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RESEARCH ARTICLE

Conversion of Agricultural Residues into Protein Biomass by Milky Mushroom Fungus *Calocybe Indica var.* Apk2 Through Solid State Fermentation

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ABSTRACT

Experiments were conducted to estimate the morphogenesis related enzymes, yield parameters, and yield of the milky mushroom fungus *Calocybe indica* utilizing different growth substrates viz., paddy straw, sorghum stalks, sugarcane baggase, maize stalks, soybean hay, blackgram hay, ground nut halums, saw dust, paddy straw compost, and coir pith compost. Palmorasa and vetiver grasses were also used along with all other substrates mentioned by enzyme production technology, Solid State Fermentation (SSF) for the fruiting body production and biodegradation of phytoconstituents. Among the substrates tested, a higher level of endocellulase (2.40%) and exocellulase (1.01%) production was observed in paddy straw, followed by sorghum stalks. Laccase (1.10%) and poly phenol oxidase (0.037%) activity showed increased levels in blackgram hay followed by maize stalks and coir pith compost, respectively. Among the substrates used for yield estimation, the Paddy straw and maize stalks gave significantly higher yields (356.5 and 354.3 g per bed, respectively) followed by sorghum stalks and vetiver grass. The high level of protein biomass was calculated in Paddy straw (2.929g/500g of substrate) followed by maize stalks (2.911 g/500g of substrate).

Key words: *Mushroom; Calocybe indica var. APK2; SSF; Biodegradation; Yield estimation; Enzymes*

INTRODUCTION

Calocybe indica P & C is native to India and was first reported by Purkayastha and Chandra (1974). The technology for commercial cultivation and the variety APK2 has been first introduced from Tamil Nadu Agricultural University, Coimbatore, India (Krishnamoorthy et al., 1998). Mushroom culture offers an excellent means for recycling agro wastes presently available in the country (Sohi, 1988a). Alam et al, (2010) have used 30% maize powder to supplement paddy straw substrate to increase mushroom yields. More promisingly, supplements like soybean and cottonseed cake gave the highest absolute mushroom yields (64.8% and 59.2% increased biological efficiency over control). Converting lignocellulosic agricultural and forest residues into protein-rich mushrooms is one of the most economically viable and sustainable biotechnology processes to address world food demand, especially protein demand (Hawksworth, 1991). The addition of rice bran to lignocellulosic substrates increased the production of soluble proteins, the enzyme activity, and the productivity of *P. ostreatus* (Luz *et al.,* 2012). The present study was undertaken to estimate the morphogenesis related enzymes, yield parameters, and yield of milky mushroom fungus *C. indica* were in different growth substrates.

MATERIALS AND METHODS

Suitability of different substrates for enzyme production and bed preparation of paddy straw, sorghum stalks, maize stalks, ragi straw, pearl millet straw, sugarcane bagasse, groundnut haulms, soybean hay, blackgram hay, and paddy straw compost were used as substrates for mushroom cultivation. They were sun-dried and chopped into one or two cm bits (except sawdust and composts) and filled in empty glucose drip bottles at the rate of 100 g per bottle. The substrates were added with 200 mL of water and soaked for 4 h. After draining excess water, the substrates were sterilized in an autoclave at 1.5 kg.cm^2. The mycelial discs of 8 mm diameter of three discs per bottle. Uninooculated control, along with suitable replications, were maintained. For bed preparation, all other substrates including, palmarosa grass vetiver grass, were soaked in water for 4 h, except paddy straw compost. After draining excess water, the materials were treated in hot water (80°C) for 60 min. Paddy straw compost was prepared following a long method of composting

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based on IIHR (1986) formula. Beds were prepared following "polybag method" described by Baskaran *et al*. (1978), using different growth substrates. The and poly bags were incubated at room temperature 30 ± 2°C, and the mycelial growth was measured at 5, 10, 15, 20, and 25 days intervals.

Extraction of enzymes

The enzyme extracted was estimated by the following method of Maxwell and Bateman, 1967.

Assay of endo and Exocellulases

Dinitro salicylic acid (DNS) method was used for determining the activity of endocellulase and exocellulase. By measuring the reducing sugar as glucose, the enzyme activity was estimated (Miller, 1972).

Assay of laccase in vitro

Assay of laccase was carried out as per the method suggested by Frochner and Eriksson (1974). But the carbon source was substituted with sawdust and sources (2:1 w/w) in the medium.

Assay of Polyphenol Oxidase Activity

Polyphenol oxidase activity was determined by the method described by Sadasivam and Manickam (1992) using catechol as substrate at 495 nm. Changes in OD followed at 30 sec. interval was recorded and the enzyme activity was expressed as units/ml of the extract.

Estimation of total nitrogen in substrates: Total nitrogen content of the samples was estimated by 'Kjeldahl Method" (Piper, 1966).

RESULTS AND DISCUSSION

C. indica produced cellulases, laccases, and polyphenol oxidases during their growth on substrates. The activity of all these enzymes was found to increase on 25th d after inoculation.

Table 1. In-vivo estimation of morphogenesis related enzymes and yield parameters of C.indica APK2

Mean of Enzyme activities /5 days interval					**Yield performance of *C. indica* on different growth substrates**				
Different growth substrates	Exo cellulase	Endo cellulase	Laccase	Polyphenol oxidase	Different growth substrates	Nitrogen content of growth substratesin %	Yield (g/500 g of the substrate)	Bioefficiency / 500 g of substrate (%)	Protein Biomass (g/500g of substrate)
Paddy Straw	1.01	2.40	0.97	0.021	Paddy Straw	1.20	356.5	142.6	2.929
Sorghum stalks	0.90	2.35	0.80	0.027	Sorghum stalks	0.80	325.8	130.3	2.688
Maize straw	1.00	2.28	0.91	0.024	Maize straw	1.30	354.3	141.7	2.911
Sugarcane bagasse	0.84	2.15	0.75	0.018	Sugarcane bagasse	1.70	278.0	111.2	2.290
Soybean hay	0.71	1.90	1.04	0.033	Palmorasa grass	0.70	246.8	98.7	2.030
Blackgram hay	0.66	1.92	1.10	0.037	Vetiver grass	0.75	305.0	122.0	2.510
Groundnut haulms	0.64	1.84	.94	0.037	Groundnut haulms	1.60	235.3	94.1	1.941
Saw dust	0.47	1.84	0.50	0.046	Saw dust	1.50	237.8	95.1	1.962
Coir pith compost	0.54	1.41	0.26	0.035	Coir pith compost	1.50	205.0	82.0	1.691
Paddy straw compost	0.53	1.12	0.60	0.020	Paddy straw compost	1.12	118.0	47.2	0.973

Among the substrates tested, a higher level of endocellulase (2.40%) and exocellulase (1.01%) production was observed in paddy straw, followed by sorghum stalks. Laccase (1.10%) and poly phenol oxidase (0.037%) activity showed increased levels in blackgram hay followed by maize stalks and coir pith compost, respectively. Doshi *et al.*, (1987) estimated endocellulases in spawn bottles of C.*indica* and found its maximum activity on 29th day, almost the time of button formation. Matsumoto (1998) found that the cellulase and xylanase activities increased during the development of the fruiting bodies, with the highest levels during mushroom maturation. The increase in the enzyme activities during fructification may be due to the fungus's need to mobilize large amounts of carbon for mushroom formation.

The present study proved that the Paddy straw and maize stalks gave significantly higher yields (356.5 and 354.3 g per bed, respectively) followed by sorghum stalks and vetiver grass.

The high level of protein biomass was calculated in Paddy straw (2.929g/500g of the substrate) followed by maize stalks(2.911 g/500g of the substrate). Bioefficiency shows that the lignocellulolytic enzyme activity is dependent on the composition of

1. Paddy Straw
2. Sorghum stalks haulms
3. Maize straw
4. Sugarcane bagasse compost
5. Palmorasa grass compost
6. Vetiver grass
7. Groundnut
8. Saw dust
9. Coirpith
10. Paddy straw

Figure 1. Growth of mushroom on various substrates

the substrate and on the C/N ratio, as reported by Kahraman and Gurdal (2002). Purkayastha (1984) used chopped rice straw, presoaked for 18 to 24 h in water and put in hot water for 2-3 h. Vijaykumar *et al.* (2013) proved that wheat straw substrate as the best substrate for the cultivation of *C. indica* followed by paddy straw. The increase in the biological efficiency for substrates supplemented with rice bran was verified when *Pleurotus* and *L. edodes* were cultivated on different agricultural wastes, such as eucalyptus sawdust and bark, corncobs, coffee husks, and sugar cane (Ribeiro, 2009).

CONCLUSION

The present study was carried out to estimate the morphogenesis related enzymes by using different growth substrates *viz*., paddy straw, sorghum stalks, sugarcane baggase, maize stalks, soybean hay, blackgram hay, ground nut halums, saw dust, paddy straw compost, and coir pith compost. Among the substrates, the increased level of morphogenesis related enzymes and better growth of C.indica was observed in paddy straw. The higher level of endocellulase (2.40%) and exocellulase (1.01%) production was observed in paddy straw, followed by sorghum stalks. Laccase (1.10%) and poly phenol oxidase (0.037%) activity showed increased levels in blackgram hay followed by maize stalks and coir pith compost, respectively. The present study proved that the paddy straw and maize stalks gave significantly higher yields when compare to all the substrates.

Further, research is warranted to analyse the role of each and every enzymes in the growth of milky mushroom at different growth stages.

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