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Evaluation of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleurotus sajor-caju* (Fr.) Singer

G.V. Thomas,* S.R. Prabhu, M.Z. Reeny and B.M. Bopaiah

The lignocellulosic biomass from coconut palm (*Cocos nucifera* Linn.) such as bunch waste (spathe + spadices), leafstalk (petiole), leaflets and coir pith (by-product from coir processing industry) were evaluated as substrates for cultivation of oyster mushroom, *Pleurotus sajor-caju* (Fr.) Singer. A low-cost mushroom shed built exclusively of coconut materials such as coconut wood and plaited coconut leaves inside a coconut plantation was used for spawn run and cropping. Leafstalk and bunch waste were superior to leaflets and coir pith in producing significantly more edible biomass of mushrooms. Biological efficiency of 58.9% was obtained in leafstalk, followed by bunch waste (56.9%), coir pith (39.7%) and leaflets (38.2%). The yield of sporophore was positively related to cellulose content and the cellulose : lignin ratio of the substrates.

Key words: Coconut lignocellulosics, mushroom, *Pleurotus sajor-caju*.

Various agricultural by-products are being used as substrates for the cultivation of the oyster mushroom (Jandaik & Kapoor 1974; Chang *et al.* 1981; Rajarathnam & Banu 1987). Though paddy straw is considered as the best substrate in terms of yield, it has become necessary to find cheap and alternate substrates due to the higher cost of paddy straw and its non-availability in certain areas, particularly in the plantation sector.

The coconut palm (*Cocos nucifera* Linn.) is a perennial oil seed crop cultivated in the tropical belt in an area of 11.1 million hectares (Thampan & Venkatachelam 1996). In India, the palm is reported to be grown in 1.63 million hectares with an annual production of 12 355 million nuts (Aravindakshan 1996). The cultivation and processing of coconut result in the accumulation of a large quantity of by-products which are rich in lignin and cellulose (PCA 1979) and are available without any cost throughout the year not only in large plantations but also in a large number of homestead gardens in the coconut-growing areas. Cultivation of edible mushrooms is one of the most economically viable processes for the bioconversion of lignocellulosic wastes. The present investigation was undertaken with a view to finding the feasibility of utilizing lignocellulosic by-products of coconut palm as substrates for cultivation of the oyster mushroom, *Pleurotus sajor-caju* (Fr.) Singer.

The authors are with the Central Plantation Crops Research Institute, Kasaragod 671 124, Kerala, India; Fax: 0091-499-430322; E-mail: cpcri@x400nicgw.nic.in. *Corresponding author.

Material and Methods

Substrate Preparation and Spawning

Pleurotus sajor-caju (Fr.) Singer strain M_2 obtained from Tamil Nadu Agricultural University, Coimbatore, India was used in the study. Spawn of the culture was prepared on *Sorghum vulgare* grains. Bunch waste consisting of spathes and spadices and partially dried leaves removed during the harvest of nuts from palms belonging to the West Coast Tall coconut cultivar were collected from the farm of the Central Plantation Crops Research Institute, Kasaragod. Leafstalk (petiole) and leaflets were separated and chopped to 5–7 cm long segments. Bunch wastes were also chopped similarly. Coir pith, the waste material after extraction of coir fibres collected from a nearby coir processing factory, was also used. The byproducts were soaked in fresh water for 16 h after sun drying. The excess water was allowed to drain off and 9 kg substrates were sterilized at 1.02 kg cm^{-2} pressure in each batch in an autoclave for $1\frac{1}{2}$ h. The sterilized substrates were allowed to cool to room temperature and were

filled in perforated (having 8–10 holes of 1 cm size) polythene bags of 60 × 45 cm (100 gauge) at the rate of 3 kg substrate per bag. Multilayered spawning technique was followed using 3% spawn. Rice bran was added as organic supplement at 6% level on wet substrate basis. A layer of sterilized substrate was spread to a height of about 5 cm at the bottom of the polythene bag. Later one-fourth portion of sterilized rice bran was spread followed by a layer of spawn. Similarly, four layers were prepared with substrate, rice bran and spawn and covered with a layer of substrate at the top. The open portion of the polythene bag was tied with a jute/plastic thread. Eight replications were maintained for each treatment.

Spawn Run

Spawn run and cropping were done in a low-cost mushroom shed of 5.1 m length × 3.1 m breadth × 3.1 m height built exclusively of coconut materials such as plaited coconut leaves and coconut wood, inside an adult coconut plantation. The bags were kept in a framework of racks made of coconut wood inside the shed. The temperature inside the mushroom shed was 20–30 °C and relative humidity was 80–85%. Cross ventilation was provided by ventilators of 0.6 × 0.3 m on all sides of the mushroom shed.

Cropping

After a complete spawn run, the polythene bags were removed, exposing the total surface area of compact mass for fruit body development. The compact mass of substrate and mycelium was watered daily from the second day of opening of the bags. Mature fruit bodies were harvested at different periods and the fresh weight recorded immediately after the harvest. A solution of 1% urea and 1% superphosphate was sprayed at the rate of 100 ml when there was delay in fructification. Biological efficiency (BE) was calculated as percentage yield of fresh mushroom in relation to dry weight of the substrate. Variance analysis was done to compare mushroom yield, BE and cropping period in different substrates. Transformation of percentage values of BE has been done to satisfy the conditions for analysis of variance (Snedecor & Cochran 1956).

Analysis

The substrates were powdered and analysed for constituents. Cellulose was estimated by the procedure of Updegroff (1969), lignin by the method of Zadrazil & Brunnet (1980), total phenols by the procedure of Bray & Thorpe (1954) and nitrogen by the microKjeldahl method. Four replications were maintained for each treatment. Correlations were worked out between mushroom yield and constituents of the substrates, to find out the relative contribution of each constituent in the development of fruit bodies.

Results

The mycelium of the fungus colonized the substrates within a period of 20 days of spawn run. Compact mass was formed due to the complete impregnation of mycelium with the substrates. Mycelial ramification was comparatively less in leaflets and coir pith. The primordia started appearing 2–3 days after the removal of polythene bags in all the substrates uniformly. The data on the quantity of sporophore harvested in different flushes are presented in Table 1. Maximum yield of 709.5 g edible biomass of mushroom per bag of 3 kg wet substrate was obtained in five harvests in leafstalk, followed by 611.7 g in bunch waste. The first flush of crop gave a maximum yield of 314.7 g in leafstalk followed by 247.8 g in bunch waste. More than 70% of the yield was obtained in the first two flushes in all the waste materials tested.

Table 1. Fresh weight of mushroom harvested from different substrates.

	Yield of fresh sporophores (g bag^{-1})					
	Harvest					
Substrate	1	2	3	4	5	Total
Leafstalk	314.7	162.1	151.7	81.0	9.9	709.5
Bunch waste	247.8	135.8	120.9	69.3	38.0	611.7
Leaflets	159.3	104.4	82.9	41.1	15.7	403.4
Coirpith	125.5	28.3	20.3	2.5	0.0	178.6
LSD (P = 0.05)	–	–	–	–	–	123.2

The first flush appeared uniformly in all the substrates on the sixth day after opening of the beds. The second flush appeared within 10 days after the first flush in bunch waste, leaflets and coir pith. In leafstalk the second flush of crop was obtained only after 30 days of the first harvest. The third flush was also delayed in leafstalk when compared to the pattern of production in all other substrates. Bunch wastes also took more days to yield mushrooms than leaflets and coir pith. Fructification was over in leaflets and coir pith within a short period of 30–36 days after the removal of polythene bags.

Biological efficiency (BE) of mushroom production and time for primordia formation varied in different substrates (Table 2). Among the four substrates tested, maximum biological efficiency of 58.9% was obtained in leafstalk during a cropping period of 63 days after removal of polythene bags, followed by 56.9% in bunch waste within a period of 58 days of cropping. BE was

Table 2. BE and time for primordia formation of *P. sajor-caju* in different substrates from coconut palm.

Substrate	Biological efficiency (%)	Time for primordia formation (days)
Leafstalk	58.9(50.2)*	25.8
Bunchwaste	56.9(49.3)	25.8
Leaflets	38.2(38.0)	24.8
Coirpith	39.7(38.9)	22.5
LSD (P = 0.05)	7.31	2.2

* Transformed values obtained by the formula $\sin^{-1}\sqrt{P}$ where P is proportion of BE.

only 38.2% and 39.7% in leaflets and coir pith, respectively. However, the yield in these two substrates was obtained in a short cropping period of 36 and 30 days, respectively. Analysis of mushroom yield and BE of production also revealed significant difference among the substrates. Leafstalk and bunch waste were superior to the other two substrates in total yield of mushrooms per bag and BE of production. Mushrooms obtained from leafstalk, bunchwaste, leaflets and coir pith had a protein content of 17.2, 22.1, 25.7 and 24.1%, respectively on dry weight basis. The data on the chemical constituents of the byproducts tested for the mushroom cultivation are presented in Table 3. The by-products differed significantly in the concentration of constituents such as lignin, cellulose, nitrogen and phenol. Leafstalk and bunchwaste had significantly high cellulose content and cellulose : lignin ratio. Leaflets had higher nitrogen and phenol content than the other by-products tested.

Correlation studies between mushroom yield and constituents of different substrates (Table 4) revealed a significant positive relationship of mushroom yield with cellulose content ($r^2 = 0.57$) and cellulose : lignin ratio ($r^2 = 0.66$). However, sporophore production was negatively related to lignin and phenol contents.

Discussion

Among the different byproducts of coconut palm tested as substrate for cultivation of *P. sajor-caju*, leaf stalk and bunch waste supported best growth of the fungus as evidenced by complete colonization of substrates forming a compact block within 20 days of spawn run. The yield of the mushroom is directly related to the spread of the mycelium into the substrates. The quantity of edible biomass of mushroom harvested was significantly more in leafstalk and bunch waste when compared to leaflets and coir pith. The superiority of the two substrates was also evident in biological efficiency (BE) of conversation, with leaf stalk showing 58.9% BE in a period of 63 days and bunch waste 56.9% BE in 58 days of mushroom production after removal of the polythene bags for cropping. However, the pattern of mushroom production in leafstalk and bunch waste showed variation with extended intervals between the appearance of flushes after the first harvest. The substrates which supported high mushroom yield in the present study have not been tried earlier.

Variable ranges of BE have been reported when different lignocellulosic palm wastes were used as substrates for production of oyster mushrooms. BE of 37.7% was reported by Chandramohanan & Moorthy (1991) when leafsheath of arecanut palm was used for cultivation of *P. sajor-caju*. While comparing mushroom production by different species of *Pleurotus* on mesocarp waste of oil palm, Kochu Babu & Ramachandran Nair (1991) obtained 58.4 and 55.7% conversion by *P. florida* and *P. sajor caju*, respectively. Patil & Jadhav (1991) reported fresh mushroom yield of 583 g per kg dry weight of coconut leaves in a comparative study with fourteen substrates.

Pleurotus spp. are reported to be efficient colonizers and degraders of lignocelluloses (Rajarathnam & Banu 1989). The fungi accomplish the enzymatic degradation of lignocellulosic portion of substrates by elaborating enzymes such as endoglucanase, β-glucosidase, xylanase, laminarinase, laccase and polyphenol oxidases which are involved in the degradation of lignocelluloses (Saxena & Rai 1992). The positive relationship obtained in the present study between mushroom yield and cellulose content and cellulose : lignin ratio revealed that the cellulose content of the substrates is an important factor for fruit body development. Cellulose-rich organic materials were reported to be good substrates for cultivation of mushrooms (Sivaprakasam 1986). Cellulase production was positively correlated with yield of sporophores (Ramasamy & Kandaswamy 1976).

The selection of substrate for cultivation of mushroom is largely determined by the abundance and cost of the substrate. The widely used substrate for cultivation of oyster mushroom is paddy straw. But its shortage and non-availability are constraints for the spread of mushroom technology in coconut-growing areas. The advantage with the by-products of coconut are their availability throughout the year at little or no cost not only in large

Table 3. Chemical constituents of the by-products.

By-product	Cellulose (%)	Lignin (%)	Cellulose : lignin ratio	Nitrogen (%)	Phenol (%)
Leaf stalk	31.73	25.08	1.31	0.31	2.84
Bunch waste	29.18	31.28	0.97	0.55	2.26
Leaflets	23.83	38.68	0.58	1.00	8.45
Coir pith	22.00	34.73	0.70	0.46	1.40
LSD ($P = 0.05$)	5.93	8.74	0.06	0.41	1.28

Table 4. Correlation between mushroom yield and constituents of the substrates.

Constituents of substrates	Mushroom yield
Lignin	−0.3868
Cellulose	0.5709*
Cellulose : lignin ratio	0.6580**
Phenols	−0.3749

* Significant at 5%
** Significant at 1%

plantations but also in a large number of homestead gardens. The leaf dry matter production by the tall coconut palm is reported to be 32.2 kg palm^{-1} year^{-1} (Kasturi Bai *et al*. 1986) and hence the availability of leaf from a hectare of coconut plantation can be estimated as 5.6 tonnes per hectare per year. Similarly, the availability of bunch waste from a hectare of coconut plantation can be estimated as 1 tonne per hectare per year.

The present study has also revealed the suitability of mushroom sheds constructed with easily available coconut materials inside the coconut garden for spawn run and cropping of oyster mushroom. The shaded condition provided by the canopy of the coconut palms inside the plantation helps to maintain congenial atmospheric conditions such as temperature, humidity and aeration inside the mushroom shed for fruit body development.

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