the subject (Freitas et al., 2018; Fayssal et al., 2021).

Because of the inhibitory biomolecules and the higher acidic pH they produced, the substrate enriched with fruit waste produced more looser mycelium and decreased mycelial density, that eventually resulted in inferior harvests. Nevertheless, it might be feasible to effectively incorporate fruit waste or by-products as a low-cost supplementary resource within the substrate formulation for *Pleurotus* mushrooms growing (Rashad et al., 2009, Otieno et al., 2022).

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