

Original Research Article

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Comparative Studies Using Various Substrates for Enhancing Yield of *Pleurotus* spp

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ABSTRACT

Four species of oyster mushroom including *P. djamor*, *P. eous*, *P. florida* and *P. citrinopileatus* were tested for their yield performance, of them, the most promising yield was obtained in *P. eous* and *P. djamor* with minimum spawn running period. On subjecting them with additional substrates, sorghum grain was superior over other spawn substrates for quality spawn production in terms of earliness in growth of spawn and total yield followed by wheat grain. For, sporophore production, paddy straw was the best substrate followed by maize stalk. Additionally, Paddy chaffy grain spawn supplementation with bengal gram at 60g/kg increased the yield of *P. djamor* and *P. eous* with minimum spawn running time followed by green gram. Exocellulase, endocellulase and laccase activity was found to be maximum in paddy straw followed by maize stalk. *P. djamor* reached the maximum enzyme activity at 20 days after inoculation followed by *P. eous* and *P. florida*. The enzymes such as cellulase and laccase are responsible for degradation of cellulose and lignin present in the substrates. The efficiency of the enzyme production was positively correlated with the yield of mushroom. *P. eous* recorded more dry weight of mushroom than other species of *Pleurotus* spp. when dried at 55 to 60°C in an oven. Shelf life of *P. eous* was longer than that of *P. djamor*, *P. florida* and *P. Citrino pileatus* under different storage conditions.

Keywords

Pleurotus spp.,
Substrate, Yield,
Enzyme, Shelf life,
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Introduction

Since life began on earth, fungi have evolved since then. In Greek the term “mushroom” means “Sphonggos” meaning, sponge like structure. The term mushroom represents fungi, having stem (stipe), cap (pileus), hymenium (lamellae) and spores on the underside of the cap (Masarirambi *et al.*,

2011). The edible mushroom has been consumed by people since ancient times. Mushroom consumption have been increasing day by day due to its significant role in human health and nutrition (Khan *et al.*, 2005). Mushroom world production accounts for 29,000 Mt, off which *Agaricus*, *Pleurotus*, *Lentinus*, *Auricularia*, *Flammulina* and *Volvariella* are the dominant cultivated taxa

constituting 85 per cent of the world's mushroom supply. Among them, *Pleurotus* spp. ranks second and constitute 27 per cent among the commercially cultivated mushrooms (Royse, 2014). At present, the annual production of oyster mushroom ranks 2nd in India with production of 21, 272 mt. *Pleurotus* spp. are the most popular ones and is widely cultivated throughout the world due to its tropical conditions (Mane *et al.*, 2007; Alam and Raza, 2001; Shah *et al.*, 2004; Flores, 2006). The genus *Pleurotus* is the most important mushroom cultivated among other genus due to its adaptation to various agro-climatic conditions (Zadrazil and Dube, 1992). Oyster mushroom is widely studied due to its flavor, nutritional and medicinal properties. As nutrient source of protein, carbohydrates, vitamins, calcium and iron it can be used in a variety of applications (Correa *et al.*, 2016). A high nutritional value of dried oyster mushrooms has been reported with protein (25-50%), fat (2-5%), sugars (17-47%), mycocellulose (7-38%) and minerals (potassium, phosphorus, calcium, sodium) of about 8-12% (Stanley, 2011). Edible mushrooms also increase immune system to our body and has rich source of vitamins including ascorbic acids, niacin, riboflavin, pantothenic acid, thiamine, pyridoxine and calciferol (Khatun *et al.*, 2015; Ahmed *et al.*, 2009).

Oyster mushrooms are mostly cultivated on lignin and cellulose based substrate. Agricultural byproducts are rich in lignose and cellulose and are discarded as waste (Marlina *et al.*, 2015). These waste materials have been used as substrates for cultivation of various mushrooms. Cultivation has been made using paddy and wheat straw (Yang *et al.*, 2013; Rezanian *et al.*, 2017), sugarcane bagasse (Hasan *et al.*, 2015), empty fruit bunch (Marlina *et al.*, 2015), date-palm leaves (Alananbeh *et al.*, 2014), tomato tuff (Ananbeh and Almomany, 2008), banana

leaves and pine needles (Ananbeh, 2003), jowar straw and groundnut pod (Khandar *et al.*, 1991), rubber wood waste (Mathew *et al.*, 1991), wheat straw (Gupta and Langer, 1988) and cotton stalk (Chang *et al.*, 1981). Addition of pulse powder for increasing the production of oyster mushroom has also been carried out by various researchers. (Singh, 1998) observed enrichment of sugarcane bagasse with chickpea bran at five per cent to produce highest yield of sporophores of *P. abalonus* and *P. florida* followed by bran of pigeon pea, lentil and pea. (Dubey, 1999) reported that supplementation of substrate (paddy straw) with pigeon pea dal powder at five per cent on a dry weight basis during spawning gave the highest number of sporophores and yielded maximum biological efficiency in *P. sajor-caju*, *P. flabellatus*, *P. ostreatus* and *P. cystidiosus* followed by gram dal and karanj cake. (Permana *et al.*, 2000) observed that sugarcane bagasse supplemented with soybean meal or wheat bran served as valuable substrate for production of *P. sajor-caju* and *P. eryngii*. Wheat bran concentrations of 7.5% to 12.5% gave higher yield when compared to the control (saw dust). 12.5% of wheat bran when added to the saw dust substrate gave the highest total yield by 683.5g of harvested mushroom (Victor and Ifeanyi, 2013). (Kumar *et al.*, 1997) obtained maximum yield with bengal gram powder and wheat bran @ 100g /kg with 155% biological efficiency in *P. sajorcaju*. (Singh and Shandilya, 2004) reported that supplementation of cotton seed meal, cotton seed cake and soybean meal increased the yield of button mushroom (*Agaricus bisporus*). The combination of wheat straw, leave and pigeon peas stalks and cotton stalks were found to be superior compared to soyabean straw and groundnut haulms. An increase in yield was recorded by the addition of rice bran and gram powder in the substrate (Mane *et al.*, 2007).

Thus, the present study was carried out with the objective to determine the high yielding *Pleurotus* spp. using different spawn substrates including pearl millet, sorghum grains, ill-filled paddy grains, finger millet, maize grains, wheat, banyard millet and foxtail millet. Additionally, sugarcane trash, maize stalk, banana leaf waste, paddy straw, maize shelled cob and foxtail straw were also used in the study as bed substrates. The substrates in combination along with supplements of pulse powder were also investigated in this study to produce high yielding *Pleurotus* spp. Also, enzymes analysis was made in order to determine the substrate utility. The post harvest technology including dehydration and shelf life of the high yielding mushroom was also studied for long term storage and transport.

Materials and Methods

Collection, isolation and purification of *Pleurotus* species

The following oyster mushroom species including *P. djamor* (Rumph) Boedijn., *Pleurotus eous* (Berk) Sacc., *P. florida* Eger were purchased from Agro Mushrooms, Thrissur, Kerala, India and *P. citrinopileatus* Singer was purchased from Jupiter Biotech, Tiruvannamalai, Tamil Nadu, India. The fungi were isolated by making a cut at the junction of pileus and stipe from the freshly harvested sporophore. The tissue bits were collected, surface sterilized with 95 percent ethyl alcohol thrice for two minutes followed by sterile distilled water. The tissue bits were placed in center of Potato dextrose agar medium (PDA). The plates were incubated at room temperature for seven days and mycelial mat radiating from the tissue bit was taken at its end and transferred to PDA slants using single hyphal tip method (Rangaswamy *et al.*, 1975). The PDA slants containing pure culture were maintained as stock culture at

4°C with periodic transfer throughout the period of study.

Effect of media on the growth of oyster mushroom species

To find out the best medium for the *in vitro* growth of oyster mushroom species, various solid medium including oatmeal agar, potato dextrose agar, Czapek's (Dox) agar, rose Bengal agar, malt extract agar and Richard's agar were used. The culture discs of nine mm diameter were cut out from a seven day old culture of oyster mushroom species and were inoculated at a temperature of $30\pm 2^{\circ}$ C and the colony diameter was measured seven days after incubation.

Preparation of mother and bed spawn

Sorghum grain spawn was prepared using the isolated cultures of *P. djamor*, *P. eous*, *P. florida* and *P. citrinopileatus* by adopting the method described by (Sivaprakasam, 1980). Sorghum grains were partially cooked with water for 40 min, drained, and grains were mixed two per cent with calcium carbonate. The grains were filled upto three-fourth volume of 750 ml glucose drip bottles and tightly plugged with non-absorbent cotton wool, wrapped and sterilized at 1.5 Kg/cm^2 for two h. The bottles were inoculated with pure cultures of nine mm mycelial disc of the fungi and incubated at room temperature ($30\pm 2^{\circ}$ C). The nature of growth and the time taken for complete colonization in the spawn were recorded. For bed spawn, sorghum grains were processed similarly to mother spawn except that 5" × 12" heat resistant polypropylene bags (200 gauge thickness) were used. From the mother spawn bottle, 10 g of fully grown mushroom mycelia was transferred to sterilized polypropylene bags under aseptic condition. Inoculated spawn bottles were incubated for 15 days at room temperature. Thirty bed spawn bottles were

prepared from one mother spawn of oyster mushroom viz., *P. djamor*, *P. eous*, *P. florida* and *P. citrinopileatus* separately and used for cultivation.

Cultivation of oyster mushroom and yield performance

The beds were prepared by adopting the polybag method described by Baskaran *et al.*, (1978) using paddy straws. Polypropylene bags of 100 gauge thickness and 60× 30 cm size were used to prepare the beds. Two holes of one cm diameter were made at the center to promote air circulation. For each bed, 500 g of the straw and 150g of spawn were used. Straw bits were placed at the bottom of the bag to a height of five cm and the spawn was spread to a thin layer.

This forms the first layer. A second layer of straw bits to a height of 10-15 cm was placed over the first layer and spawned. Likewise, four layers were formed; the final layer (fifth) was formed by placing to a height of five cm and tied to form a compact bed. The bags were kept for spawn run and sporophore production under high humid atmosphere. The observations were made for initial appearance of the sporophore, days taken for spawn run, and total yield.

Suitability of different substrates for spawn production

To find out the best medium for spawn production, different substrates including pearl millet, sorghum grains, ill-filled paddy grains, finger millet, maize grains, wheat, barnyard millet and foxtail millet were tested for spawn production of *P.djamor* and *P.eous*.

The grains were partially cooked with water for about 40 min, drained, mixed with two per cent calcium carbonate and filled upto three-fourth volume of 750 ml glucose drip bottles,

plugged with non-absorbent cotton wool, wrapped the mouth and sterilized at 1.5 Kg/cm² for two h. The sterilized bottles were inoculated with pure culture of the fungus and incubated at room temperature for 10-15 days. Observations on number of days required for spawn running, number of sporophores per bed, and yield per bed were recorded.

Effect of pulse powder on spawn production

Pulse grains including red gram, black gram, bengal gram, green gram, horse gram, cowpea and soyabean were sun dried, ground to powder form and 60 g/kg at 60% moisture level were added to the spawn substrate along with 20 g/kg calcium carbonate. The substrates were filled in polypropylene bags, sterilized and were inoculated with 9 mm mycelial disc and incubated at room temperature for 10 -15 days. Observations on number of days required for spawn running, number of sporophores per bed and yield per bed were recorded.

Suitability of different substrates for bed preparation

Various agricultural substrates including sugarcane trash, maize stalk, banana leaf waste, paddy straw, maize shelled cob and foxtail straw were used as substrates. These substrates were dried in sun and chopped into three to five cm bits and soaked in water for overnight. The excess water was drained off and boiled for 1 h.

The substrates were shade dried to get 60% moisture and the beds were prepared. After air drying, beds were laid out using these substrates. The observations on the number of days required for the complete coverage of the mushroom beds with the mycelium, number of harvest and the weight of the sporophores were recorded.

Assay of enzymes

In vitro assay of cellulase, exocellulase and laccase

Czapek's Dox broth was prepared and sterilized in autoclave for 1.5 kg/cm² for 1 h and used as basal medium in enzyme assay. Three mycelial discs (9 mm dia) from a seven day old culture were inoculated into 100 ml of sterile broth in conical flasks and incubated for 10 days at room temperature. The mycelial mat in the broth was filtered through Buchner funnel using Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 40 min at 4 °C. The supernatant was collected and the enzyme activity was estimated (Bateman, 1964). The samples from various substrates were drawn at 10, 20 and 30 days interval and the enzyme activities were estimated. Five g of substrates was ground with 20 ml phosphate buffer using a pestle and mortar, filtered through muslin cloth and filtrate was centrifuged in refrigerated centrifuge at 18,000 rpm for 20 min at 4 °C. The supernatant was collected and used as enzyme source (Maxewell and Bateman, 1967). Dinitrosalicylic acid method was followed for estimating endocellulases (C_x) activity by measuring the reducing sugar. The absorbance of the sample was read at 540 nm using colorimeter (Miller, 1972). For assay for exocellulase (C₁), enzyme was isolated and assayed using methods described by (Miller, 1972). The enzyme laccase was assayed by the oxidation of guaiacol. Enzyme was assayed using 10 mM guaiacol at pH 6.0 and absorbance was measured at 470 nm between an incubation period of 30 to 180 seconds. The enzyme activity was expressed as change in A₄₇₀ by 0.01/min/mg protein (Frochner and Eriksson, 1974).

Post-harvest technology

For dehydration, 100 g of fresh oyster mushroom of *P. djamor* and *P. eous* of

uniform size, maturity and weight were selected and subjected to drying in an oven at 55 to 60 °C. The dry weight of mushroom, loss in weight and change in colour of the dried mushrooms were recorded. For keeping quality, the mushrooms were processed as above and stored Under refrigerated condition, packed in thermocol boxes with ice cubes and normal conditions. The shelf life of the oyster mushroom species was recorded.

Statistical analysis

The results were statistically analysed using completely randomized design (single factor experiment) (Rangasamy, 1995) by AGRES software. The experimental data from each test was subjected to analysis of variance to determine the least significant difference among the treatment means.

Results and Discussion

Optimization of medium for myceliul growth on growth medium

Among the different media tested for the growth of mycelium among the four oyster mushroom species including *P. eous*, *P. djamor*, *P. florida* and *P. citrinopileatus*, oats meal agar medium recorded the maximum mean mycelial growth of 8.96 cm while potato dextrose agar medium showed close mean of 8.53 cm. 6.82 cm and 6.27 mean mycelia growth was observed in rose bengal agar medium and Czapeks medium respectively. An average yield of 5.47 mean myceliul growth was obtained using malt extract agar medium. The least growth were observed in Richard's agar medium 3.97 cm. *P. eous* recorded the maximum mycelial growth of 6.92 cm followed by *P. djamor* 6.84 cm, *P. florida* (6.47 cm) and *P. citrinopileatus* (6.24 cm) (Table 1 and Fig. 1). Comparatively, the complete growth of mycelium was found to be quicker in 9.33 days for *P. eous* followed by *P. djamor*

growing in 12.33 day. The other two species *P.florida* and *P. citrinopileatus* showed slower mycelial growth at 13.33 and 14.67 days respectively.

Yield parameters for mushroom species before substrate enhancement

Among the four species tested, *P. eous* recorded minimum days for completion of spawn run (10.33 days) followed by *P. djamor*, *P. florida* and *P. citrinopileatus* (13.33, 14.33 and 15.67 days). The time taken for the appearance of sporophore was rapid in *P. eous* (11.33 days) followed by *P. djamor* and *P. florida* with 13.67 and 14.67 days respectively. The maximum yield of 603.38 g/bed was obtained in *P. eous* followed by *P. djamor* with 584.16 g/bed while it was a minimum of 519.60 g/bed in *P. citrinopileatus* in 3 harvests (Table 2 and Fig. 2). Hence the high yielding *P. djamor* and *P. eous* was taken for improving its yield using additional substrates and factors for the study.

Effect of spawn substrates on production of spores and spawn yield

Studies on assessment of the efficacy of different spawn substrates of *P. djamor* revealed that sorghum grain is superior to other spawn substrates in yield parameters viz., days for completion of spawn run, initial appearance of sporophore, number of harvests per bed and yield (12.00 days, 13.33 days, 3.33 no. and 588.16 g respectively) followed by wheat grain (13.00 days, 14.00 days, 3.33 no. and 575.67 g respectively). The least values were observed in the case of finger millet (17.67 days, 19.00 days, 2.67 no. and 464.70 g respectively). For *P. eous*, sorghum grain supported the maximum yield of 599.38 g/bed with minimum spawn running period of 9.33 days and other yield parameters viz., initial appearance of sporophore and number of harvests per bed (11.33 days and 4.00 no.

respectively) followed by wheat grain (592.12 g, 10.33 days, 12.00 days and 3.33 no. respectively). The finger millet was found to record maximum spawn running period of (14.67 days) and minimum number of harvest and yield of 2.33 no. and 484.08 g/bed respectively (Table 3 and Fig. 3).

Effect of pulse powder as additive for production of spores and spawn yield

The results indicated that supplementation of spawn substrate with bengal gram powder reduced the days to complete spawn growth (13.33 days), first harvest (14.33 days) and maximum number of harvest (4.00 nos.). The total yield of *P.djamor* was higher in beds prepared from the spawn supplemented with bengal gram powder (593.75 g/bed) while in other treatments it was ranged from a minimum of 393.50 g/bed (horse gram) to 580.67 g/bed (green gram). The result for *P. eous* revealed that supplementation with bengal gram powder reduced the days to complete spawn growth and first harvest (10.33 days and 11.00 days) than control (12.33 days and 13.00 days). The maximum number of harvest was recorded in bengal gram powder (4.00 nos.) Supplementation of spawn with bengal gram powder also gave the highest yield of 613.12g/bed. Supplementation with horse gram powder delayed the days to first harvest (13.67 days) and drastically reduced the yield (458.75g/bed) (Table 4 and Fig. 4).

Effect of bed substrates on production of mushroom

Six different substrates were tested for the yielding ability of *P. djamor* by using sorghum grain spawn and the result revealed that paddy straw supported the maximum yield of 594.16 g/bed with minimum spawn running period of 13.67 days followed by maize stalk (497.96 g/bed), maize hulled cob

(473.50 g/bed) and banana leaves (440.28 g/bed). The other factor viz., shorter duration for the appearance of the sporophore (15.25 days) and maximum number of harvest (3.33 nos.) were also noticed in paddy straw while in all other substrates the appearance of sporophore was delayed by 2 to 8 days and the number of harvest was in between 2 to 3 numbers. The lowest yield (367.15 g/bed) and increased duration for colonization (20.33 days) were obtained in foxtail straw. Paddy straw registered the minimum number of days for colonization (10.33 days), initial appearance of sporophore (11.33 days) followed by maize stalk which recorded the 11.33 and 13.67 days respectively in *P. eous*. Maximum number of harvest and yield were obtained in the paddy straw (4.00 no. and 610.38 g/bed). This was followed by maize stalk with 3.00 no. and 586.85 g/bed respectively. The lowest yield of 465.02 g/bed and increased duration for colonization (18.33 days) were obtained in foxtail straw (Table 5 and Fig. 5).

Utilization of available resources by mushroom and its enzyme activities

The activity of enzymes viz., exocellulase, endocellulase and laccase gradually increased from 10 days after incubation and reached maximum on 20 days after incubation in all four species of oyster mushroom. Among the four species tested, *P. citrinopileatus* showed maximum exocellulase activity (C_1) and endocellulase activity (C_x) at 20 days after incubation (0.63, 1.53, 0.22) followed by *P. florida* (0.61, 1.17, 0.22), *P. djamor* (0.60, 1.02, 0.25) and *P. eous* (0.54, 1.19, 0.22) (Table 6.1). *P. djamor* showed maximum C_1 activity was in paddy straw after 20 days of incubation (0.66) followed by maize stalk (0.62), hulled maize cob (0.52) and banana leaves (0.51). C_x enzyme activity was found to be higher in paddy straw (1.63) followed by maize stalk (1.53) and sugarcane trash

(1.53). The activity of laccase enzyme was also more in the case of paddy straw (0.26) followed by maize stalk (0.25) and maize hulled cob (0.24). Exocellulase activity by *P. eous* was higher in paddy straw (0.55) followed by maize stalk (0.52). Maximum endocellulase activity was recorded in maize stalk (1.36) followed by paddy straw (1.31). The activity of laccase enzyme was maximum in sugarcane trash (0.24) followed by paddy straw (0.21). The activity of all the three enzymes were increased upto 20 days of incubation and thereafter was a decreasing trend. Among the substrates, paddy straw recorded maximum enzyme activity and it was low in foxtail millet straw (Table 6.2).

Post harvest parameters for *Pleurotus* sp

The different species of oyster mushroom were stored under ordinary conditions remained fresh for 72 h, 24 h, 18 h and 12 h respectively for, *P. eous*, *P. djamor*, *P. citrinopileatus* and *P. florida*. While those stored in under refrigerated condition remained fresh for 168 h, 96 h, 72 h and 48 h respectively for *P. eous*, *P. djamor*, *P. citrinopileatus* and *P. florida*. Those stored in thermocol box with ice cubes remained fresh for 96 h for *P. eous* followed by 36 h for *P. djamor*, 30 h for *P. citrinopileatus*. The least shelf life was observed in *P. florida* (24 h). Among the four species of oyster mushroom tested *P. eous* ranked first in keeping quality under different types of storage conditions. The mushrooms of *P. djamor*, *P. eous*, *P. florida* and *P. citrinopileatus* were dried in oven at 55 to 60° C and the results of dry weight were presented in the table 7. After drying, the mean weight was 10.75 g, 12.25 g, 10.00 g and 10.78 g respectively for *P. djamor*, *P. eous*, *P. florida* and *P. citrinopileatus*. The loss in weight was least (87.75 g) in *P. eous* as compared to other species viz., *P. djamor* (89.75 g), *P. florida* (90.00 g) and *P. citrinopileatus* (89.22 g). After drying, *P.*

eous showed a light brown with rose tinge discoloration, while *P. djamor*, *P. florida* and *P. citrinopileatus* showed a yellow discoloration (Table 7).

Mushroom cultivation has been upraised and much more awareness of its food values has been understood. The cultivation has been found to be increasing year by year. In India, much emphasis has been made on cultivation of temperate or white button mushroom. But greater part of the country comes under tropical region and great potential exists for cultivation of suitable tropical mushrooms. There is a vast potential for the cultivation of oyster mushroom due to its tropical conditions and ability to grow on any kind of agricultural byproducts that are rich in cellulose, hemicelluloses and lignin. Oyster mushrooms are lignicolous fungi that have capabilities to fight hunger, malnutrition and environmental pollution by producing protein rich food and recycling the substrates. *Pleurotus* spp, a well known white rot edible basidiomycete produce a wide range of extracellular enzymes to degrade complex lignocelluloses of substrates into soluble products. The present study focused on improving the yield of *Pleurotus* spp through suitable spawn materials and substrate supplementation. The initial studies were conducted to identify the suitable medium for efficient growth of mycelium. Oats meal agar medium recorded the maximum mean mycelial growth of all species (8.96 cm), followed by potato dextrose agar (8.53 cm) and rose bengal agar medium (6.82 cm).

The least growth was observed in the case of Richard's agar medium (3.97 cm) and these significantly differ from each other. Earlier, (Suharban and Nair, 1993) also reported that oats broth was the better medium for growth of *P.ostreatus* and *P.citrinopileatus*. Potato dextrose agar (PDA) and yam dextrose agar (YDA) were found to be suitable media for

the mycelium growth of oyster mushroom among the other two mediums including sweet potato dextrose agar, and malt extract agar medium. The medium were found to be suitable for *P. ostreatus* and *P. cystidiosus* (Hoa and Wang, 2015). But the oyster mushroom species of *P. djamor* and *P. oeus* showed better mycelial growth in oats meal agar medium. (Rakeshkumar and Kushwaha, 2014) reported that the maximum radial growth of mycelium on oat meal agar (OMA) medium in PL-3 and PL-1 strain. In our findings we found oats meal agar medium to be high mycelial yielding medium that could serve as alternate medium to increase yield in oyster mushroom. It is well known that the type of spawn and age of spawn influence the yield of sporophores (Sivaprakasam, 1980; Suharbanetal., 1997). The fastest growth of colonization in spawn was observed for *P. eous* followed by *P. djamor*. (Kotwaliwale *et al.*, 2007) compared different *Pleurotus* spp. and observed faster growth of *P. sajor-caju* and *P. florida* and slow growth of *P. ostreatus* in spawn bottles. On initiation, cultivation of *Pleurotus* spp, the maximum yield was obtained from *P. eous* that showed a quicker first harvest followed by *P. djamor*. Similar observation was reported by (Muthusamy *et al.*, 1995, wherein he observed *P. eousto* yield higher amount.

The yield performance of other mushroom species were reported by several workers including *P.citrinopileatus* (Sivaprakasam *et al.*, 1986), *P. sajor-caju* (Baskaran *et al.*, 1978; Sivaprakasam, 1980) *P. djamor* (Geetha, 1993) and *P. flabellatus* (Karthikeyan, 1999). (Patra and Pani, 1995) observed *P. florida* to have highest yield followed by *P. sajor-caju*, *P. citrinopileatus*, *P. sapidus* and *P. flabellatus*. (Ingale and Ramteke, 2010) tested different *Pleurotus* spp. viz., *P. sajor-caju*, *P. florida* and *P. eous* and found the highest yield for *P. eous*.

Table.1 Mycelial growth of inoculated mushroom and it's time for complete colonization

S. No.	Species	Diameter of mycelial growth (cm)*						Mean	No of days for complete colonization (days)
		Oats meal agar	Potato dextrose agar	Czapek's (Dox) agar	Rose Bengal agar	Malt extract agar	Richard's agar		
1	<i>Pleurotus djamor</i>	8.91	9.33	7.02	6.94	6.33	3.42	6.84	12.33
2	<i>Pleurotus eous</i>	9.00	13.33	6.65	7.84	6.21	3.86	6.92	9.33
3	<i>Pleurotus florida</i>	8.94	14.67	5.97	6.83	5.11	3.76	6.47	13.33
4	<i>Pleurotus citrinopileatus</i>	9.00	8.78	5.02	5.69	4.13	4.86	6.24	14.67
	Mean	8.96	8.53	6.27	6.82	5.45	3.97		0.33

*Mean of four technical replications

Table.2 Yield performance of different species of oyster mushroom

S.No.	Species	Days for completion of spawn run	First appearance of sporophore (days)*	Yield (g/bed)*	Bio-efficiency (%)
1	<i>Pleurotus djamor</i>	13.33	13.67	584.16	116.83
2	<i>Pleurotus eous</i>	10.33	11.33	603.38	120.67
3	<i>Pleurotus florida</i>	14.33	14.67	564.77	112.95
4	<i>Pleurotus citrinopileatus</i>	15.67	16.33	519.60	103.92
	CD (P=0.05)	0.43	0.39	21.85	

*Mean of four technical replications

Table.3 Effect of different substrate on spawn sporulation and yield

S. No.	Spawn substrates	<i>P. djamor</i>				<i>P. eous</i>			
		Days for the complete colonization	First appearance of sporophore (days)*	Yield (g/bed)*	Bio-efficiency (%)	Days for the complete colonization	First appearance of sporophore (days)*	Yield (g/bed)*	Bio-efficiency (%)
1.	Pearl millet	13.67	14.33	526.08	105.21	11.00	12.67	578.15	115.63
2.	Sorghum	12.00	13.33	588.16	117.63	9.33	11.33	599.38	119.87
3.	Paddy chaffy grains	15.67	17.33	520.83	104.16	12.33	13.00	575.33	115.06
4.	Finger millet	17.67	19.00	464.70	92.94	14.67	16.33	484.08	96.81
5.	Maize grains	16.33	17.67	519.12	103.82	13.00	15.00	570.77	114.15
6.	Wheat	13.00	14.00	575.67	115.13	10.33	12.00	592.12	118.42
7.	Barnyard millet	17.00	18.33	482.86	96.57	14.00	15.67	499.11	99.82
8.	Foxtail	14.33	15.00	502.72	100.54	13.67	14.33	556.15	111.23
	CD (P= 0.05)	0.58	0.45		14.09	0.29	0.49	15.52	

*Mean of four technical replications

Table.4 Effect of Pulse Powder on Spawn growth and yield potential

S. No.	Spawn substrates	<i>P. djamor</i>				<i>P. eous</i>			
		Days for the complete colonization	First appearance of sporophore (days)*	Yield (g/bed)*	Bio-efficiency (%)	Days for the complete colonization	First appearance of sporophore (days)*	Yield (g/bed)*	Bio-efficiency (%)
1.	Red gram	14.67	15.33	565.63	113.13	12.67	11.33	583.55	116.71
2.	Black gram	15.33	16.00	427.3	85.46	13.67	14.00	494.78	98.96
3.	Bengal gram	13.33	14.33	593.75	118.75	10.33	11.00	613.12	122.62
4.	Green gram	14.00	15.67	580.67	116.13	11.00	12.33	608.03	121.61
5.	Horse gram	16.33	17.67	393.5	78.77	13.67	14.33	458.75	91.72
6.	Cowpea	16.00	17.33	490.61	98.12	13.33	15.00	527.67	105.53
7.	Soyabean	15.00	17.00	551.60	110.32	13.00	14.67	577.54	115.51
	CD (P= 0.05)	0.47	0.43	16.64		0.36	0.29	15.27	

*Mean of four technical replications

Table.5 Effect of different bed substrates on mushroom

S. No.	Bed substrates	<i>P. djamor</i>				<i>P. eous</i>			
		Days for the complete colonization	First appearance of sporophore (days)*	Yield (g/bed)*	Bio-efficiency (%)	Days for the complete colonization	First appearance of sporophore (days)*	Yield (g/bed)*	Bio-efficiency (%)
1.	Sugarcane trash	19.67	21.33	404.82	80.96	18.67	21.00	495.53	99.11
2.	Maize stalk	15.33	18.33	497.96	99.59	11.33	13.67	586.85	117.37
3.	Banana leaves	18.67	20.33	440.28	88.05	14.33	16.67	503.64	100.73
4.	Paddy straw	13.67	15.25	594.16	118.83	10.33	11.33	610.38	122.08
5.	Maize shelled cob	17.33	19.67	473.50	94.7	12.67	15.00	520.34	104.04
6.	Foxtail straw	20.33	22.67	367.15	73.43	18.33	21.33	465.02	93.00
	CD (P= 0.05)	0.53	0.33	19.82		0.52	0.39	18.91	

*Mean of four technical replications

Table.6.1 *In vitro* activity of exocellulase (C₁), endocellulase (C_x) and laccase

S.No	<i>Pleurotus</i> spp.	C ₁			C _x			Laccase		
		Days after incubation			Days after incubation			Days after incubation		
		10	15	20	10	15	20	10	15	20
1	<i>P.djamor</i>	0.49	0.55	0.60	0.99	1.00	1.02	0.16	0.20	0.25
2	<i>P.euos</i>	0.47	0.50	0.54	1.10	1.15	1.19	0.16	0.21	0.22
3	<i>P.florida</i>	0.46	0.52	0.61	1.05	1.14	1.17	0.15	0.19	0.22
4	<i>P.citrinopileatus</i>	0.53	0.61	0.63	1.15	1.50	1.53	0.17	0.23	0.22
	CD (P=0.05)	0.01	0.02	0.02	0.02	0.03	0.05	0.01	0.02	0.01

*Mean of four technical replications

C₁ - Exocellulase

C_x - Endocellulase

C₁ and C_x – mg of sugar released per 5gm of substrate

Laccase – change in absorbance of 0.01 per minute

Table.6.2 Exocellulase (C₁), endocellulase (C_x) and laccase activity in different substrates

S. No	Substrates	<i>P. djamor</i>									<i>P. eos</i>								
		C ₁ Days after incubation			C _x Days after incubation			Laccase Days after incubation			C ₁ Days after incubation			C _x Days after incubation			Laccase Days after incubation		
		10	20	30	10	20	30	10	20	30	10	20	30	10	20	30	10	20	30
1	Paddy straw	0.61	0.66	0.64	1.22	1.63	1.31	0.27	0.26	0.19	0.47	0.55	0.52	1.17	1.31	1.21	0.24	0.21	0.17
2	Maize stalk	0.57	0.62	0.60	1.17	1.53	1.21	0.24	0.25	0.18	0.46	0.52	0.48	1.28	1.36	1.24	0.17	0.19	0.12
3	Sugarcane trash	0.33	0.41	0.28	1.07	1.53	1.21	0.17	0.19	0.18	0.28	0.37	0.26	1.06	1.17	1.12	0.22	0.24	0.19
4	Banana leaves	0.47	0.51	0.48	1.20	1.37	1.24	0.18	0.20	0.17	0.44	0.48	0.45	1.08	1.18	1.20	0.17	0.19	0.12
5	Maize shelled cob	0.46	0.52	0.48	1.04	1.14	1.00	0.22	0.24	0.18	0.45	0.49	0.46	1.17	1.29	1.18	0.21	0.20	0.16
6	Foxtail straw	0.31	0.37	0.27	1.27	1.34	1.22	0.16	0.18	0.13	0.22	0.26	0.15	1.14	1.16	1.11	0.12	0.14	0.11
CD (P=0.05)		0.15	0.16	0.15	0.39	0.41	0.36	0.19	0.16	0.04	0.11	0.13	0.12	0.42	0.41	0.37	0.16	0.14	0.01

*Mean of four technical replications, C₁ - Exocellulase, C_x - Endocellulase, C₁ and C_x - mg of sugar released per 5gm of substrate, Laccase - change in absorbance of 0.01 per minute

Table.7 Post harvest technology for mushroom

S. No	<i>Pleurotus</i> spp.	Keeping quality			Dehydration			
		Ordinary Condition (h)	Under refrigerated condition (h)	Packing in thermocol boxes with ice cubes (h)	Weight of fresh mushroom (g)	Mean dry weight, after drying (g)	Loss in weight (g)	Change in colour
1	<i>P.djamor</i>	24	96	36	100	10.78	89.22	Yellowing
2	<i>P.euos</i>	72	168	96	100	12.25	87.75	Light browning with rose tinge
3	<i>P.florida</i>	12	48	24	100	10.75	89.75	Yellowing
4	<i>P.citrinopileatus</i>	18	72	30	100	10.00	90.00	Yellowing

*Mean of four technical replications

Fig.1 Mycelial growth on nutrient growth mediums

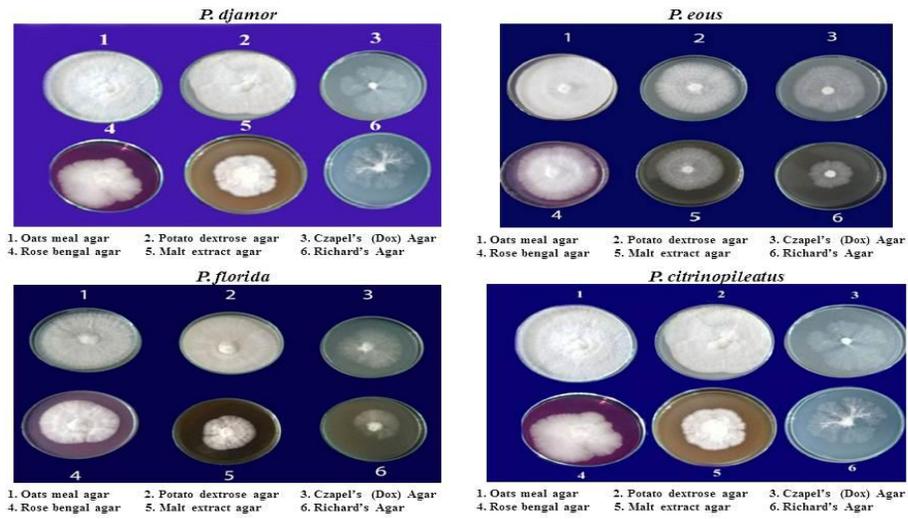


Fig.2 Yield performance of *Pleurotus spp* using natural substrates

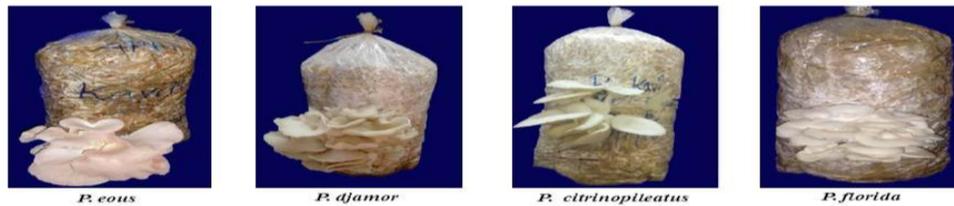
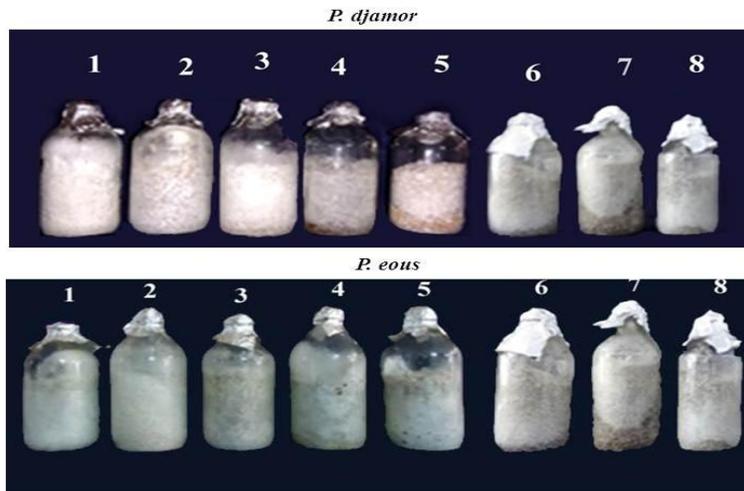


Fig.3 Effect of different spawn substrates on complete colonization by *P. djamor* and *P. eous*



1.Pearl millet 2. Sorghum 3. Paddy chaffy grain 4.Finger millet5. Maize 6.Wheat 7. Barnyard millet 8. Foxtail millet

Fig.4 Effect of pulse powder on spawn growth and yield of *P.djamor* and *P.eous*

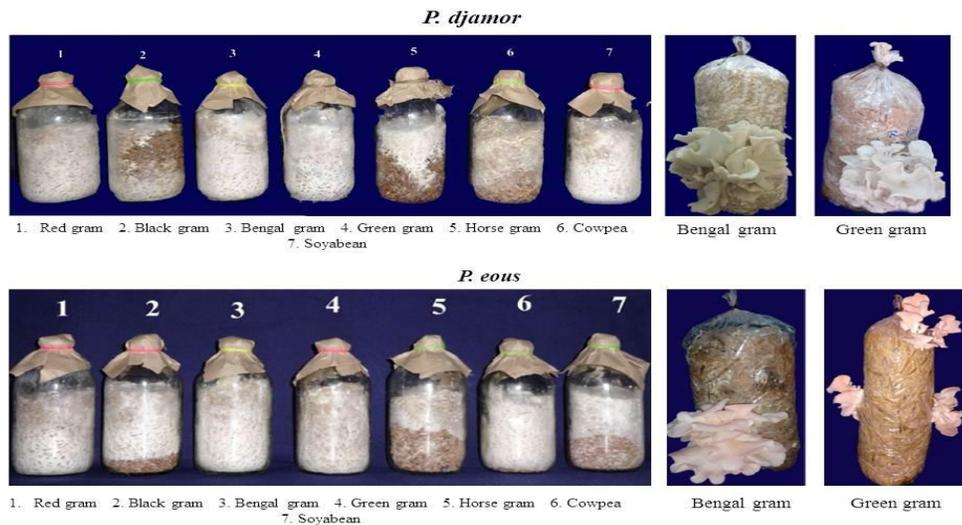


Fig.5 Effect of bed substrate on production of mushroom



In mushroom culture, spawn is comparable to seed in crop plants and the yield of mushroom depends on the type of spawn used. In the present study, sorghum grain was found to be the best preferred substrate for spawn making as the spawn growth was the fastest with highest yield of mushroom that was followed by wheat grains. Earlier reports indicated that the spawn of mushrooms can be easily prepared on a variety of grains such as wheat, bajra, jowar and maize with equal advantage

(Sivaprakasam 1980; Karthikeyan, 1999). Sorghum grain was the best substrate for spawn production due to its spherical shape and its compactness. It provides larger surface area for colonization of the mushroom mycelium and is rich in nutrients (Pakela and Eicker, 1997). Considering the above points, sorghum grain was found to be the best substrate for quality spawn production in terms of earliness in growth of spawn, total yield and number of crops and wheat grain

would definitely proved to be profitable for commercial spawn production. (Rehana *et al.*, 2007) showed that sorghum grain was the best substrate as compared to wheat and oat for mycelial growth of *P.sajor-caju*. Full mycelial growth was obtained in 7.8 days on sorghum grains which was followed by wheat grains which took 11.8 days for full mycelial growth and finally oat grains which took 13.1 days for full mycelial growth. (Khare *et al.*, 2010) reported that the wheat, barley, sorghum and millet grains could be equally used in the production of good quality spawn for the cultivation of oyster mushrooms. (Jiskani *et al.*, 2011) also grown spawn on sorghum grains and reported near about similar results. Thulasi *et al.*, (2012) reported sorghum grains, paddy grains and chopped areca nut leaf sheath supported *P. eous* for 100% colonization in 10, 16 and 10 days respectively. Poonam and Deepak, (2015) reported that wheat grains spawn are best for cultivation of *P sajor-caju* followed by sorghum grain spawn.

The yield of the sporophore of *Pleurotus* spp. depends on the nature of substrate used as bedding material (Zadrazil, 1978). In the present study, paddy straw as substrate recorded the maximum yield and earliness to first harvest followed by maize stalk and maize shelled cob in *P.djamor* and *P.eous*. Superiority of paddy straw for the cultivation of *Pleurotus* had also been reported by many workers (Bano *et al.*, 1993; Sivaprakasam, 1980; Nallathambi, 1991; Krishnamoorthy *et al.*, 2006; Ashrafi *et al.*, 2014). Similarly (Eyini *et al.*, 1995) reported higher yield of *P.ostreatus* in paddy straw pretreated with lime. The use of different combinations of wheat straw with other substrates enhanced the yield of *P. florida* (Jain and Vyas, 2002). Randive (2012) reported the development of both grey and pink oyster mushroom production to be highest paddy straw and wheat straw substrates. Patel (2012) reported

the yield, biological efficiency (B.E.) of the *P. sajor-caju* cultivated on different agro waste showing maximum yield on paddy straw (836.66 gm/kg straw) with 83.66 % B.E., while the least was recorded with Pigeon pea stalk (716.33 gm/kg straw) respectively. The maximum yield of *P.florida* was obtained when it was cultivated on soybean straw (875.66gm/kg straw) with 87.56% B.E., this was followed by yield on soybean + paddy straw (852.00gm/kg straw) with 85.20% B.E. (Syed Abrar Ahmed *et al.*, 2009). Yang *et al* (2013) reported that the wheat straw and rice straw gave the faster mycelia growth rate, time to primordial formation, time to first crop and gave high yield. The maximum numbers of fruit bodies with large diameter (9 cm) of pileus were found in case of combined substrates of rice and wheat straws. (Borkar *et al.*, 2014) found maximum biological efficiency of *P.pulmonarius* on paddy straw (76.305) followed by wheat straw (74.53%). (Patel and Trivedi, 2016) revealed that the maximum yield of mushroom under paddy straw with biological efficiency of 85.9%. 183.1 g, 151.8 g, 111.5 g, 87.8 g, 49.5 g, 23.5 g and 13.0 g of yield were observed for rice straw; maize stalk, corn husk, rice husk, fresh sawdust and elephant grass respectively (Obodai *et al.*, 2012).

In terms of using pulse powder for spawn production, the study revealed an increased yield of *Pleurotus* spp. Supplementation with bengal gram and green gram flour decreased the days required for spawn growth and first harvest and also increased the yield of *P. djamor* and *P. eous*. (Kumar *et al.*, 1997) obtained maximum yield with bengal gram powder and wheat bran at 100g/kg with 155% biological efficiency in *P. sajorcaju*. (Kathe *et al.*, 1996) found soyabean flour at three per cent concentration to be better than other nutrients with increased yield of *Pleurotus* spp. significantly.

Enzyme activity

Enzyme plays an key role in degradation of most of agro-byproducts by utilizing its nutrients. Cellulase and laccase are key enzyme for efficient degradation of cellulose and ligning respectively present in those substrates. The amount of enzyme produced while mushroom cultivation has direct positive correlation to that of the nutrient uptake from the byproducts and its degradation. The present study revealed that exocellulase (C₁) and endocellulase (C_x) activity was higher in paddy straw after 20 days of incubation. Similar trend were observed in different substrate including maize stalk, maize shelled cob, banana leaves, sugarcane trash and foxtail straw.

The maximum activity was found in paddy straw followed by maize stalk. Higher activities of the cellulase enzymes complex were exhibited during cultivation of *P.ostreatus* (Sharma *et al.*, 1999).(Singh *et al.*, 2003) observed the highest cellulase production during the fruiting phase of the *Pleurotus* spp. Cellulase activity of 0.607 U/ml and of 0.490 U/ml were observed in *P.ostreatus* and *Calocybe indica* respectively (Karthikeyan, 2015).The highest cellulase activity was obtained in mixture of substrate consisting rice bran, rice husk and sodium nitrate in button mushroom *Leucoagaricus meleagris* (Santhaya *et al.*, 2014).

In the present study, it was found that laccase activity reached maximum during initial phase while decreased after 20 days of incubation in paddy straw. Similar observations were observed in *P.ostreatus* showing 0.015 U/g of laccase activity during initial phase with increased production of biomass followed by decline (Navarro *et al.*, 1998). Ghosh and Nandi (1995) found laccase activity of *P. ostreatus* and *P. sajor- caju* to

be maximum in 24 days with water hyacinth as substrate. *In vitro* and *in vivo* activities of exo and endocellulases reached maximum after 20 days of inoculation with *P. djamor* (Geetha and Sivaprakasam, 1998). The maximum activity of C₁ and C_x enzyme was observed in paddy straw inoculated with *P. djamor* (Ravichandran, 2001). Sebnem (2010) experimental results also showed increased laccase levels with the increased nitrogen contents. Maximum laccase activity was exhibited by *P. djamor* in paddy straw substrate at 10 days after inoculation (Ravichandran, 2001). Similarly *Volvariella volvacea*, strain V14, produced multiple forms of extracellular laccase on the 16th day, when grown in submerged culture with glucose as sole carbon source with cotton waste (Shicheng *et al.*, 2004). In *Pleurotus* spp, assay of fungal laccase activity showed maximum activity on the 19th day (Sunil *et al.*, 2011).

The postharvest technology remains one of the major importance since it has to be dried as earliest and can be stored without any colour change for longer durations. The dehydrated mushrooms were found to be acceptable even after a storage period of six months, as was also reported by (Riaz *et al.*, 1991). The mushrooms of *P. djamor* and *P. eous*, were dried in an oven at 55 to 60° C. After drying, the mean weight was 10.25 g and 11.25 g respectively. (Pal and Chakraverty, 1997) found reduction of about 40 % in the process time, when the drying temperature of *P. ostreatus* fruiting bodies was increased from 45 to 60 °C. An increase in the drying speed with drying temperature was when *A. bisporus* and *P. florida* were dried. When the species were subjected to a temperature of 40 °C, it showed 43.7 % reduction time and at 60 °C it showed reduction of 28.6 % (Apati *et al.*, 2010). Similar results have been found by (Arora *et al.*, 2003; Krokida *et al.*, 2003).

The mushrooms are highly perishable as these start deteriorating within a few hours after harvest, due to enzymatic action. Therefore, proper processing and preservation of mushrooms are of vital importance. (Minamide et al., 1981) reported that the shelf life of fresh oyster mushroom could be prolonged four times when these were stored in an atmosphere of 40 per cent carbon dioxide and one to two per cent oxygen at 20° C. In the present investigation storage of fresh mushrooms in refrigeration, thermocol boxes with ice cubes and ordinary condition were tried for increasing the shelf life of mushrooms *P. djamor* and *P. eous*. From these, *P.eous* ranked first in keeping quality under different types of storage conditions which offers great scope for promotion in the marketing potentialities. The oyster mushroom packed in polyethylene terephthalate box with aluminium foil and banana leaves stored under refrigeration maintained shelf-life with respect to colour (L* value, 64.23), PLW (5.54%), hardness (149.7gf), nutritional parameters of protein (25.83%), fat (1.94%), fibre (9.31%), ash (10.35%) and organoleptic score (7/9) and microbial load (4.19×10³cfu/g) up to 16 days storage (Avinash, 2012). To ensure high quality mushrooms in the market place with enhanced shelf-life, these must be cooled as quickly as possible after picking and kept cool throughout the cold chain (Rai et al., 2003). (Cano-Chauca et al., 2002) also reported that cutting force, a measure of hardness, increased as drying of banana progressed. The shelf life of oyster mushroom was about 8-11 days at 0 °C, about 4-6 days at 5 °C, about 2-3 days at 10 °C and about 1-2 days at 20 °C. During storage, film packaging prevented or retarded the deterioration of mushroom appearance, texture and discoloration (Choi et al., 2003). They attributed this to increase in concentration of other components with moisture removal during drying. Similar results were reported by (Argyropoulos et al.,

2008) and (Arumuganathan et al., 2009) on drying of button mushroom.

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