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PROPAGATION OF *PLEUROTUS SAJOR-CAJU* (OYSTER MUSHROOM) THROUGH TISSUE CULTURE

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Abstract

Mushrooms have been recorded as a source of vegetable and medicines for human beings throughout the world. The oyster mushroom (*Pleurotus sajor-caju*) is edible and an important ingredient of pizza and many other popular bakery dishes. Oyster mushroom is cultivated on different agricultural wastes due to its compatibility and produce high yield in diversified climate. Studies revealed that the joint portion of cap and stipe produced vigorous mycelium growth in minimum time, The average maximum growth was obtained on Malt Extract Agar (MEA) than on Potato Dextrose agar (PDA) medium at 25 ºC under humid (65 – 80 RH) conditions. For the substrate, out of three types of grains viz., wheat, sorghum and oat; sorghum was found to be best for mycelium propagation and the time period for optimum growth was 7 days.

Introduction

The cultivated mushrooms mostly belong to the family Agaricaceae of class Basidiomycetes. Mushrooms may be saprophytic, parasitic and mycorrhizal in their mode of living. Most of the cultivated mushrooms are saprophytic; they feed on organic matter which has already been manufactured by plants or animals. In nature they grow on fallen leaves, animal droppings and stumps of dead wood (Bilgrami & Verma, 1978).

In nature mushrooms grow wild in every country from snowy mountains to sandy deserts on all types of soils, pastures, forests, cultivated fields or water lands. They appear in all seasons, chiefly during the rainy weather, wherever organic matter or its decomposition products are available (Kapoor, 1989).

Mushroom a food of high quality, flavour and nutrition value have high content of protein, low content of fat (4%), vitamins (B_1, B_2, C, niacin, biotin etc), minerals (P, Na, K, Ca) and high content of fibers and carbohydrates (Peter, 1991). Mushrooms are also used for chronic catarrh diseases of the breast and hinges, lower the cholesterol level of blood, improves circulation, remedy for night sweating in tuberculosis, rheumatism, gout, jaundice, dropsy, intestinal worms and have anti-tumor, anti-viral and anti-cancer agents.
The most well known species of *Pleurotus* are *P. ostreatus, P. florida, P. eryngii, P. cystidiosis, P. flabellatus, P. cornucopie,* and *P. sajor-caju. P. sajor-caju* is recognized as an excellent mushroom. It can be cultivated within a wide range of temperatures on different natural resources and agricultural wastes. The total production of mushroom crop residues from the cereal crops in Pakistan is estimated to be about 36 million tons and this waste can be used for mushroom cultivation by recycling mechanism (Rehman, 1993).

The cultivation of oyster mushroom is simple as compared to other varieties. The mycelial growth variation of six species of *Pleurotus* at different temperatures was studied by Mehta & Bhandal (1988). The six species were propagated on PDA medium at 10, 15, 20, 25, 30, 35, 40ºC. They found that the time taken for complete colonization was 16 days and suitable temperatures were 15, 20, 25 and 30ºC while no growth was observed at 10, 35 and 40ºC.

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The sporophores of *P. sajor-caju* have 26.9 % protein having high digestibility values and all essential seventeen amino acids in good concentration. The five species of *Pleurotus* cultivated on cotton seed hulls, wheat, rice or maize straw are different in composition of crude protein. Among the substrates, rice straw was best for mushroom growth (Qin *et al*., 1989). *P. sajor-caju* can be successfully grown on paddy straw at temperature range of 19.1-30.5ºC and relative humidity 65.5-80%, there is a decline in yield above and below this temperature (Singh, 1981). Oyster mushroom can be grown on most of the agricultural wastes of which sugarcane bagasse proved to be the best substrate for sporophore production (Khan & Khatoon 1982).

Pleurotus sajor-caju can be grown on wheat straw, paddy straw, stalks and leaves of sorghum, pearl, millet and maize for commercial cultivation. The cotton stalks and leaves induced high yield (2361 gm/10Kg substrate) followed by sorghum stalk and leaves (1463 gm/10 Kg substrate) of oyster mushroom (Patil *et al*., 1989). Four strains of oyster mushroom on cotton waste showed fastest spawn running in blue gray strain of *P. ostreatus* with highest yield in first and third flush while *P. sajor-caju* gave highest yield in second flush (Muhammad & Khan, 1993). The cultivation of *P. sajor-caju* on bagasse medium showed vigorous growth and highest yield of 174 Kg FW/100 Kg medium than wheat straw and wheat bran (Shi, 1994).

Keeping in view the importance of edible mushrooms, the main objective of this study was to propagate *P. sajor-caju* at faster growth rate using tissue culture techniques. In order to achieve this objective, there was a need to find out the most suitable portion of the fruiting body for culture studies as to get the best growth, the selection of media and substrates were required.

Materials and Methods

The propagation of *Pleurotus sajor-caju* (oyster mushroom) was carried out in the mushroom unit of Horticulture Research Institute (HRI), National Agricultural Research Centre (NARC) Islamabad, Pakistan during October-December, 2002. One litre of PDA medium contained 20 gm potato starch, 20 gm dextrose, 20 gm agar and 1 gm Peptone in distilled water. Similarly one litre of MEA medium contained 20 gm malt extract, 20 gm dextrose, 20 gm agar and 1 gm peptone in distilled water.

Both PDA and MEA media were sterilized in an autoclave at 15 psi at 121ºC for 30 minutes and then poured in 90 mm Petri dishes under the laminar flow hood to avoid contamination. Media were cooled to 40ºC. The joint, stalk and veil of the fresh mushrooms were inoculated on culture media. The cultures were incubated at 25ºC for 12 days. Radial growth of mycelium of different portions was observed until the Petri dishes were filled with it. The experiment was repeated for 6 times.

Dried and clean grains of wheat, sorghum and oat 500 gm each, were separately used for multiplication of pure culture of *P. sajor-caju*. Grains were soaked for 1-2 hours in water and then boiled for 10-15 minutes. Upon softening grains were spread on newspaper and amended with 1-2% lime. Glass bottles were filled with grains having 70% moisture content and sterilized at 15 psi at 121ºC for 30 minutes. The bottles containing grains were inoculated with pure culture of mushrooms and incubated at 25ºC. Percent mycelial growth was observed daily until the bottles were filled.

Results and Discussion

To find out the best portion of mushroom for mycelial growth, our results revealed that the colony diameter of joint portion showed more vigorous growth than the other

portions in 12 days to fill the of 9cm diameter Petri dish containing PDA medium (Table 1). So the most suitable portion of mushroom for tissue culturing was joint portion as it has both the properties of stalk and veil.

The analysis of variance showed that means were significantly different at 5% level of significance (Table 1). Mehta & Bhandal (1988) studied the mycelial growth of number of species of *Pleurotus* at different temperatures on PDA medium and observed complete colonization of a Petri dish (7 cm in diameter) in 8-11 days on PDA medium at 25ºC. Our results are in consistence with those of Mehta & Bhandal (1988).

In order to find out best medium for spawn growth among PDA and MEA, linear growth of pure culture of *P. sajor-caju* was studied at 25 ºC (Table 2). This study revealed that MEA was the best for faster mycelial growth with average colony diameter of 8.60 cm in 7 days (Table 2). The analysis of variance showed that both medium and time intervals had significantly affected the size of colony diameter.

To find out the best grain substrate for faster mycelial growth, wheat, sorghum and oat grains were used. The results of this study revealed that sorghum grain was the best substrate as compared to wheat and oat for mycelial growth (Table 3). Full mycelial growth was obtained in 7.833 days on sorghum grains which was followed by wheat grains which took 11.83 days for full mycelial growth and finally oat grains which took 13.167 days for full mycelial growth. Our results are similar to that of Awasthi & Pande (1989) who reported that highest yield of fruiting bodies was obtained on sorghum grains followed by wheat grains and maize grains. The findings of this study revealed that joint portion of mushroom was the best source for propagation. MEA medium and sorghum grains substrate was also best to grow *Pleurotus sajor-caju.*

Table 1. Colony diameter of different portions in hours at 25°C on PDA medium.

Interval	Portions of mushroom		
Hours	**Joint (cm)**	**Stalk (cm)**	**Veil (cm)**
48	1.8	0.6	0.2
96	3.2	1.4	0.6
144	5.3	2.2	1.2
192	6.2	3.0	2.8
240	7.5	4.3	3.6
288	9.0	6.5	4.6
Means	5.5 A	3.0 B	2.1 C

Any two means carrying the same letter(s) are not significantly different at *P*=0.05 by LSD.

Table 2. Comparison of MEA and PDA media for spawn preparation at 25°C.

Intervals (days)	MEA (cm)	PDA (cm)	Means (cm)
2	2.150 e	0.440 f	1.795 D
4	4.137 d	2.473 e	3.305 C
6	6.470 b	3.987 d	5.228 B
7	8.600 a	5.643 c	7.122 A
Means	5.339 A	3.386 B	

Means followed by different letters are significantly different at *P*= 0.05 by LSD.

Table 3. Spawn running in wheat, sorghum and oat grains.

S. No.	Spawn run (%)	Wheat	Sorghum	Oat	Means
1	25	2.833 h	18.331 a	3.833 g	8.330 D
2	50	5.833 f	3.833 g	7.833 f	5.833 C
3	75	9.167 d	5.833 f	10.670 c	8.556 D
4	100	11.83 b	7.833 e	13.170 a	10.994 A
Means		7.417 B	4.633 C	8.875 A	

Means followed by different letters are significantly different at $P = 0.05$ by LSD.

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