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Development of an easy and efficient technique for cultivation of different species of *Pleurotus*

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Abstract
Oyster mushroom is one of the most popular mushrooms in North Bengal and a large number of growers are now cultivating oyster mushroom throughout the year. There are mainly three species of oyster mushroom (*Pleurotus ostreatus, P. sajor-caju, P. florida*) which are being cultivated in this region. Out of these three mushrooms, *P. ostreatus* and *P. sajor-caju* are generally cultivated in summer as it grows in a temperature between 25-33°C while *P. florida* is generally cultivated during winter as it requires 15-20°C for its growth. Bottle cultivation is a technique of mushroom cultivation where it reduces the cost of plastic bags and the plastic bottles can be recycled. Besides plastic bottles, other used laboratory chemical plastic containers were also tested and gave good results.

Key words: *Pleurotus ostreatus, P. sajor-caju, P. florida*, Bottle Cultivation.

Introduction:

Mushrooms are prized for their delicacy and distinctive flavor. Because of their unique nutritional status, they are known as "the ultimate health food" (King, 1993). They are non-conventional sources of human food and are delicious, nutritionally rich and have their own importance as medicines. Mushroom has been defined as a "Macro-fungus with a distinctive fruiting body which can be either epigeous (above ground) or hypogeous (underground) and large enough to be seen with the naked eye and to be picked by hand" (Chang and Miles, 1993). Mushrooms are rich in protein, some essential amino acids, fiber, potassium, and vitamins and have low cholesterol and fat levels (Rafique, 1996). Mushroom cultivation represents the only current economically viable biotechnology process for the conversion of waste plant residues from forests and agriculture (Wood and Smith, 1987). It is a highly efficient method of disposing of agricultural residues as well as producing nutritious food (Chang *et. al.*, 1981).

Oyster Mushroom belongs to the genus *Pleurotus* from the class Basidiomycetes with a shell like fleshy stipe and white, brownish, pink or dark grey in colour. It is now ranks second among the cultivated mushrooms in world (Chang and Miles, 1991). Almost all the available lignocellulosic substances can be used as substrate for *Pleurotus* sp. It is a popular mushroom due to its nutritional, medicinal and potential commercial value (Saidu *et. al.*, 2011). They have ability to grow at wide range of temperatures and utilize various lignocellulose substrates (Khan and Garcha, 1984). Species of *Pleurotus* are usually found to be most efficient in the degradation of lignocellulose substrates among all types of white rot fungi (Das and Mukherjee, 2007). It is composed of 90% water and 10% dry matter (Morais *et.al.*, 2000; Sánchez, 2004). Fruiting bodies as well as active mycelia of *Pleurotus* sp. possess a number of therapeutic properties like anti-inflamatory, immuno-stimultory and immuno-modulatory (Asfor*set. al.*, 1993), anticancer activity (Wasser, 2002), and many more medicinal activities. Wide spread malnutrition with ever increasing protein gap in our country has necessitated the search for alternative source of protein because the production of pulses has not kept pace with our requirement due to high population growth. Animal protein is beyond the reach of most people in this country. Edible mushrooms are recommended by the FAO as food, contributing to the protein nutrition of developing countries dependent largely on cereals. Human have been eating different food groups such as meat

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and plant-based including fungi or edible mushrooms for thousands of years. Edible mushrooms have been consumed as food and medicine in many cultures (Bobek *et. al*., 1997; Yang *et al*., 2001; Chocksaisawasdee *et. al*., 2010; Wan Rosli *et al*., 2011). Mushrooms are fungi which commonly grown in the shady area and prolifically propagated through its spores. They are versatile and may be eaten fresh or cooked entirely. Mushrooms are eaten by people for their unique flavour, texture as well as for the health benefits they accord. Mushrooms have been consumed and appreciated for their flavour, economical and ecological values and medical properties for many years (Sanchez, 2010). Mushrooms are healthy foods, low in calories and fat, but high in vegetable proteins, chitin, vitamins and minerals (Manzi *et al*., 1999). Some species of wild mushrooms are sold in the local wet markets as vegetable and many researchers focused on their therapeutic effects and cultivation methods (Tan and Wahab 1997; Pathmashini *et. al*., 2008; Rashad *et al*., 2009; Beluhan and Ranogajec 2011).

Materials Methods

Preparation of Spawn

Wheat grains are used for the preparation of spawn. Wheat grains were boiled in for 20 min and the water drained off. Then it was allowed to dry for overnight in a clean place after which 0.5% (w/w) $CaCO_3$ and 2% (w/w) $CaSO_4$ were added and mixed well. The grains (200gm) were filled in each polypropylene bag and it was autoclaved at 20lb pressure for 1hour. The grains were inoculated with actively growing mycelium of the *Pleurotus ostreatus*, *P.sajor-caju* or *P.florida* from PDA slant and incubated at 25-28°C for mycelial growth for 14 days until the mycelium fully covered the grains. Completely covering the grains with mycelium rapidly colonizes the bulk growing substrate (Sánchez, 2010).

Cultivation of mushroom

Maintenance of mother culture

Mother culture of the fungal mycelia was maintained by preparing proper selective media and sub-culturing the same following the method suggested by Fritsch (1978) and Jong (1978).

Substrate preparation

Rice straw was used for the cultivation of oyster mushroom. Chopped (2-4 cm long) rice straw was washed and soaked in water for overnight. The straw was again cleaned and pasteurized at 55-65°C for 20-30 min. then it was allowed for cooling at room temperature. Spawning was done using the polypropylene bags, waste bottles of chemicals, waste water bottles, and broken laboratory glass goods.

Spawning

Polypropylene bags are generally used for spawning while using the bottles is a new introduction in the cultivation practices in North Bengal. Besides, used plastic bottles and empty laboratory chemical bottles were also tested as container. Layer spawning was done using the cooled pasteurized straw. 100 gm of spawn was used for 1kg of substrate for spawning. The bottles and bags were then filled using the substrate and spawn and the bags were closed tight and the bottles were closed using the lid. Small holes were made in each bottle and bag for aeration. The bags and bottles were then incubated at room temperature (20-30° C) for 10-12 days. After 10-12 days, the white mycelia covered the whole substrate. Then the plastic was removed and the lid of the bottles was opened. 80-90% moisture was maintained by spraying water on the substrate for 2-3 times in a day for the initiation of pinhead (Sarker*et. al*. 2007).

Initiation of Fruiting body

The pinhead appeared after 4-5 days of opening the bags and the lids. Fruiting body was developed at room temperature and 80-90% relative humidity. The fruiting body was harvested from the base carefully so that there should be no injury of the mycelia. Humidity was maintained again after the harvest for further flushes.

Determination of total protein

Protein was extracted from the mushroom using Phosphate buffer (pH7.2) and protein content was determined following the methods as described by

Lowry *et al.*, (1951) using BSA as standard.

Determination of total sugar

1gm of fresh mushroom tissue was crushed with 95% ethanol and the alcoholic fraction was evaporated in boiling water bath. Then the fraction was collected and the volume made upto 5 ml using distilled water. Then it was centrifuged at 10,000 rpm for 15 min and the supernatant was collected for estimation.

1 ml of extracted sample was taken and 4ml of Anthrone's reagent was added and incubated on boiling water bath for 10 min. Then it was cooled down in tap water and observed at 620 nm in colorimeter.

Determination of reducing sugar

Ethanol (80%) extract was used for estimating the reducing sugar. Extract (2 ml) was mixed with 2 ml of Alkaline copper tartrate and boiling was done. Determination of reducing sugar using Arseno-molybdate was carried out at 620 nm following Nelson-Somogyis' Method (1952).

Results and discussion

Three species of *Pleurotus* cultivated in different season. It was observed that the optimum temperature for the cultivation of *Pleurotus sajor-caju* is 20-28° C, *P. ostreatus* 20-25° C and *P. florida* was 18-20° C as reported by Dhar *et. al.* (2011). They also stated that the Fruit body of *P. sajor-caju* fan shaped with thick texture grey in colour, 3-4 inches long when mature while in case of *P. ostreatus* the mature pileus is quite dark and small as compared to *P. sajor-caju* and in case of *P. florida*, the mature pileus is small and white in colour with thin margin and smooth fleshy. *P. florida* look like a white disc growing on a thick stipe with decurrent gills extended up to the base of the stipeunlike *P. sajor-caju* and *P. ostreatus* (Figure 1). It was also observed that the amount of production of *P. ostreatus* is higher followed by the production of *P. sajor-caju* and *P. florida* (Figure 2).

Cultivation of oyster mushroom using waste bottle is a new introduction in North Bengal and showed a significant result. Using of waste plastic bottles as reported by Hyunjong Kwon is a sustainable technique for the mushroom cultivation and it was observed that the rate of contamination is being reduced when plastic bottles are used. Using waste chemical bottles and other waste bottles of water and cold drinks could be another way to recycle

Fig 1: Different stages of three species of oyster mushroom

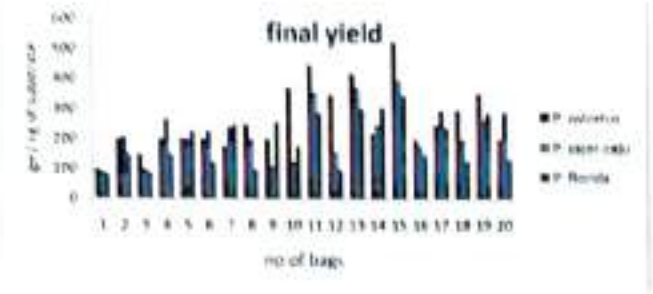

Fig 2: Final yield of three species in bags

the waste bottles in a good way and it reduces the cost of plastic bags. Different species of oyster mushroom was cultivated and it was observed that the production of *P. ostreatus* and *P. sajor-caju* was much higher than *P. florida* (Figure 3).

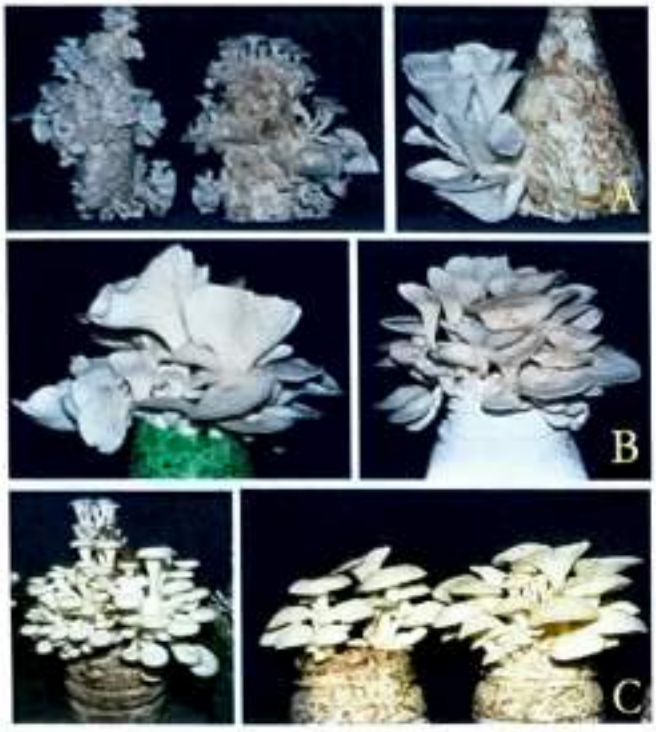

Fig 3: Bottle cultivation of three different species, A: *P. ostreatus*; B: *P. sajor-caju*; C: *P. florida* using waste plastic bottle, laboratory chemical bottles.

Total protein content was estimated and it was observed that total protein is quite higher in *P. ostreatus* and *P. sajor-caju*in comparison to *P. florida* (Table 1). It was also observed that in case of *Pleurotus ostreatus* the protein content was high in mature pileus (390 mg/gm tissue) and young pileus (230 mg/gm tissue) while in case of mature stipe and young stipe the total protein content was lower (190-280 mg/gm tissue). Pinhead of *P. ostreatus* also shows significant amount of protein content.

In case of *Pleurotus sajor-caju* the total protein content is high in mature pileus (370 mg/gm tissue) while it was observed that the protein content is lower in mature stipe (170 mg/gm tissue) and other stages. On the other hand, it was observed that the protein content was quite higher in pinhead stage (265 mg/gm tissue) of *P. florida*. While in *P. florida* the total protein content was lower in mature pileus (220 mg/gm tissue). It was observed that the total protein content was higher in *P. sajor-caju* as described by Khan *et. al.* (2008).

Table 1: Activity of total soluble protein content of different stages of the three species

Stages	Protein content (mg/gm tissue)		
	P. ostreatus	*P sajor caju*	*P. florida*
Pin head	270	240	265
Young Stipe	190	185	135
Young Pileus	230	220	230
Mature Stipe	280	170	190
Mature Pileus	390	370	220

It was also observed that the total sugar content and reducing sugar was higher in *P. sajor-caju* followed by *P. ostreatus* and *P. florida* as reported by Alam *et. al.* (2008). In case of *P. ostreatus*, total sugar content ranges from 100-300 mg/gm tissue and maximum total sugar content observed in young pileus (300 mg/gm tissue) while in case of reducing sugar, it ranges from 20-50 mg/gm tissue and maximum activity found in young pileus (Table 2).

In case of *P. sajor-caju* total sugar content ranges from 120-310 mg/gm tissue and maximum activity observed in young pileus (310 mg/gm tissue). While in case of reducing sugar it was observed that the activity ranges from 29-55 mg/gm tissue and maximum activity showed by the young pileus and pinhead stage (55 mg/gm tissue). On the contrary, in case of *P. florida* total sugar content ranges from 140-290 mg/gm tissue and reducing sugar content ranges from 26-60 mg/gm tissue. It was also observed that the maximum total sugar content showed by young pileus (290 mg/gm tissue) and maximum reducing sugar activity showed by young pileus (60 mg/gm tissue).

Table 2: Estimation of Total sugar content and reducing sugar activity in different stages of three mushrooms

Stages	Reducing sugar (mg/gm tissue)			Total sugar (mg/gm tissue)		
	P. ostreatus	*P sajor-caju*	*P. florida*	*P. ostreatus*	*P sajor-caju*	*P. florida*
Pin head	52	55	49	100	120	140
Young Stipe	49	48	45	210	225	210
Young Pileus	50	55	60	300	310	290
Mature Stipe	32	39	33	170	180	185
Mature Pileus	20	29	26	300	280	270

In conclusion, the chemical composition of edible mushrooms determines their nutritional value and sensory properties as also mentioned by Manzi *et. al.* (2001). They differ according to the species and also on the atmospheric conditions, age and part of the fructification. In our study we found that the nutritional values differ in different parts of the fructification. These data suggests that the three species cultivated in North Bengal *P. ostreatus, P. sajor-caju* and *P. florida* are a good source of nutrients specially in protein and sugar. These data also indicated that the mushrooms have a good nutritive value for human.

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