MUSHROOM CULTIVATION ON PALM WASTES

CENTRAL PLANTATION CROPS RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
KASARAGOD 671 124, KERALA

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Introduction

Mushrooms are fleshy fruit bodies of fungi technically called the sporophore. Mushrooms collected from the natural habitats are being consumed by man as a delicious food item from the time immemorial due to their flavour and texture. Of the large number of mushroom species growing wild in nature, many are edible while some are mildly to deadly poisonous. Artificial cultivation of edible mushrooms has been undertaken due to the realization of their nutritional, medicinal and commercial values. In India, three types of mushrooms viz. white button mushroom (*Agaricus bisporus*), paddy straw mushroom (*Volvariella volvacea*) and oyster mushroom (*Pleurotus* spp.) are commonly cultivated. Lower temperature of 15-20°C is required for the cultivation of white button mushroom and is ideally cultivated in winter season in northern India. The paddy straw mushroom can be cultivated under tropical conditions, but it has not become popular due to low productivity and perishable nature of fruit bodies. Oyster mushroom is an ideal mushroom for tropical regions due to its ability to grow at a wide range of temperature from 15 to 31°C.

Cultivation of oyster mushroom is being done mostly using paddy straw as substrate. Paddy straw has become costly due to the heavy demand as cattle feed and it is not available in many areas particularly in the palm growing tracts of the country. In order to make mushroom cultivation popular among rural masses, it is necessary to find out cheap substitutes which are available in plenty throughout the year. The cultivation and processing of products of palms such as coconut, arecanut and oil palm result in the accumulation of large quantities of by-products which are not being properly utilized now. Research carried out at Central Plantation Crops Research Institute revealed the suitability of utilizing by-products of coconut, arecanut and oil palm as substrates for cultivation of oyster mushroom, and bunch refuse of oil palm for cultivation of paddy straw mushroom, *Volvariella volvacea.* The production technology of the two mushrooms on palm wastes is described in this bulletin.

Availability of palm wastes

The coconut palm (*Cocos nucifera* L.) is perennial oil yielding plantation crop cultivated in India in 1.83 million hectares and the annual production is 22,355 million nuts. The major coconut growing states in India are Kerala, Tamil Nadu, Karnataka and Andhra Pradesh. It is estimated that 3.2 million tonnes of coconut leafstalk and 1.0 million tonne of bunch wastes are available in the country. There is lot of scope for utilizing these wastes for oyster mushroom cultivation as they are rich in lignin and cellulose.

Arecanut palm (*Areca catechu* L.) occupies a prominent place among the cultivated crops in the states of Kerala, Karnataka, Assam, Meghalaya, Tamil Nadu and West Bengal and is of considerable economic importance for the entire country. Fallen areca leaves, areca bunch wastes and arecanut husk are the major by-products of areca cultivation which are available in large quantity in the areca growing areas of India. About 0.1317 million tonnes of dried areca leaf, 0.0832 million tonnes of arecanut bunch waste and 0.2224 million tonnes of dry husk are estimated to be available annually in India. Among these areca wastes, areca leaf sheath and arecanut bunch waste are very promising substrates for oyster mushroom cultivation.

Oil palm (*Elaeis guineensis* Jacq), the highest oil yielding crop per unit area is now cultivated in over 30,000 hectares in the states of Andhra Pradesh, Kerala, Karnataka, Goa, Maharashtra and Tamil Nadu and in the Union Territory of Andaman and Nicobar islands.

Large quantities of wastes viz. bunch refuse (BR) and mesocarp waste (MW) are available in an oil palm factory. These wastes are obtained in sterile form due to sterilisation of fresh fruit bunches at 3 kg/cm^2 pressure for one hour to arrest lipase enzyme associated with free fatty acid formation.

At an average annual yield of 20 tonnes of FFB/ha under irrigated conditions of oil palm cultivation, about 7 tonnes of bunch refuse and 3 tonnes of mesocarp waste become available after processing. In an oil palm factory with the capacity of one tonne/hour, about 5 tonnes of FFB will be processed in a shift of 8 hours and about 1750 kg bunch refuse and 750 kg of mesocarp waste become available.

I. OYSTER MUSHROOM

Advantages

- *Pleurotus* mushroom can utilize a variety of agricultural wastes containing lignin, cellulose and hemicellulose, as substrates.
- Among the cultivated mushrooms, *Pleurotus* has larger number of commercially cultivated species suitable for different situations.
- Low initial investment, simple technology and limited space requirement for the cultivation.
- Fruit body has longer shelf life and can be easily dried and stored.
- It has the highest productivity as compared to other cultivated mushrooms.

Biology

Oyster mushroom known as "Dhingri" in north India are fruit bodies of basidiomycetous fungi belonging to the genus *Pleurotus*. It is a lignocellulolytic fungus which grows naturally on decaying wood logs and is endowed with ability to secrete a wide spectrum of hydrolysing and oxidative enzymes. In nature, the mycelium of the fungus lives buried in the soil or substratum (Fig.1). When conditions are favourable, the reproductive structures (fruit bodies) are formed. The fruit bodies are only a stage in the life cycle of the fungus and a mature mushroom bears spores. The spores are released in the air and they germinate to form primary mycelium when they find a suitable substrate. From the primary mycelium, secondary mycelium develops, from which the fruitbody develops. The fruit body of an oyster mushroom has three distinct parts - a fleshy shell or spatula shaped cup (pileus), a short or long lateral or central stalk called stipe and long ridges and furrows on the lower side of the pileus called gills or lamellae which bear the spores. The colour of the sporophore vary from white, cream, grey, yellow, pink or light brown depending upon the species. Commercially cultivated species of *Pleurotus* include *P. sajor caju, P. ostreatus, P. flabellatus, P. florida, P. eous, P. djamore, P. sapidus, P. eryngii, P. cornucopiae, P. citrinopileatus* etc.

Cultivation

Oyster mushroom cultivation is done by the following four steps;

1. Preparation or procurement of spawn
2. Substrate preparation
3. Spawning of substrate
4. Crop management

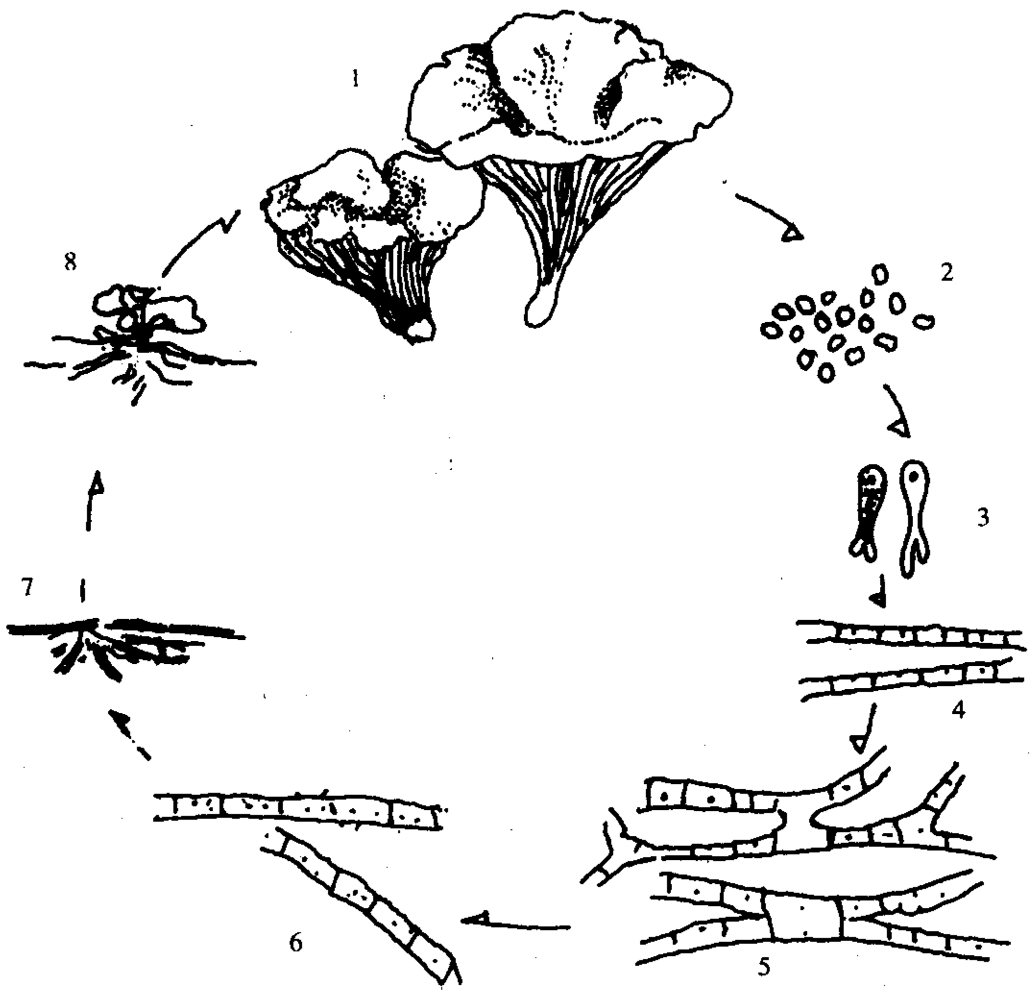


Fig. 1. Life Cycle of the Oyster Mushroom

1. Fully mature mushroom
2. Basidiospores
3. Germinating spore
4. Primary mycelium
5. Hyphal fusion
6. Secondary mycelium

7 & 8. Fruit bodies develop from secondary mycelium

1. Preparation of spawn

Spawn is a pure culture of a mushroom, used as the seed material for cultivation of mushroom. The most important requirement in spawn production is a pure culture from high yielding mushroom. This can be obtained from agencies engaged in mushroom production or from research laboratories. Cultures of the fungus can also be isolated from fresh sporocarps (Fig. 2.) The culture thus obtained can be stored in a refrigerator without losing its productivity.

Potato dextrose agar medium (PDA), petridishes, alcohol, cotton, forceps, inoculation needle, spirit lamp, test tubes and glucose/saline bottles are the materials required for spawn production. The culturing work is to be carried out under sterile conditions in an inoculation chamber or laminar flow or in a small room fitted with ultra violet (UV) lamp. The table in the inoculation room is to be cleaned with alcohol before inoculation. Once in a week this room is to be fumigated with formalin. Addition of a teaspoon of potassium permanganate to 25 ml of formaldehyde in a petridish will result in the release of formalin gas.

The stipe or pileus portion of young sporocarps (mushrooms) free from damage and fungal contamination from high yielding beds are to be used for isolation. The sporocarp is surface-sterilised with 70% alcohol and kept on a sterile petridish. The peripheral tissues are trimmed with a clean blade (surface sterilised using alcohol) and sliced into small bits. These bits are transferred using sterile forceps into sterile petridishes having solidified Potato Dextrose Agar (PDA) and incubated at room temperature (26 ± 3°C) for 7 days. White mycelial strands which grow from the tissues are to be subcultured on PDA slants in test tubes and incubated at room temperature (26 ± 3°C). After full coverage of mycelium on slants, these mother cultures are to be maintained in refrigerator and periodically sub-cultured.

Sorghum, paddy, maize or wheat grains are commonly used as substrates for spawn production. The grains are cooked for 30 minutes, excess water drained and allowed to cool by spreading over a clean surface/wire-net frame. Calcium carbonate @ 20-30 g/kg of grain is added and mixed well. These grains are filled in empty glucose bottles up to 3/4th level and plugged tightly with cotton. A paper cap is placed over the cotton plug and sterilized at 1.02 kg/cm^2 (15 lb/in^2) for a period of two hours. The bottles after cooling are inoculated with discs (6-8 mm diameter) of fungal growth using a sterilized cork borer and inoculation needle in inoculation room. The inoculated bottles are incubated at room temperature for 15 to 20 days. Complete coverage of white mycelial growth on the grains indicates that mother spawn is ready for use.

From the mother spawn, further spawn can be produced upto third or fourth generation. It is possible to prepare 30 spawn bottles from a single mother spawn bottle.

2. Substrate preparation

The major steps in oyster mushroom cultivation from the by-products of coconut, arecanut and oil palm are presented in Figure 3.

Partially dry leaves and bunch waste (spathe + spadics) of coconut removed during the harvest of nuts from palms are to be collected. They are to be chopped to 5-7 cm long pieces and sun dried, if necessary. Chaffcutters which are capable of cutting coconut leaves including stalk and bunch waste very fast are available at a lower cost. They can be operated either by

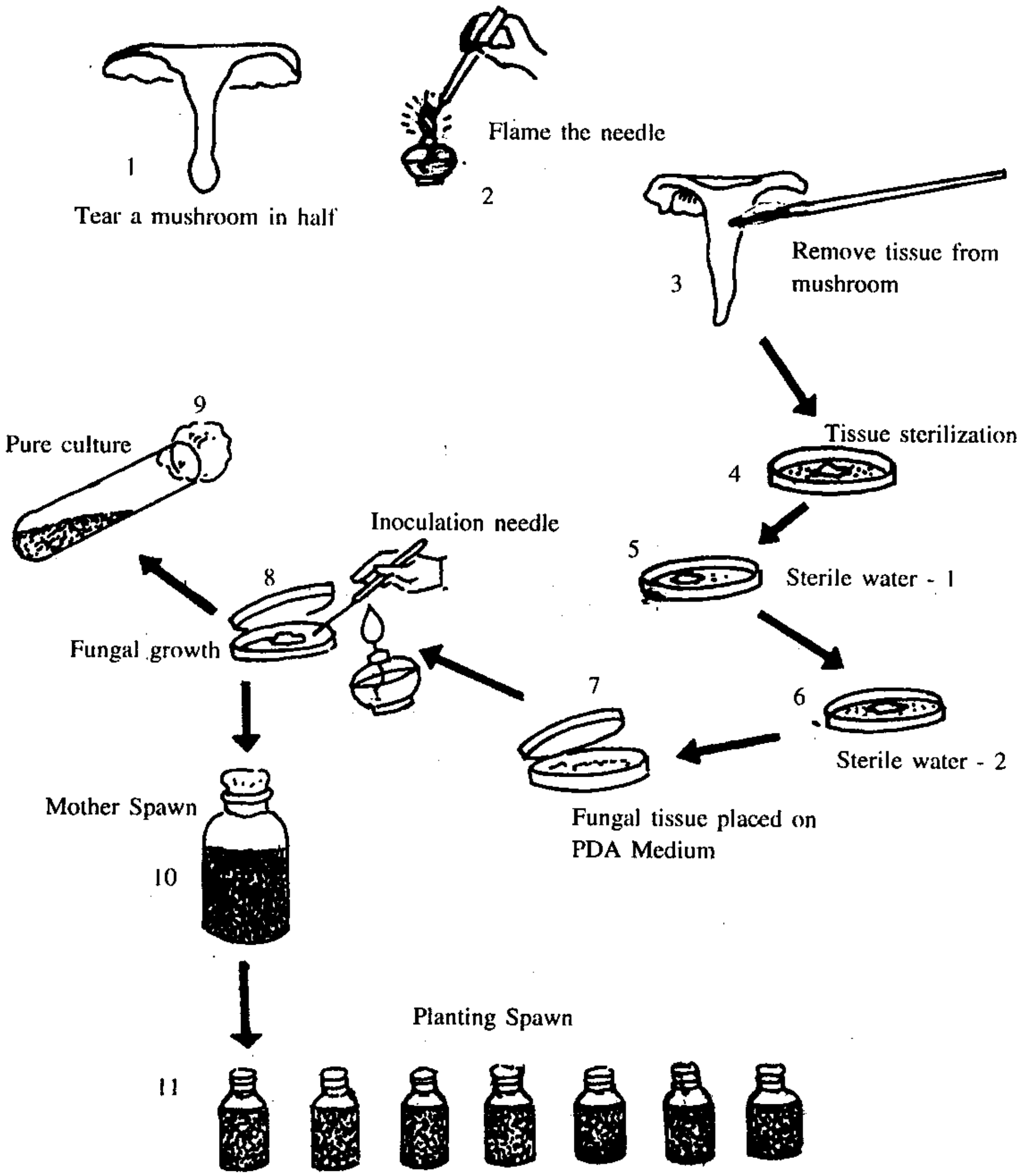


Fig. 2. Isolation of pure culture from mushroom tissue and spawn preparation

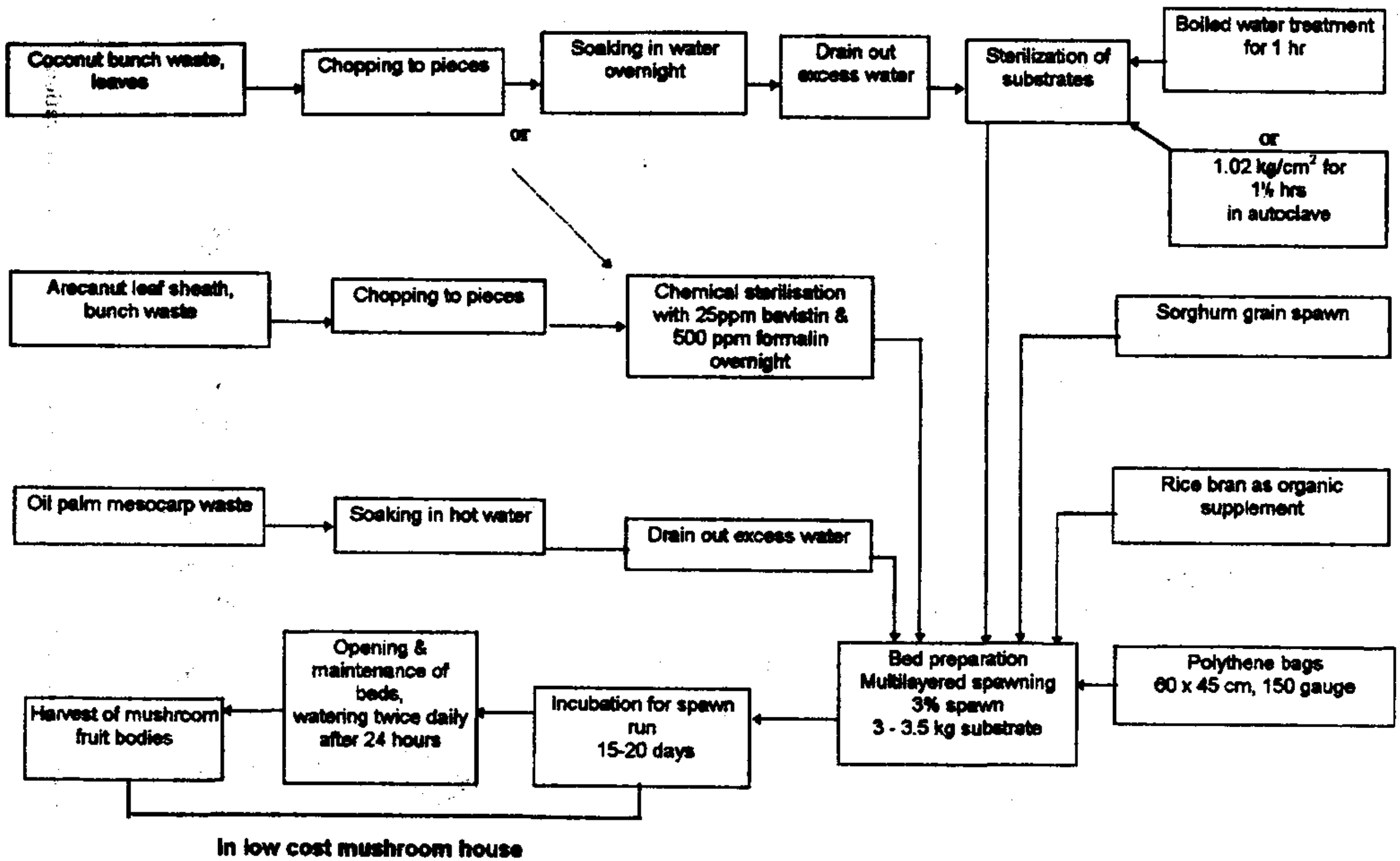
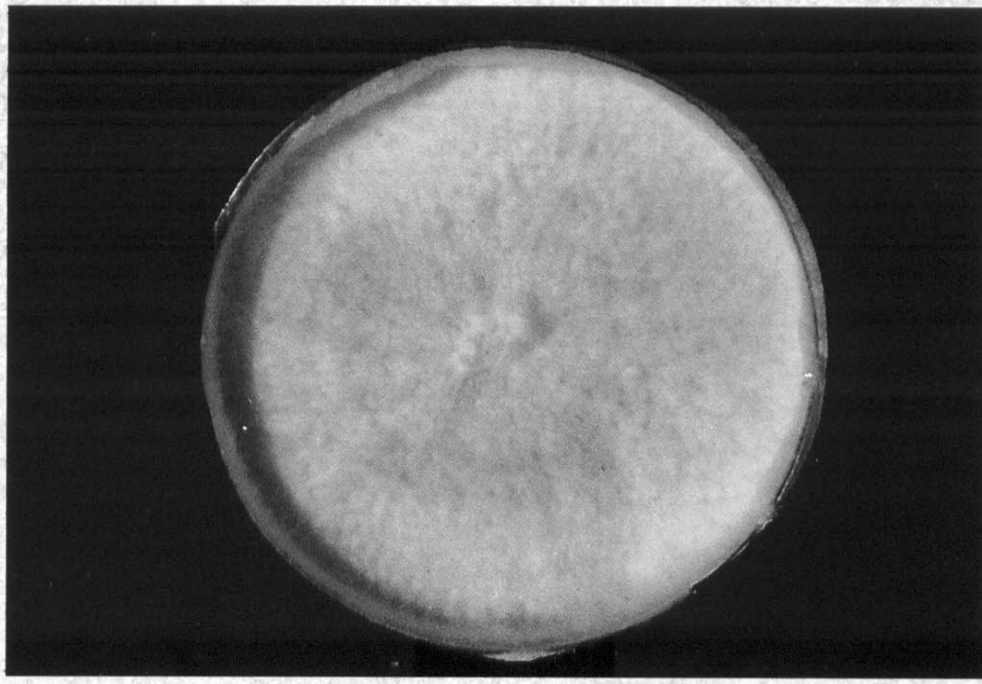


Fig. 3. Major steps in oyster mushroom cultivation using palm wastes as substrates



P. sajor-caju on Potato Dextrose Agar

Spawn of *P. sajor-caju*

Chaff cutter for cutting coconut wastes

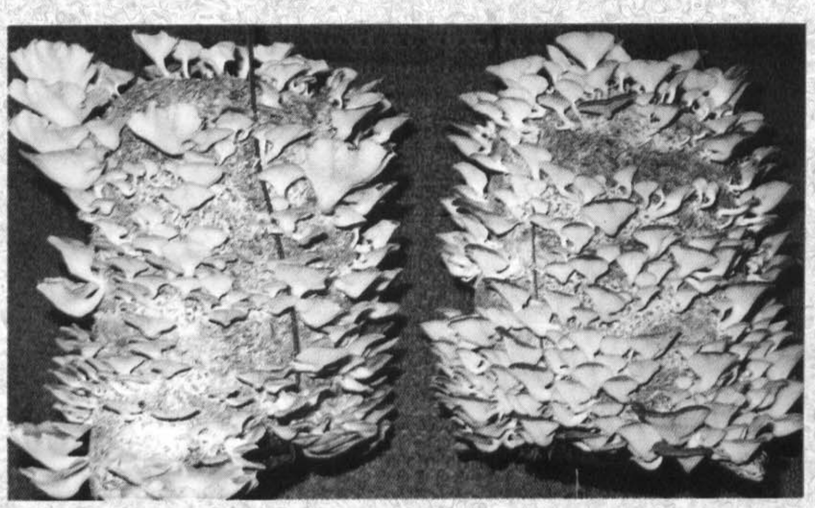
Coconut leaf stalk
fully colonised by *Pleurotus*

P. flabellatus on
coconut leaf stalk

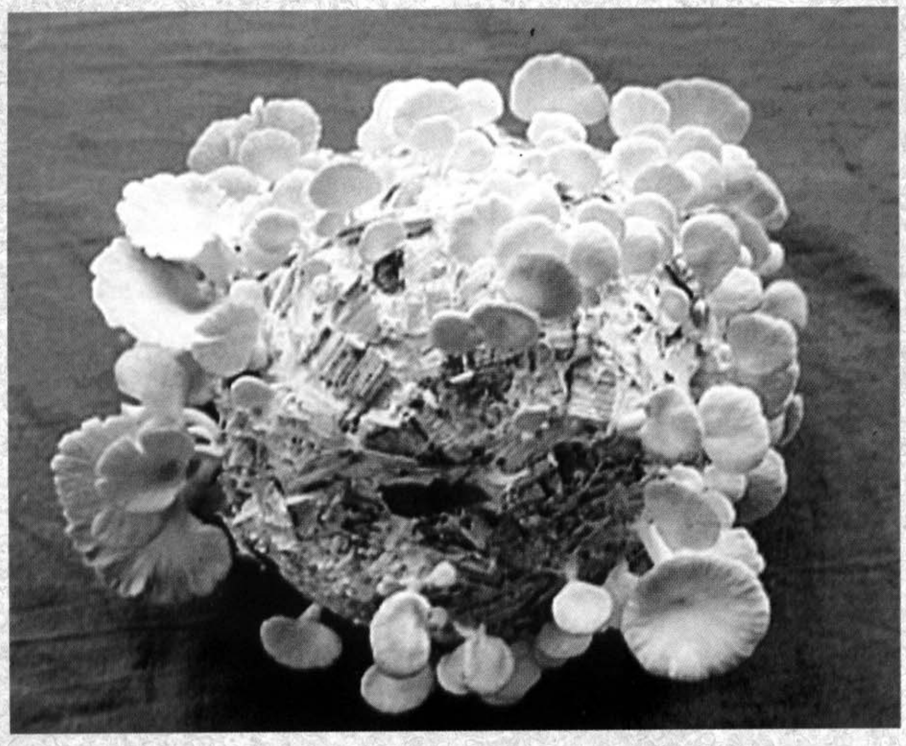
P. eous on coconut bunch waste

P. sajor-caju
on coconut leaf stalk

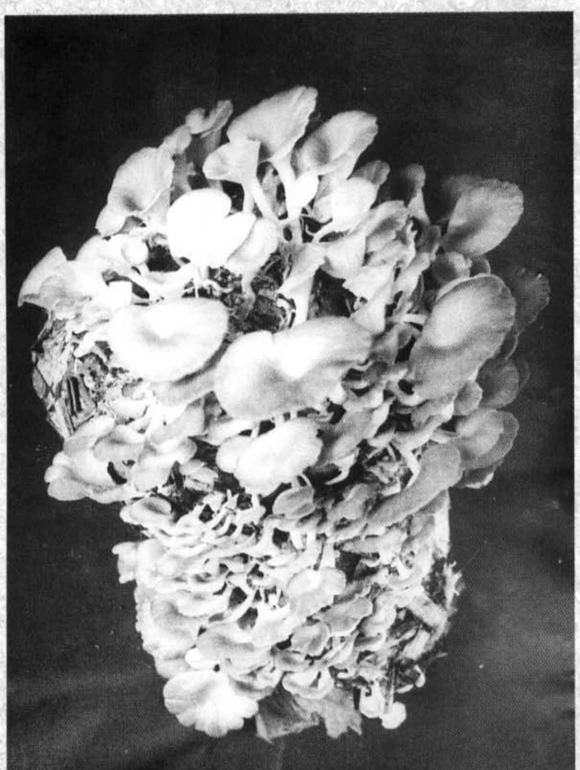
Low cost mushroom house built exclusively of coconut materials



P. sajor-caju
on oil palm mesocarp waste



P. sajor-caju on areca leaf sheath



P. sajor-caju on areca leaf sheath

Low cost auto clave for sterilising substrates hand or with the help of a motor. The substrates are soaked in clean water overnight. Excess water is drained off in the next morning. Sterilization of substrate is necessary to make it free from harmful micro-organisms and to favour the growth of *Pleurotus*. Substrates are sterilized by one of the methods; steam pasteurization, hot water treatment or chemical treatment. Steam sterilization can be done in an autoclave at 1.02 kg cm^{-2} pressure for 1 1/2 hrs. Hot water treatment is given by immersing pre soaked wastes in boiling water for one hour. Substrates can also be sterilized using a simple and low cost autoclave which can be locally made by the farmers using 200 L capacity petrol drums. A detachable lid is to be provided. Coconut materials such as shell or husk can be used as fuel to generate steam.

Naturally dried areca bunch wastes (ABW) are to be chopped to a size of 5-6 cm and leaf sheath to a size of about 3.5 x 2.5 cm. Chemical sterilization technique was found to be effective in sterilizing areca wastes. Areca bunch and leaf sheath are to be filled in gunny bags and soaked in a solution of formalin (formaldehyde solution 37-41% w/v) 500 ppm + fungicide Bavistin (Carbendazim) 25 ppm. The chopped substrate can be soaked @ 10 kg substrate in 100 L chemical solution which can be prepared by adding 5g Bavistin and 125 ml formalin. After soaking, the gunny bags are taken out from the tank and allowed for the excess water to drain off.

The fresh mesocarp waste of oil palm from factory is soaked in hot water for removing oil remnants as well as for increasing moisture content. For utilization of bunch refuse, the empty bunches are to be chopped to 5-6 cm pieces after separating individual inflorescence rachilla and soaked in water for 5 to 10 minutes.

Excess water in the substrates is to be drained by spreading on wire net frame. The moisture content in the substrate should be about 70% to avoid contamination.

Containers

Polythene bags of 60 x 45 cm or 45 x 35 cm size (100-200 gauge) can be used as containers. About 10 small holes (0.5 cm dia) should be made on all sides and bottom. Tying the bottom of the bag with jute thread helps to provide flat circular bottom for the bed.

3. Spawning of substrate

Twenty to thirty days old grain spawn may be used for spawning. Spawn is taken out from spawn bottle using a hooked iron rod cleaned with Dettol. The spawn is collected in a plastic tray cleaned with Dettol solution (1ml Dettol in 100 ml of water). The spawn from one bottle (300 g) is divided into three equal parts and again one portion can be apportioned to four parts for preparing a bed.

Multilayered spawning technique may be followed to inoculate the substrate with spawn @ 100g per bag containing 3-3.5 kg substrate. It is better to add rice bran at the rate of 5% to the coconut and oil palm wastes as an organic supplement to quicken mycelial ramification. The substrate is filled in polythene bags to a height of 5 cm and one portion of spawn is sprinkled along the periphery and a layer of rice bran is added over this. The second layer of substrate is spread to a height of 10 cm and the second portion of spawn and rice bran are applied. Four such layers are made with 3-3.5 kg of substrate and the tip of the bag is tied with jute thread.

4. Crop management

After spawning, the bags are incubated for spawn run in a mushroom house.

Mushroom house

Low cost mushroom sheds can be built inside areca or oil palm or coconut garden in between two rows of palms, with coconut/areca stem and coconut leaves.

It should have only one door in the front. The construction of mushroom shed inside coconut/areca garden has several advantages like inexpensive maintenance of low temperature and high humidity during summer months. Inside the shed there is a central frame work of areca or coconut stem with five pairs of shelves. This multitier rack can accommodate several bags. Small racks can also be built lengthwise on both sides keeping space for walking in between the central rack and side rack.

Inside of the shed is lined with good gunny sheets. Ventilators with insect proof plastic net are to be provided on all sides of the shed. The floor of the shed is filled with river sand to a height of about 8 cm. By periodical watering of the floor and gunny bags on the sides, high percentage of humidity can be maintained.

Spawn running

The filled up polybags or beds are kept at a distance of 10 cm in racks in the spawn run room in mushroom shed. It is ideal to maintain the temperature between 20-28°C and relative humidity of above 85% in the mushroom shed. The spawn grows as white mycelium and covers the entire bed. The contaminated beds are to be removed as and when observed. During the spawn run period, it is better to close the ventilators. Spawn running will be complete in 15-20 days. After the spawn run, the polythene cover is ripped open and the compact cylindrical bed is placed on hangers or racks at a spacing of 20 cm in the cropping room.

Cropping

After 24 hours of removal of bags, water is sprayed two or three times daily with sprayer or rose can. The ventilators of mushroom shed are to be opened to get diffused light and aeration. The first flush will be ready in 5 to 10 days after opening of the bag. The mushrooms are to be harvested just before the up-curving of the pileus and shedding of spores.

It is better to pluck the mushrooms in the morning itself before spraying water. Scrapping and removing of about one cm deep layer of substrate from the entire surface of the bed is necessary after each harvest to obtain the next flush within a short period. Three to four crops can be harvested from each bed. The interval between flushes was 7-10 days normally.

Mushroom yield

Coconut bunch waste, and leaf stalk were better substrates yielding higher quantities of mushrooms when tested individually (Table 1). Other by-products such as coir pith and leaflets were found to be poor substrates. However, mixing of coir pith and leaflets with other by-products resulted in higher yield of mushrooms. Bunch waste + leaflets combination yielded higher quantities of mushrooms in a shorter cropping period of 51.4 days. Biological efficiency (BE) is calculated as the yield of fresh mushrooms per 100 g dry weight of the substrate. Biological efficiency of conversion was high when coir pith + leafstalk and bunch waste + leaflets were used as substrates. The yield of mushroom was obtained in 3-4 harvests from each bed. Cropping period was more when leafstalk was the substrate due to the extended interval between harvests. The first flush was also delayed in some cases on leaf stalk substrate. Spraying the beds with a solution of 1% urea and 1% superphosphate

Table 1: Mushroom production in coconut by-products and mixtures of by-products

Substrate	Mushroom yield (g/bed)	Biological efficiency (%)	Cropping period (Days)
Bunch waste	611.7	56.9	59.3
Leaf stalk	709.5	58.9	72.6
Bunch waste + leaf lets	556.7	52.0	51.4
Coir pith + leaf stalk	356.0	60.0	75.0

Mixtures were tested at 1:1 ratio

helps to reduce interval between harvests to some extent.

Among the six species of *Pleurotus* tested on coconut bunch waste, *P. eous* yielded maximum quantity of mushrooms which are pink coloured (Fig. 4). *P. flabellatus* also produced higher quantities of mushroom followed by *P. sajor-caju*. Mushroom flushes were obtained at regular intervals in leafstalk with *P. flabellatus.*

Among the by-products of arecanut palm tested as substrates, bunch waste and leaf sheath were superior. The average yield of mushroom per bag containing 3.5 kg each of arecanut bunch waste and leaf sheath were 645.5 g and 418g, respectively. Biological efficiency of 69% and 49.8% was recorded in arecanut bunch waste and arecanut leaf sheath respectively. The total cultivation period i.e. number of days from spawning to third harvest was 47 to 52 days.

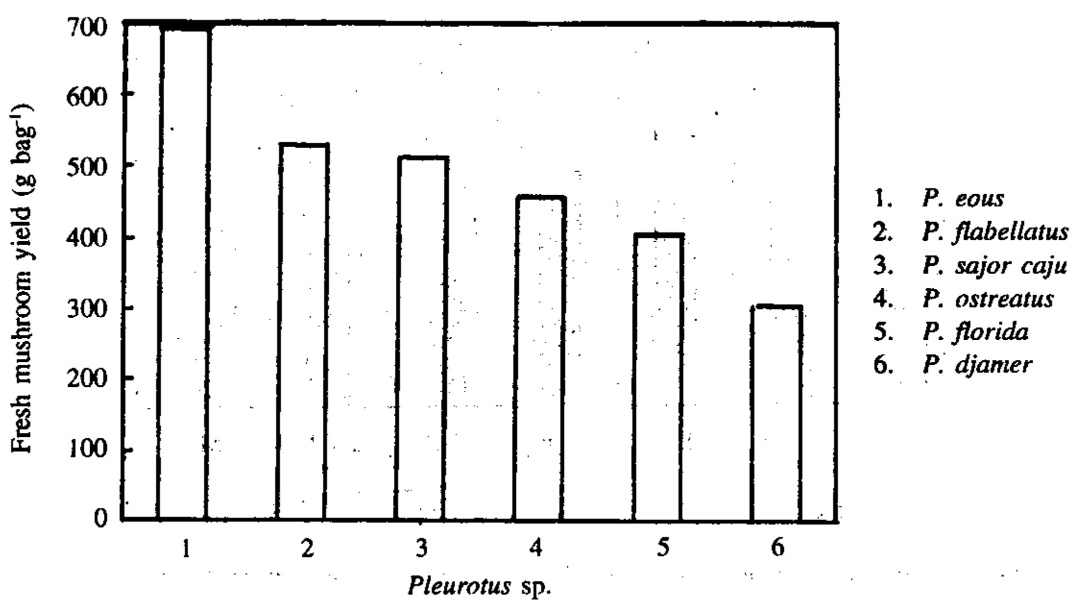


Fig. 4. Mushroom yield different species of *Pleurotus* in coconut bunch waste

Mesocarp waste of oil palm was found to be excellent substrate for oyster mushroom production. In addition to *Pleurotus sajor-caju*, mushroom production by other species of *Pleurotus* viz. *P. pulmonarius, P. ostreatus, P. flabellatus, P. citrinopileatus* and *P. florida* was also tested. Maximum yield of 1167 g fresh mushroom per bed of 2 kg mesocarp waste was obtained with *P. sajor-caju* (Fig. 5). Biological efficiency of 58.4% and 55.7% were obtained with *P. florida* and *P. sajor-caju*, respectively. With most of the species, more than 75% of mushroom yield was obtained within four weeks after the spawn run.

Post harvest practices

Mushrooms after harvest should be packed in polythene or polypropylene bags with air holes and sold the same day. If there is no immediate market, it can be stored in refrigerator upto three days. Mushrooms can be stored for 3-4 months after drying in bright sunlight or by mechanical drying in hot air oven at 40-50°C. Mushrooms dried in sunlight retain the colour. The dried mushroom can be rehydrated in luke warm water within 20-30 minutes.

Nutritive value

Mushrooms are highly nutritious food item which can be included in the diet of both vegetarians and non-vegetarians. Mushrooms contain more protein (2-3%) than most of the vegetables, but have less carbohydrate and fat contents. They contain most of the mineral salts required by the human body such as potassium, sodium, phosphorus, iron and calcium. Mushrooms are also rich in Vitamins such as niacin, folic acid, thiamin and riboflavin. The protein in the mushrooms contain all the essential amino acids required by the human body. It can be safely included in the diet of patients with hypertension, obesity and diabetics because of its low sodium: potassium ratio, starch and caloric value and high crude fiber contents.

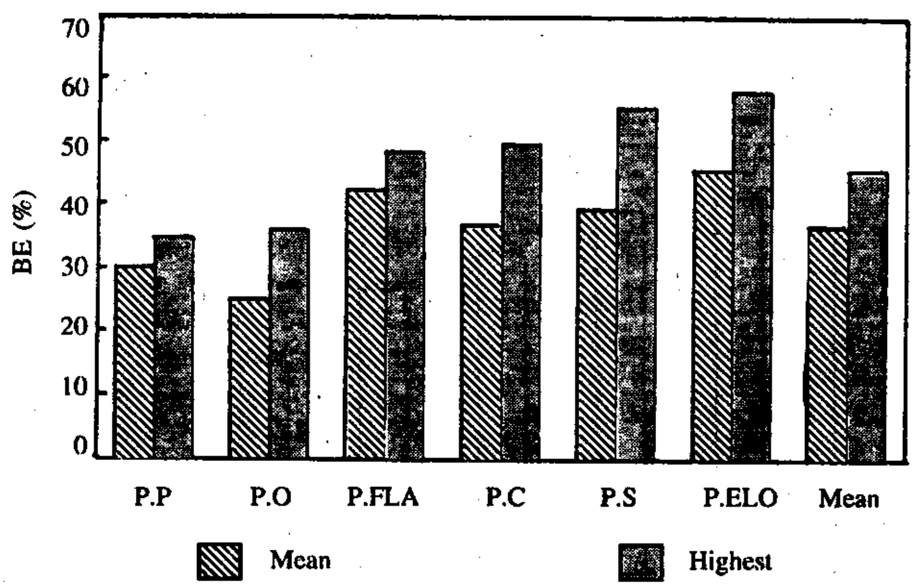


Fig. 5. Biological efficiency (BE) of *Pleurotus* spp. on mesocarp waste of oil palm

PP - *P. pulmonaris*, PO - *P. ostreatus*
PFLA - *P. flabellatus*, PC - *P. citrinopileatus*,
PS - *P. sajor caju*, PFLO - *P. florida*

II. PADDY STRAW MUSHROOM

Paddy straw mushroom comes up well in regions where the temperature ranges from 25 to 35°C. However, its cultivation on paddy straw has not become popular due to high cost of paddy straw, low-BE and low keeping quality of mushroom compared to oyster mushroom.

The bunch refuse of oil palm is found to be an ideal substrate to substitute paddy straw. Its cultivation can be done in thatched sheds, varandas, corridors and under shade of trees. Raised platforms of size 150 x 60 cm are to be prepared with deal wood planks or bricks. Before bed preparation, the bunch refuse is watered to increase the moisture level. Three rows of bunches are arranged over the platform with 15 to 18 bunches. The spawn is applied over this, preferably in the peripheral portions. Red gram powder (coarse) is dusted over this and water is sprinkled. The second layer is made by arranging 8 to 10 bunches in two rows to get a bed size of 150 x 60 x 45 cm approximately. The spawn can be applied to the entire top surface and the whole bed is to be covered with thick transparent polythene sheet to provide humidity for mycelial growth. If the moisture levels are found to be low during spawn run, watering may be done using a rose can.

After 10 days of spawn run, the polythene sheet is removed and watered twice daily. Buttons appear from all sides in a few days which advance to egg stage in 2 to 3 days. The mushrooms are to be harvested at this stage. After removal of the adhering substrate partially, they are packed in polypropylene bags with air holes for sale. Four to five cropping can be done in five weeks. After this, rearrangement of bunches is be done to get one or two crops.

For bulk utilization of bunch refuse, mushroom cultivation can be taken up in the basins of oil palms or in inter-rows of oil palms above ten years of age.

Direct exposure of beds to sunlight should be avoided as far as possible. Watering is to be done with a hose to the bunches before preparation of bed. Beds of 150 x 60 x 45 cm (two layers) can be prepared on ground without platform. Utilization of bulk bunch refuse dumped around the factory is also possible for mushroom cultivation. A shady area is to be chosen for this and small heaps of bunch refuse are made. Spawning is to be done on the top surface followed by red gram powder dusting and covering with transparent polythene sheet. At a BE of 3%, about 30 kg of paddy straw mushroom can be produced from one tonne of bunch refuse (Fig. 6).

Economics

Based on the studies conducted during different seasons it has been estimated that an average yield of 643 kg and 258.85 kg fresh mushroom can be produced annually using leaf sheath and bunch waste available from one hectare areca plantation. Thus by utilizing these two waste materials, mushroom worth Rs. 45,092 (@Rs. 50/- per kg) can be produced annually. For this, the present cost of production is Rs. 28,033/-. Thus an areca cultivator can get an additional net income of Rs. 17,059/- annually from one hectare areca plantation. If the family labour is utilized for mushroom cultivation, the cost of production can be brought down considerably.

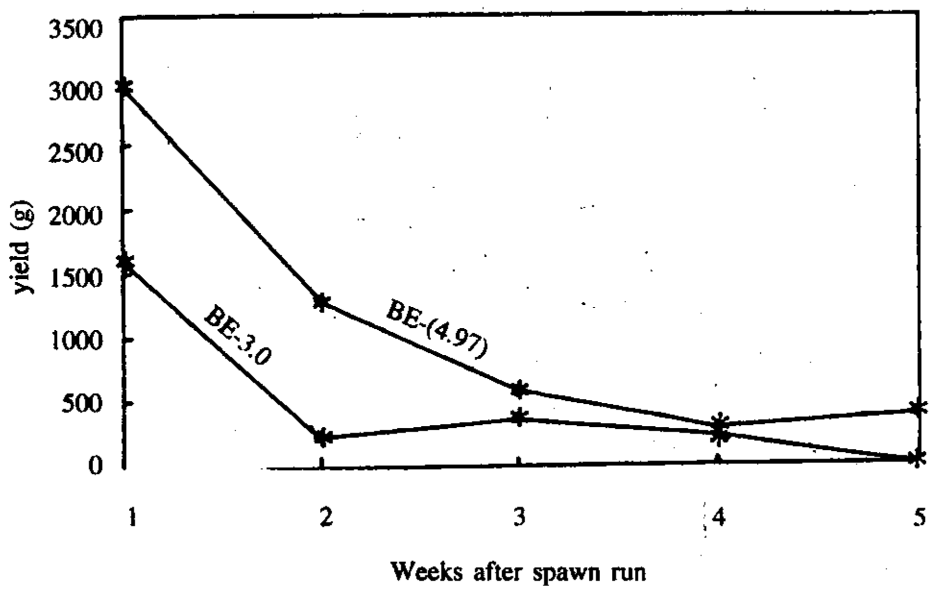


Fig. 6. Mushroom yield of *Volvariella volvacea* on bunch refuse of oil palm.

+ - Outdoor cultivation (Mean substrate wt. - 82 kg)
* - Indoor cultivation (Mean substrate wt. - 112 kg)
BE - Biological Efficiency

From the coconut leafstalk and bunch waste available in a hectare of coconut plantation, 1660 kg of fresh mushroom can be produced annually. The income from sale of mushroom is estimated as Rs. 66,444/- (@ Rs. 40/- per kg) and cost production is Rs. 39,919/-. Thus a coconut cultivator can get an additional income of Rs. 26,525/- from the waste materials available in a hectare of coconut plantation.

For setting up an oyster mushroom production unit with a capacity of producing 8 kg mushroom/day from oil palm wastes, a capital investment of Rs. 49,100/- and working expenses of Rs. 43,565/- are needed. Annual income of Rs. 80,530/- is obtainable from this unit.

For establishing a paddy straw mushroom unit with capacity of producing 17 kg mushroom/day from oil palm bunch refuse, capital investment of Rs. 52,700/- and working expenses of Rs. 1,02,800/- are required. Net income of Rs. 1,66,485/- is obtainable annually from this unit. If cultivation of both *Pleurotus* and *Volvariella* are done in the mushroom unit, an expenditure of Rs. 26,700/- under capital expenses can be saved.

The spent substrate obtained after the cultivation of oyster mushroom can be used as organic manure.