"Study of Spawning response of Pleurotus sajor-caju in two different substrates (wheat and rice)"

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ABSTRACT: The *Pleurotus sajor-caju* is chosen as the sample for the study because of its availability throughout the year in Manipur and also the people of the state owing to its good taste and nutrition mostly consume them. In wheat, spawn begins to appear after 15-16 days whereas the spawn started to appear after 18-19 days when rice was used as a substrate. Wheat seems to produce more spawn in a shorter period of time than rice. Therefore wheat seems to be the more appropriate substrate for the commercial production of spawn. However because of high contamination rate and difficulty in the preparation of substrate in case of wheat, rice can be considered as a better option. Also in the context of Manipur rice being the staple diet is available in abundance and at low price throughout the year. Therefore rice will be the better option as far as commercial production of spawn is concerned .We can conclude that *Pleurotus* spawn can be easily produced using rice as a substrate and allow farmers for the mushroom cultivation throughout the year thus helping in food availability.

Key Words: commercial production, spawn, mushrooms, mycelium, paddy straw, *Pleurotus sajor-caju,* pure-culture, wheat.

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Introduction

Mushrooms are the member of the kingdom fungi, which belongs to the class Basidiomycetes or Basidiomycota. They lack chlorophyll and hence depend on organic decays or living plant for their nutrition. They grow saprophytically as a weak parasite and symbiotically with other organisms.
As a member of Basidiomycetes, they have the ability to degrade cellulose, hemi cellulose, lignin and inturns produce edible fruiting body which posses appreciable flavor, texture and better nutritive value. It contains a high percentage of proteins which is higher than any other vegetative protein, but it is low in calories (carbohydrate), high in some vitamins (B-complex) and minerals like Ca, P, Fe, Cu, which are essential for formation of bones and teeth. Being remarkably low in starch content, it is a good diet for people suffering from diabetic.

The use of fungi for the conversion of lignocellulose into food and feed rich in protein offers an alternative for developing unconventional source of proteins as food/feed. Yeast and algal proteins require sophisticated techniques and heavy inputs whereas the beauty of mushroom cultivation lies in its ability to grow on cheap lignocellulosic materials with minimum inputs and a high yield of valued food protein for direct human consumption.

A wide range of diverse cellulosic substrates, are used for cultivation of *Pleurotus sajor-caju*. Amongst various cereal straws, paddy straw was reported to be the best substrate for the cultivation of oyster mushroom (Bano and Srivastava, 1962; Jandaik and Kapoor, 1974; Khanna and Garcha, 1982), whereas, next to the paddy straw, wheat straw proved to be the best substrate for the cultivation of *Pleurotus* spp. (Bano and Rajarathnam, 1982; Bhatti et al., 1987; Thampi et al., 1996; Bonatti et al., 2004). Sorghum straw was also effectively used to cultivate *P. sajor-caju* (Bahukhandi and Munjal, 1989; Patil et al., 1989). Similarly, Garcha et al.(1984) and Diwakar et al.(1989) reported the utility of pearl millet stalks in the cultivation of *P. sajor-caju*. Rye straw waste (Pal and Thapa, 1979), lawn grass (Yamashita et al., 1983), maize cobs (Bhatti et al., 1987), Banana waste (Bonatti et al., 2004) and maize straw (Bahukhandi and Munjal, 1989) were reported as suitable substrates for cultivations of different *Pleurotus* spp. Bhandari et al.(1991) successfully cultivated *P. sajor-caju* on straws of millets viz. *Echinochloa frumentacea* (Poaceae) and *Eleusine coracana* (Poaceae), and grasses viz. *Heteropogon contortus* (Poaceae) and *Andropogon purtuses* (Poaceae). Many other types of substrates were also reported to be useful for the cultivation of various species of *Pleurotus* spp .

Supplementations of main substrates with nutrient or combination of two or more substrates were reported to increase the yields of *P. sajor-caju* (Jadhav et al., 1998). Amendments or mixtures of various straws were tried to assess possibilities for main substrates to increase the yield of *P. sajor-caju*. The second objective was to determine an effective substrate combination for the cultivation of *Pleurotus* spp. in the paucity of wheat or paddy straws.

Lignocellulosic materials are generally low in protein content, insufficient for the cultivation of mushrooms, which requires nitrogen, phosphate and potassium. Since the C:N ratio plays an important role in spawn running and the growth of fruiting body, nitrogen supplementation is an important factor for the growth and yield of mushrooms.

Based on earlier studies and local availabilities of the agricultural wastes, in the present study we utilize paddy straw for the cultivation and production of *P. sajor-caju*. We also study the effects of spawning growth in rice and wheat substrate.

For commercialization, *Pleurotus spp.* (*sajor-caju)* have been chosen for cultivation and advantages from other types of mushroom are as follows:

- Growing technique is easy.
- It can be grown throughout the year.
- It can be grown on a variety of agricultural or industrial waste.
- Growing media are cheap and easily available.
- It is suitable for small/ large-scale cultivation.
- Infrastructural facilities required lesser inputs.
- It is not labour intensive.

Materials and Methods

Pure Culture Preparation of *Pleurotus*

Pure culture of fleshy fungi/mushrooms can be prepared by multi- spore culture or tissue culture. Multi- spore culture is obtained by placing a fresh fruit body after surface sterilization with alcohol on a petriplate / sterilized paper. Millions of spores are collected within 48 hrs. Serially diluted loop full of spore are then transferred to sterile Potato Dextrose Agar (PDA). These slants are then inoculated at 25^0C $\pm 2^0$C for two weeks to obtain pure culture. For tissue culture the basidiocarp after alcohol sterilization is cut longitudinally into two halves and bits from collar region are transferred to pre sterilized PDA. The petriplates are incubated at 25^0C $\pm$ 2^0C in BOD incubator for 1 week. Mycelium from growing edges is carefully transferred to PDA slants and again incubated for 2-3 weeks to obtain pure culture.

Alcohol sterilization of the sample (*Pleurotus*)

A young and fresh mushroom of Pleurotos species is taken. The mushroom is wiped by 90% alcohol to the site where the flesh is to be taken and is transferred into the PDA/ agar slant. All these procedures should be done inside the Laminar Air Flow under sterile condition. It is required that we wipe our hands with 90% alcohol in order to prevent or for lesser contamination. If contamination is occurred the growth of mycelium is decreased and black patches can be observed inside the test tubes. The contaminated test tubes should be discarded.

Procedure

Mother Spawn Preparation

About 350g prepared substrate is filled in bottles up to 2/3 volumes and plugged with non – absorbent cotton. These bottles are then autoclaved at 22lbpsi.Pressure at 126^0C for 2 hrs. These autoclaved bottles are left in room for 24hrs so that they are cooled and kept on laminar airflow under UV tube for 20-30 minutes. A piece of growing mycelium is aseptically transferred to these bottles are gently shaken on 5^{th} and 10^{th} Day. Fully grain colonized mother spawn bottles can be used for inoculating commercial spawn banks after 2-3 weeks. Incubated bottles are incubated at 22-25^0 C.

Substrate Preparation

- Grains (paddy/wheat) were washed and boiled to the extent that no starch was released.
- Remove the excess water of grain by spreading the grain in a sieve.

- Allow the grain to surface-dry by spreading over polythene sheets.
- Mix the boiled grains thoroughly with chemicals (calcium sulphate2% and calcium carbonate 4% respectively on weight basis of the grains).
- Fill the grains-chemical mixture in 500 ml glucose/milk bottles /polythene bags about 200 – 250 gm.
- Plug the container with non-absorbent cotton and sterilize the substrate by autoclaving at 121^0 C (15psi).
- Allow the substrate container to come to room temperature now the substrate is ready for inoculation.

Inoculation of Substrate

- Inoculate the substrate (grains in containers) with mycelium of mushroom grown on specific medium (mother spawn) by transferring mycelium on the grains under aseptic conditions.
- Shake the containers after plug in to distribute fragments of the mycelium.

Incubation

- Incubate the inoculated containers in B.O.D. incubators or storage room at 25^0 C.
- Appearance of silky whitish colony completely covering with grains indicates the preparation of a spawn of mushroom. If the contaminated colors of mycelium appeared, contaminated bags were discarded.

Culture of Mushroom

Selection of Straw

Paddy straw, which is easily available, is selected as the substrate for cultivation. Chopped paddy straw forms a suitable base for the cultivation of *Pleurotus spp*. The straw is well dried free from moulds and other microorganisms.

Chopping

The paddy straw is chopped with hand chopper to a size of about 5-9 cm.

Steeping

The chopped paddy straw is soaked in cold water for about half an hour in a suitable container. The excess water is drained out.

Sterilization

Water soaked straw is then steamed sterilized by boiling in a drum with wire basket for about half an hour. The sterilized straw is then spread on a large plastic sheet thus draining away the excess water and at the same time letting it to cool.

Filling and Spawning

The well drained sterilized and chopped paddy straw is finally filled in a polythene bag (45-60) cm in layers with each layer being followed by spawning. The mouth of the filled bag is then tightened with a rubber belt. Spawning at the rate of 300g/1.5 kg of straw (dry weight) is optimum. The mycelium runs started in the next 2-3 days. It was allowed to continue for 10-25 days depending upon the species and prevailing temperature. High humidity is maintained in the culture room. The pinheads started appearing within 15-25 days of spawning. In case of punched cultivation bags, the bags were allowed to remain as such but in case of unpunched bags, the bags were torn off carefully just as the pinheads appeared. The straw should be kept sufficiently moist by sprinkling water. Usually the fruiting bodies appeared in sudden outbreaks called flushes at an interval of 8-12 days. 2-3 flushes occurred during full cropping period of one and a half month. As the harvesting period advanced, the number of fruiting bodies gradually decreases.

Harvesting

The crop is harvested when the cap started folding. The fruiting body is twisted and taken out manually so that no broken remains were left out.

Observation and Result

During the pure culture preparation it was observed that the mycelia of *Pleurotus sajor-caju* begin to appear after incubation at 25^0 C in a B.O.D. incubator. The mycelia were seen as a silky whitish fibre-like substance.

Finally during the spawn production stage the spawn begin to appear after 15-16 days when wheat was used whereas the spawn started to appear after 18-19 days when rice was used as a substrate. However contamination occurred maximally in the case of wheat as a substrate then using rice as a substrate. The spawn appeared as silky whitish grown completely covering the grains.

For the spawn production the temperature should never below 15^0 C or above 30^0 C. If the temperature is above or below the above-mentioned temperature, the spawn production is hampered. The spawn is then considered to be ready for further cultivation.

Conclusion

During spawn production wheat as a substrate seem to produce more spawn in a shorter period of time than rice. Therefore wheat seems to be the more appropriate substrate for the commercial production of spawn. However because of high contamination rate and difficulty in the preparation of substrate in the case of wheat, rice can be considered as the better option. Also in the context of Manipur, rice being the staple diet is available in abundance and at low price throughout the year. On the other hand, procurement of wheat grains will be a problem because of its high price and unavailability in the state. Therefore rice will be the better option as far as commercial production of spawn is concerned.

The *Pleurotus sajor caju* has been chosen as a sample for the study because of its availability throughout the year in Manipur and also the people of the state owing to its good taste and nutrition mostly consume them.

Therefore, from the above study, we can conclude that *Pleurotus* spawn can be easily produced using rice as a substrate and allow farmers for the mushroom cultivation throughout the year thus helping in food availability.

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