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Comparative evaluation of different *Pleurotus* spp in selected ligno-cellulosic residues from agricultural and horticultural ecosystems

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Abstract
Converting lingo-cellulosic crop residues into protein rich mushrooms is one of the most economically viable and sustainable biotechnology processes to address the growing demand for food and quality protein. In the present investigation, the large scale availability of lignocellulosic residues in wetland, garden land and horticultural ecosystems of three different substrates *viz.*, paddy straw, maize cobs and shredded are canut leaf sheath have been evaluated for the cultivation of different species of Pleurotus *viz*., *Hypsizygus ulmarius* var. CO (OM) 2, *Pleurotus eous* var. APK1, *P.florida* (Pf), *P.pulmonarius*, *P. platypus* and *Pleurotus florida* in the polybag size 35x25cm under homogenized cropping system. The results revealed that, paddy straw was more suitable for the cultivation of tested species of Pleurotus followed by shredded arecanut leaf sheath. Among the species tested, *P.platypus* recorded the maximum yield of 319.4, 478.9 and 376.4g in paddy straw, maize cob and shredded arecanut leaf sheath substrates, respectively with an average bioefficiency of 127.9, 119.5 and 125.4 per cent, respectively. Whereas, *P.florida* recorded higher yield (345.4g) in areca nut leaf sheath followed by its better performance in maize cobs.

Keywords: *Pleurotus* spp, Paddy straw, Maize cobs and Shredded arecanut leaf sheath, Yield.

Introduction
India is blessed with different agro climate, abundant agricultural residues and manpower, making it the most suitable for the cultivation of all the types of temperate, subtropical and tropical mushrooms. Most of the lignocellulosic agricultural residues constitute abundant natural resources that can be used as staple animal feed for the ruminants and as raw material for the production of industrial chemicals, bio-fuels and protein rich food. Biodegradation of these lignocellulosic substances by fungi makes an attractive approach to meet energy and food demands of growing population (Chang, 2006) [3]. In India, about 1150 million t of crop residues become available every year, which includes cereals, millets, fibre and oilseeds crop residues; rice straw; maize stover; horticultural crops residues like banana, coconut residues and areca nut residues (MoA Report, 2012) [7]. Composting of these organic wastes by mushroom fungi is one of the safest methods to eliminate polluting xenobiotic wastes (Ahlawat and Indu Rani, 2003 and Krishnamoorthy *et al*., 2005) [2, 4].
Mushroom cultivation has developed into a profitable industry in many countries of the world. Mushrooms offer vast rural employment potential. Oyster mushrooms (Pleurotus species) are the second most cultivated mushrooms after white button. They are the easiest and least expensive commercial mushrooms to grow because they convert crop residues into food protein. The ligno-cellulosic substrates used for the cultivation of Pleurotus species are coffee pulp, cotton seed, sugarcane bagasse, wheat straw, wheat husk, rice bran, gram husk, cotton seed hulls, cassava peels, paddy straw, saw dust, agricultural waste, corncobs, water hyacinth, water lily bean, oil-palm fiber, paper and cardboard (Marimuthu *et al*., 2011) [6] etc,. Pleurotus species is one of the most efficient ligno-celluloses decomposing types of white rot fungi. Mycelium can produce a group of complex extra-cellular enzymes which can degrade wastes materials and helps in reducing pollution. Keeping all these background information, present study the evaluation of selected ligno-cellulosic residues from wetland, garden land and horticultural ecosystems for the cultivation of different oyster mushroom species.

Materials and Methods
Collection of Pleurotus Cultures
Cultures of different species and varieties of oyster mushroom fungi *viz*., *Hypsizygus ulmarius* var. CO (OM) 2, *Pleurotus eous* var. APK1, *P.florida* (Pf), *P.pulmonarius*, *P. platypus* and

Pleurotus florida used in the study were collected from Mushroom Research Laboratory, Tamil Nadu Agricultural University, Coimbatore; Directorate of Mushroom Research, ICAR, Solan; Indian Institute of Horticultural Research, Bangaluru; and Haryana Agro Industries Corporation, Murthal for making comparison of their commercial potential.

Isolation and Maintenance of Pure Cultures

A well-developed sporophore was selected and cut into two equal halves. A small piece of mushroom tissue was removed with a sterile forceps exactly form the center of junction of pileus and stipe from the half cut mushroom. The tissue bit was then sterilized with 70 per cent ethanol for 30 sec, washed thrice serially in sterile distilled water, air dried near a flame to remove excess moisture and placed aseptically into Petri dishes containing PDA or MA medium. The dishes were incubated at room temperature (25 ±2º C) for seven d. Following single hyphal tip method (Rangaswamy, 1972) pure cultures were transferred to PDA or MA slants and maintained at 25 ±2º C for further studies.

Spawn Production

Pure cultures maintained in PDA medium were used for the preparation of sorghum grain spawn. Cleaned grains were thoroughly washed and soaked in water for 30 min and half cooked in an open vessel for 20 min. After draining the excess water, the grains were mixed with calcium carbonate at the rate of 20 g per kg of grains (dry weight), filled in autoclavable polypropylene bags (25 x 10 cm size) and sterilized at 1.42 kg cm^{-2} pressure in an autoclave for 1.5 h. After cooling, the bags were aseptically inoculated with the pure cultures of the respective mushroom fungus, incubated at room temperature (25 ±2^{0}C) for 15 d and used for spawning the substrate.

Substrates and Pretreatment Methods

Paddy straw, paddy chaff, maize stalks, maize cobs and arecanut leaf sheath substrate were used in various experiments. Except arecanut leaf sheath and spent substrate all other substrates were cut into small pieces of 3-5 cm and pre-treated following hot water treatment (Sivaprakasam, 1980) [10], steaming (Zadrazil, 1978) [15] and chemical substrate treatment (Vijay and Sohi, 1987) [14] methods. Partially sterilized substrates, thus prepared were shade dried until the moisture content was 60 per cent and used for spawning.

Polybag containers

Polypropylene bags size of 35 x 25 (100 G thickness) were used as containers for the preparation of cylindrical mushroom beds with layer spawning or thorough spawning following the procedure suggested by Sivaprakasam and kandasamy (1982). For each bag 300 g of substrate (dry weight basis), respectively were used. Fifteen d old sorghum grain spawn was used to seed the substrate at the rate of 2 per cent (dry weight basis). The beds after spawning were provided with 4 - 8 holes of one cm dia. depending upon the size of the bed for air circulation.

Lignocellulosic residues

Based on the large scale availability of lignocellulosic residues in wetland, garden land and horticultural ecosystems. Three different substrates *viz.*, paddy straw, maize cob and shredded arecanut leaf sheath have been selected for the cultivation of different *Pleurotus* spp.

Statistical Analysis

The statistical analyses of all the experiments conducted were laid out based on Completely Randomized Block Design (CRBD) (Gomez and Gomez, 1984). Statistical software AGRES (Developed by Dept. of Physical Science, TNAU, Coimbatore) was used for the analyses of the data. The determination of antioxidant properties and enzyme assays were carried out in triplicate and the results presented are mean values ± standard deviations. In case of zero values the data was log transformed (X+0.5) before statistical analysis.

Result and Discussion

Evaluation of Crop Residues for Oyster Mushroom Cultivation

Based on the large scale availability of lignocellulosic residues in wetland, garden land and horticultural ecosystems, three different substrates *viz.*, paddy straw, maize cobs and shredded arecanut leaf sheath have been evaluated for the cultivation of different species of Pleurotus in the polybag size 35x25cm under homogenized cropping system (Table 1). The results revealed that, paddy straw was more suitable for the cultivation of tested species of Pleurotus followed by shredded arecanut leaf sheath. Among the species tested, *P.platypus* recorded the maximum yield of 319.4, 478.9 and 376.4g in paddy straw, maize cob and shredded arecanut leaf sheath substrates, respectively with an average bioefficiency of 127.9, 119.5 and 125.4 per cent, respectively. Whereas, *P.florida* recorded higher yield (345.4g) in arecanut leaf sheath followed by its better performance in maize cobs (Table 1).

Table 1: Comparative evaluation of different *Pleurotus* spp in selected ligno-cellulosic residues

Container: PP bag (35x 25 cm)

Substrates	Substrate quantity on dry weight basis (g)	*H.ulmarius* (CO(OM)2)		*P.eous* (APK1)		*P. platypus* (Pp)		*P. florida* (Pf)		*P.pulmonarius* (Ppl)	
		Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)	Yield (g)	BE (%)
Paddy straw	250	296.83	118.73	263.66	105.07	319.49	127.79	261.16	104.46	280.33	112.13
Maize cob	400	403.66	100.66	402.99	100.75	478.99	119.54	445.66	111.41	417.3	104.32
Shredded arecanut leaf sheath	300	310.6	103.53	302.4	100.8	376.4	125.46	345.4	115.13	322.8	107.6
CD (p= 0.05)		21.23		20.48		21.23		22.38		21.52	

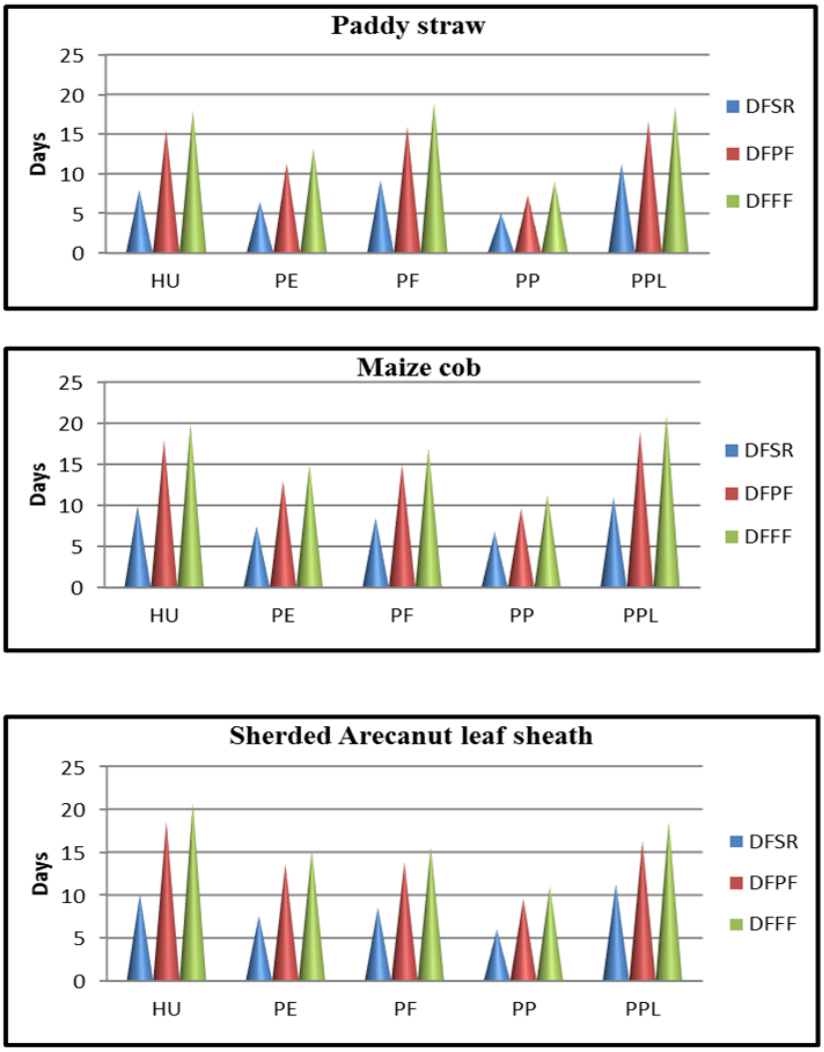
*Mean of five replications, containing six bags each

Performance Evaluation of Crop Residues

The yield of sporophores largely depended upon the nature of the bedding material used (Zadrazil, 1978) [15]. In the present study, based on the large scale availability of lignocellulosic

residues in wetland, garden land and horticultural ecosystems, three different substrates *viz.*, paddy straw, maize cobs and shredded arecanut leaf sheath have been evaluated for the cultivation of different *Pleurotus* spp. Among the substrates, paddy straw was found to be more suitable for the cultivation of Pleurotus species followed by shredded arecanut leaf sheath. *P.platypus* recorded the maximum yield of 319.4, 478.9 and 376.4g in paddy straw, maize cob and shredded arecanut leaf sheath substrates, respectively. Jandaik and Kapoor (1974) reported the cultivation of *P. sajor caju* for the first time on maize cobs and paddy straw substrates. Superiority of paddy straw for the cultivation of Pleurotus had also been reported by many workers (Bano *et al.*, 1978; Sivaprakasam, 1980; Nallathambi, 1991; Krishnamoorthy *et al*., 2006; Ashrafi *et al*. 2013; Senthilmurugan, 2016) [10, 8, 5, 1, 13]. According to Nallathambi and Marimuthu (1993) [9] paddy straw substrate gave the maximum yield of Pleurotus species when compared to wheat straw. In the present study, *P.florida* recorded higher yield (345.4g with 115 per cent bioefficiency) in shredded arecanut leaf sheath followed by maize cobs. Similar type of results were observed by Chandramohanan and Moorthy (1991), who confirmed that *P. sajor-caju* cultivated on shredded arecanut leaf sheath recorded the maximum yield of 875g / bag measuring the size 55 x 40 cm. Paddy straw is considered as the best substrate for cultivating *Pleurotus* spp in terms of yield. However, more cheap and alternate substrates for mushroom cultivation have to be identified, because the cost of paddy straw is increasing day by day. Shredded arecanut leaf sheath is one such cheap and either to unutilized source available in plenty (3800 kg / ha arecanut plantation) in India. Considering the bulk density of the substrate and container as prime declining factors, PP bag size 35x25 cm would be more convenient for the cultivation of different *Pleurotus* spp.

Fig.1. Comparison of spawn run and flushing patterns of *Pleurotus* spp cultivated in different ligno-cellulosic residues



DFSR -Days for spawn run

DFPF - Days for pin head formation

DFFF – Days for first flush

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