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Effect of Substrates of Spawn Production on Mycelium Growth of Oyster Mushroom Species

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Abstract: Spawn quality is the most important factor at production edible mushroom. In order to determine the effects of substrates spawn preparation on mycelium growth of oyster mushroom species, the experiment was conducted in a factorial experiment design at randomized completely with three replications. In the experiment, first and second factors respectively were substrates (wheat, corn and millet) and oyster mushroom species (*Florida*, *Citrinopileatus* and *Ostreatus*). The results clearly demonstrated that between various substrates used, maximum and minimum mycelium growth rate were recorded for corn and millet, respectively and between various used species, maximum and minimum mycelium growth rate was at *Florida* and *Ostreatus* species, respectively. Also the results showed that spawn dry matter has different considerably after completed maturation and between substrate maximum and minimum dry matter brought by corn and millet substrate, respectively, but it had not significant between various species. Similarly substrates by species interaction showed that the maximum and minimum mycelium growth rate were *Florida* with corn substrate and *Ostreatus* with millet substrate, respectively.

Key words: Mycelium growth, oyster mushroom, spawn grains

INTRODUCTION

The first stage of mushroom production is produced of strong spawns with increasing mycelium growth. It is equal to seed of higher plants. Spawn quality is counted the most important part in mushroom production (Mohammadi Goltapeh and Purjam, 2003). The production of mushroom by spore culture because of genetically segregation is leading variety in quantity and quality of crop. Whereas mycelium culture method is very suitable from the viewpoint of quantity and quality for preparation and propagation useful stocks. Because this method is not any characteristics segregation and exactly produced mushroom will be similarly of mother culture (Kashi, 1996). In the old days, farmers for spawn preparation used than lands soil which mushroom has grown there of naturally. Then it distributed surface of substrates which had prepared previously and after mycelium covered surface of substrate completely and from that used for inoculation of other substrates (Mohammadi Goltapeh and Purjam, 2003).

Nowadays, grains are very suitable of carrier, because it had hardly shell which aleurone layer was containing of protein and starch. It too will attract very much water by swelling.

Therefore grain is remained resistant and fixed. Keeping of spawn grain is possible for long time (Kashi, 1996). Spawn grains of advantages was mycelium the same growth in all grain surface and it was cause of the same distribution (Mottaghi, 2004). The emphasis of suitable substrate choosing is not less than strain choosing for crop production. Because spawn substrate type has influenced very much in growth mycelia and spawns prepared cover substrate with mycelium in the time shorter (Mottaghi, 2006). In fact, if substrate provide mycelia requirements and condition factors is suitable. Mycelia will surround substrates of cultivation, quickly. It prevails at other contaminations of substrate and crop will produce high quality and quantity. On the contrary, produced mycelia have weakened and consequently produced spawn will be lack of vigor for cropping (Momeni, 2003).

Spawn grains such as wheat, millet and corn have been reported to affect carpophores production (Nwanze *et al.*, 2005b) here are various additives that are known to stimulate fruiting. They include rice bran, cassava peels and soybean powder. Wheat, millet and corn that are used in making spawn also belong to these genera. In the letter work, Nwanze *et al.* (2005a) examined the effect of spawn grains such as wheat, millet and corn

on the culture of *Lentinus squarrosulus*. The results showed that corn spawn induced highest yield and dry weight of fruiting as compared to wheat and millet spawn. Furthermore, institutes use than the larger seeds of corn and wheat, reason which large seeds are the more nutrient for mycelia growth (Mottaghi, 2004). Environmental factors such as temperature, light, O₂, CO₂, humidity and pH have been reported also to affect on mycelia growth in the spawn preparation (Nwanze *et al.*, 2005b).

Generally, must choosing substrate for spawn preparation suit for the purpose of mycelia growth. The present study, in order to select of the best substrate for spawn preparation performed in the plant physiology laboratory (Horticulture Department, Agricultural Collage, Shahid Chamran University, Ahwaz, Iran).

MATERIALS AND METHODS

The experiment carried out in 2006 in order to study the response of three species of (*Pleurotus florida*, *P. citrinopileatus* and *P. ostreatus*) to three substrate types (wheat, millet and corn) for producing spawn in physiology lab of Horticulture department of Shahid Chamran University of Ahwaz. Three different types of grain, including corn, wheat and millet were used to produce. The spawns were prepared as described by Nwanze *et al.* (2005a) (Table 1) and kept for two weeks in order for the spawn to run. In this research, parameters for analysis were: spawn dry matter after completed maturation (g), diameter of colony extension in three stages (4, 8 and 12 days after inoculation). Statistical analysis was performed using the factorial design at completely randomized (with three replications). The data were analyzed using GLM procedure of SAS 9.1 version software package and the means were separated by Duncan's multiple range tests (Jamalzadeh and Shareghi, 2004).

Table 1: Spawn preparation using different grain and conditions

Spawn	Components	Method of preparation
Wheat	1.0 kg wheat grains, 12.0 g CaSO ₄ .2H ₂ O, 3.0 g CaCO ₃ and 1.5 L distilled water The water was poured off and 900.0 g of the cooked grains was mixed with 12.0 g gypsum and 3.0 g CaCO ₃ .	1.0 kg of wheat grains was boiled in 1.5 L of water for 15 min and left to cool for an additional 15 min. The grains were then filled into bags of polypropylene and sterilized for 20 min at 121°C. After cooling, the bags were inoculated with pieces of agar medium colonized with mycelium and incubated for 2
Corn	Same as above except for use of corn as grain	Same as above
Millet	Same as above except for the use of millet as grain	Same as above except that the grains were boiled for 5 min.

RESULTS AND DISCUSSION

The results of the analysis of variance showed that diameter of colony extension in three stages (4, 8 and 12 days after inoculation) were significantly affected by substrate and specie type. Substrate also had a significant on spawn dry matter after completed maturation, while specie had not a significant affect on spawn dry matter after completed maturation. Also, diameter of colony extension in two stages of 8 and 12 days after inoculation were significantly influenced by substrate×specie interaction. But, it had not significant affect on diameter of colony extension in stage of 4 days after inoculation and spawn dry matter after completed maturation (Table 2). The maximum and minimum of growth rate were at the corn and millet substrates, respectively (Table 3). Tinoco *et al.* (2001) found that however, the larger surface area and pore of substrates are the more mycelium growth rate. For the reason that corn seeds size are larger than wheat and millet seeds size, consequently, corn seeds pore is also larger. As a result, further it was mycelia growth rate. Probably, this method was also, increased ventilation and O₂ concentration in corn substrate. For, O₂ is one of the most important environmental factors. It performs an important role in metabolism and essential for respiration mushroom. For maximum of respiration perform in active growth (or mycelia growth). Therefore at the lowest O₂ concentrations, respiration rate relate directly with O₂ concentration of substrate (Mehravaran, 1993).

Table 2: F-values and degree of freedom (df) from an analysis of variance for effects of substrate and specie type on diameter of colony extension (4, 8 and 12 days after inoculation) and spawn dry matter after completed maturation

Source of variation	df	Diameter of variation extension after inoculation (F)			Spawn dry matter (F)
		4 days	8 days	12 days	
Substrate	2	0.0006*	0.0001	0.0001	0.0055
Specie	2	0.0001	0.0001	0.0001	0.7404
Substrate×specie	4	0.1933	0.0001	0.0001	0.0957
Error	18	2.7400	0.9800	0.9900	159.5300
CV%		10.0000	3.5800	2.7900	5.0400

*: Values of less than 0.05 were considered significant

Table 3: Simple effects of substrate and specie type on average diameter of colony extension (4, 8 and 12 days after inoculation) and spawn dry matter after completed maturation

Substrate	Diameter of colony extension after inoculation (mm)			Spawn dry matter (g)
	4 days	8 days	12 days	
Wheat	16.66±1.15*	30.77±1.54	38.15±2.60	254.41±4.76
Corn	16.88±1.41	32.85±3.94	40.04±4.94	258.50±5.50
Millet	13.66±0.80	19.32±1.71	28.91±2.94	237.44±2.96
Species				
<i>Florida</i>	19.71±0.84	32.72±1.95	44.81±2.01	249.75±6.13
<i>Citrinopileatus</i>	14.52±0.73	31.13±3.76	40.19±2.46	252.61±4.94
<i>Ostreatus</i>	12.96±0.64	19.08±1.49	22.09±1.54	248.00±5.44

*: Plus and minus values are mean±SE

Table 4: Interaction effect of substrate and specie on average diameter of colony extension (8 and 12 days after inoculation)

Substrate×species	Diameter of colony extension after inoculation (mm)	
	8 days	12 days
Wheat× <i>Florida</i>	34.85±0.55	45.30±0.61
Wheat× <i>Citrinopileatus</i>	32.64±0.44	41.05±0.56
Wheat× <i>Ostreatus</i>	24.82±0.57	28.10±0.50
Corn× <i>Florida</i>	41.89±0.70	51.47±0.58
Corn× <i>Citrinopileatus</i>	39.49±0.38	48.23±0.55
Corn× <i>Ostreatus</i>	17.18±0.79	20.42±0.94
Millet× <i>Florida</i>	26.05±0.72	37.67±0.43
Millet× <i>Citrinopileatus</i>	16.67±0.36	31.31±0.47
Millet× <i>Ostreatus</i>	15.24±0.43	17.76±0.24

*: Plus and minus values are mean±SE

This result is similar to the observation of Kenealy and Dietrich (2004) (for *Phanerochaete chrysosporium*) who reported that mycelia growth and dry matter increase to increasing O₂ concentration in substrate. Emelyanova (2005) also obtained better mycelia growth of *Coriolus hirsutus* when submerged culture was supplemented with oxygen solution. These results showed the importance of matching both metabolic activities and substrate O₂ concentration for maximum mycelia growth. Spawn dry matter after completed maturation was also significantly influenced by substrate. The most of dry matter was at corn substrate (258.5±5.50 g bag⁻¹), followed in order by wheat (254.41±4.76 g bag⁻¹) and millet (237.44±2.96 g bag⁻¹) substrate, respectively. That reason may be attributed to good mycelia growth rate at corn substrate which it caused spawn dry matter increasing. This result is in agreement with Kenealy and Dietrich (2004) (for *Phanerochaete chrysosporium*). Diameter of colony extension was significantly affected by specie. In the three stages, the best mycelia growth rate obtained at *Florida* specie, followed by *Citrinopileatus* and *Ostreatus* species. For the reason that, *Florida* specie was fast growth, the most colonization and degradation vigor of mycelia as compared to other species (Mottaghi, 2006). Also, it was appropriated the further cultivation at the tropical parts, because it was the high temperature and unfavorable resistant (Islam *et al.*, 2006). This result is similar to the observation of Nandi and Mukherjee (2004) who reported that *P. florida* was more effective *P. citrinopileatus* in delignification. But, *Ostreatus* specie had the least mycelia growth rate. For the reason of it is one of the species zone temperatures (Mohammadi Goltapeh and Purjam, 2003) and is extensively produced out in localities with average temperature of 15°C (Marino *et al.* 2003). For, this experiment performs in temperature above 25°C and consequently reduces mycelia growth. This result is similar to those obtained by Smith and Margarel (1995) (for *Agaricus*

strain (W4II)) and Jonathan and Fasidi (2003) (for *Psathyrella atroumbonata*). Also, diameter of colony extension in two stages (8, 12 days after inoculation) was significantly influenced by substrate×species interaction. Table 4 shows that *Florida* is suitable specie for three substrates of wheat, corn and millet. Also, the results showed that corn, corn and wheat substrates are suitable for *Florida*, *Citrinopileatus* and *Ostratus* species, respectively (8, 12 days after inoculation). Probably, these results are for the highest mycelia growth of *Florida* species and favorable of corn substrate.

CONCLUSION

- Mycelia growth of *Florida* specie covered faster than other species of the substrates surface.
- The best substrate for spawn preparation was corn substrate.

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