Development and Biotechnology of Pleurotus Mushroom Cultivation

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Abstract

The increasing population and the decreasing land size for crop cultivation throughout the world poses a serious problem to sufficient food production. The cultivation of *Pleurotus* mushrooms requires less elaborate technologies. The *Pleurotus* mushroom can adapt easily in rural areas since it can utilize farm wastes. The cultivation of *Pleurotus* mushroom could be an avenue to solving problems associated with deficiency of proteins, minerals and vitamins. During the last two decades, cultivation of *Pleurotus* mushrooms has become popular worldwide because of their desired attributes. These attributes include: the wide choice of species for cultivation under different climatic conditions, ability to grow on a variety of agricultural and industrial wastes, and their richness in culinary and nutritional values. In this paper, the importance, development and various biotechnological methods in respect to the cultivation of *Pleurotus* mushrooms are discussed. A simple method used by the authors for cultivation of *Pleurotus florida* on locally available substrate, rhodes grass (*Chloris gayana* L.) is also provided.

Key words: *Pleurotus* mushrooms, development, biotechnology, cultivation

Introduction

All the species of *Pleurotus* mushrooms are edible and are of high nutritional and culinary value. They are rich in proteins, minerals and vitamins (Bahl, 1998). The *Pleurotus* can be cultivated over a wide range of agro-climatic conditions (e.g. in warm and humid climate of tropical, subtropical, and even in temperate countries). They have the ability to directly break down lignocellulosic materials of agricultural wastes and convert them into viable proteins (Zadrazil, 1978). During the last two decades, the cultivation of *Pleurotus* has gained importance especially in South East Asia. Among the mushroom production worldwide, *Pleurotus* spp. ranked fourth in 1986

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yielding 169 thousand metric tons (Chang, 1987). The production had increased to 917 thousand metric tons (442%) just in five years in 1991. This is higher than the 31% increase in yield of commonly cultivated temperate mushroom, *Agaricus bisporus* over the same period (Chang *et al*., 1993). This increase in yield of *Pleurotus* spp. may be attributed to adoption of their cultivation by many people in rural areas because of their rapid mycelial growth, cheap production techniques and wide choice of species for cultivation under different climatic conditions (Quimio *et al*., 1990).

Pleurotus spp. are known by their common names in different countries. In Europe and America this mushroom is known as the 'oyster mushroom' (commonly grown species: *P. ostreatus, P. florida),* in China it is called '*abalone*' mushroom (*P. abalonus, P. cystidiosus)* and '*phoenix*' mushroom (a white variety of *P. sajor-caju*), and in India, '*dhingri*' *(P. flabellatus, P. sajor-caju).* There are many other species of *Pleurotus, which* can be cultivated. These are: *P. sapidus, P. eryngii, P. columbinus, P. cornucopiae P. tuberginum, P. fossulatus, P. opuntiae, P. citrinopileatus, P. membranaceus, P. platypus,* and *P. petaloids.* In this paper, development of *Pleurotus* cultivation in different countries and biotechnological methods in regards to their cultivation are discussed.

Literature Review

Historical Perspective on *Pleurotus* Mushrooms Cultivation

Pleurotus mushrooms grow on dead woody branches or on trunks of trees as saprophytes, and are called wood-rotting fungi. Falck (1917) first described the cultivation of *Pleurotus* on tree stumps and logs. Etter (1929) and Kaufert (1935) produced sporocarps of *P. ostreatus* in culture. Block *et al*. (1958, 1959) first described requirements of this mushroom for sawdust cultivation. Kalberer and Vogel (1974), Zadrazil (1978) and Kurtzman (1979) established the industrial production of *Pleurotus* in U.S.A and Europe. In India Bano and Srivastava (1962) were the first to cultivate *Pleurotus,* while Jandaik (1974) laid foundation for substrate preparation and fruit bodies production on a commercial scale, and also introduced the now popular tropical species, *P. sajor-caju*. Studies on cultivation of *P. flabellatus* (Bano *et al.,* 1978; Bano and Nagarajan, 1979; Bano and Rajarathnam, 1982; Bano *et al.*, 1987) and that of *P. sajor-caju* (Jandaik, 1974; Jandaik and Kapoor, 1976; Bhaskaran *et al.,* 1978; Chakravarty and Maliki, 1979) established the cultivation of *Pleurotus* in India. Information on history and development of *Pleurotus* cultivation has been provided by Jandaik (1997).

The *Pleurotus* industries involving the cultivation of *P. cystidiosus*, *P. sajor-caju* and *P. florida* in the Philippines have now become popular following the work of Quimio (Quimio 1978a, 1978b, 1979, 1981, 1986 and 1988). *Pleurotus* spp. were also introduced to Thailand (FAO, 1983) where they are cultivated throughout the year. Jong and Peng (1975) first reported the commercial cultivation of *P. cystidiosus* in Taiwan. Roxon and Jong (1977) first introduced the cultivation of Indian species, *P. sajor-caju* to U.S.A. In Mexico, Guzman and Martinez (1986) established *Pleurotus* cultivation and developed a technology which is now used in a semi- industrialised plant utilizing coffee-pulp as a substrate. Silanikove *et al*. (1988) and Danai *et al. (*1989) developed a technology in Israel utilizing cotton straw as a substrate for commercial cultivation of *Pleurotus* mushrooms. Zadrazil and Kurtzman (1982) discussed *Pleurotus* cultivation in tropical countries. There are only a few scattered reports on cultivation of *Pleurotus* mushrooms in Africa (Table 1).

Theoretical Analysis of Biotechnology of Pleurotus Mushroom Cultivation

Spawn Production

The mushroom seed is generally referred to as spawn. Spawn is a living ramified mycelium of a mushroom, multiplied on a suitable sterile base material under aseptic condition. The base materials that can be used for spawn production are chopped rice straw, sawdust, tealeaves, coffee hull, cotton waste and cereal grains (Oei, 1996). In the appendix, steps involved in spawn production have been outlined. Different grains like millets, wheat, rice, barley, rye and sorghum are now commonly used as base materials for spawn production (Quimio *et al.*, 1990).

The choice of grains and amount of additives used has been reported differently. Stoller (1962) preferred rye grain and cottonseed meal for spawn production and advised the use of 6g of gypsum and 1.5g chalk per pound of grain to avoid sectoring and clumping of grains.

Table 1: Some *Pleurotus* species and substrates used in their cultivation in Africa

Species	Substrate	Country	Source
P. sajor-caju	Cotton hull waste	Kenya	Nout and Keya (1983)
P. sajor-caju and *P. flabellatus*	Rice and wheat straw	Mauritius	Peerally (1989)
P. ostreatus and *pulmonarius*	French bean straw	Rwanda	Rusuku (1989)
P. tuber-regium	Loam soil and oil palm fibre	Nigeria	Okhuota and Etugo (1993)
P. ostreatus	Wheat straw	South Africa	Labuschagne *et al.*, (2000)
P. ostreatus	Rice straw	Ghana	Obodai *et al*., (2003)

Stamets (1993) also recommended rye grain for spawn production and used 1g of gypsum per 200g rye grain. Munjal (1973) recommended the use of sorghum grains whereas Hu and Lin (1972) used grain hull powder for making granular spawn. Kumar *et al.* (1975) reported that sorghum grains have better growth of mycelium after mixing 2% gypsum and 6% $CaCO_3$ with the boiled sorghum grains. Sawdust can also be used for spawn production. Sawdust spawn is generally prepared according to Quimio *et al.*, (1990).

Substrates

The material on which the mycelium grows to produce mushroom is called substrate. *Pleurotus* spp. can readily be grown on various lignocellulosic farm waste materials. They may be grown on a mixture of sawdust and rice bran or wheat bran, rice straw and rice bran, wheat straw and wheat bran, and combination of other waste materials. Quimio (1986) and Quimio *et al.*, (1990) suggested corn cobs, cotton waste, sugarcane bagasse and corn leaves as good substrates for growing these mushrooms.

Different agricultural wastes have been used and found superior for the cultivation of *Pleurotus* spp. in different countries. Table 1 and 2 show some of these agricultural wastes (substrates) that have been used in Africa and elsewhere, respectively. The substrates used for the cultivation of these mushrooms, however, depend upon the availability of agricultural wastes in areas where mushroom cultivation is to be set up. Locally available agricultural and forest wastes with better yields should be identified otherwise the transport of substrates from one region to another would increase the cost of cultivation. Most of these agricultural wastes are chopped into 1-2cm pieces and soaked in water with formalin (100ml/100l water) and bavistin (0.1%) for 1-2 days. Thereafter excess water is drained off. In cases where sawdust is to be used as substrate, it must be composted (Zadrazil and Kurtzman, 1982; Quimio *et al*., 1990). Sawdust is mixed with 1% each of urea and CaCO3. The mixture is made thoroughly wet with water and then piled up to 1m high. The heap is covered with plastic to avoid loss of water. During composting, the heap is turned every week over a period of 30-40 days. At the end of this period, the sawdust mixture becomes soft without any unpleasant smell. When the sawdust compost is ready, 20% rice bran is added. Prior to sterilization and spawning the substrates must be supplemented with various materials for better yield of *Pleurotus* mushrooms. These materials include; oatmeal (Jandaik, 1974); wheat bran and cotton meal (Seth, 1976) horse gram powder (Bano *et al*, 1979); starch (Easwaramoorthy *et al*., 1983); sterilized chicken manure (Vijay and Upadhay, 1989); wheat and rice bran (Bahukhandi, 1990; Quimio *et al*., 1990).

Table 2: Some *Pleurotus* species and agricultural wastes used in their cultivation in different non-African countries

Species	Substrate	Country	Source
P. florida	Corn cobs	Hungary	Toth (1969)
P. ostreatus *P. sajor-caju*	Coffee pulp	Mexico	Guzman and Martinez (1986)
P. sajor-caju	Cotton hull waste	Australia	Cho *et al*. (1981)
Pleurotus spp.	Wheat straw and corn cobs	U. S. A., Europe and Korea	Royse and Schisler (1980)
Pleurotus spp.	Cotton waste and paddy straw	Singapore	Leong (1982)
P. cystidiosus *P. sajor-caju* *P. florida*	Paddy straw and sawdust	Philippines	Quimio *et al*. (1986)
Pleurotus spp.	Cotton straw	Israel	Silanikove *et al*. (1988) Danai *et al.* (1989)
P. sajor-caju *P. flabellatus* *Pleurotus* spp.	Corn cobs, straw of cereals, cotton wastes, banana pseudo-stems grasses, sugarcane by-products, water hyacinth.	India	Jandaik (1974); Jandaik and Kapoor; Chakrabarty and Malik (1979); Bano *et al.* (1979, 1987); Tewari and Sohi (1979); Sivaprakasam *et al.* (1979); Bhandari *et al*. (1991); Das *et al*. (1988); Patra and Pani (1997); Singh and Kaushal (2001); Chandrashekhar *et al*. 1979.
Pleurotus spp.	Apple pomace	India	Upadhyay and Sohi (1988)
P. sajor-caju	*Jowar* straw and ground nut pods	India	Khandar *et al*. (1991)
Pleurotus spp.	Rubber wood and sawdust	India	Mathew *et al*. (1991)
Pleurotus spp.	Palm factory waste	India	Babu and Nair (1991)
Pleurotus spp.	Tea leaf wastes	India	Upadhyay *et al*. (1996)

Various types of containers are in use for filling the substrates. These are earthen pots, bamboo baskets, wooden trays, wide-mouthed bottles or jars and heat resistant polyethylene bags. Perforated polyethylene bags are commonly used in many countries for straw and sawdust substrates (Quimio *et al.*, 1990; Oei, 1996). Wide-open bottles are commonly used for sawdust substrates, particularly in the cultivation of *P. eryngii* (Oei, 1996). In Rwanda, wooden cages covered with transparent plastic paper or straw have been used (Rusuku, 1989).

Sterilization, Spawning and Incubation

Most of the substrates require sterilization or pasteurization before using them for cultivation (Quimio *et al.*, 1990; Oei, 1996). In the past *Pleurotus* substrates used to be sterilized under pressure. This is currently not recommended since it kills beneficial microorganisms, and also breaks down organic substances into forms more favourable for the growth of contaminants (FAO, 1983). Steaming of substrates or immersion into hot water is recommended for *Pleurotus* substrates. The substrate is steamed at 100^{o}C for 2-3 hours or at 70^{o}C for 6-8 hours, depending on the volume and size of the bags containing the substrates (Quimio *et al.*, 1990). Oei (1996) provided information on duration of steaming/heat treatment of substrates used in different countries.

Spawning is the inoculation of spawn into the substrate free of any contaminant. Different spawning methods have been discussed by Bahl (1998). Double layer and thorough spawning methods are preferred over others (Sivaprakasam and Ramraj, 1991). Grain spawn or sawdust spawn is commonly used to inoculate the substrate in polyethylene bags. Fresh spawn is used for spawning the substrate for better yield. Spawn stored in the refrigerator for more than 2 months and used thereafter comparatively reduced the yield of *P. sajor-caju (*Heltay, 1959; Bahl, 1998). Amount of spawn inoculated per kg of substrate is variable and is dependent on the substrate being used. Guzman and Martinez (1986) used 2.5% spawn in pasteurized coffee pulp. In Hungary 3% spawn was used on corncob while in China 7-10% spawn has been used on cotton seed hulls (Oei, 1996). Quimio *et al.* (1990) recommended one bottle of grain spawn or sawdust spawn as sufficient to inoculate 40 to 50 bags, each of 15-30 cm size. However, it is better to find out locally the minimum amount of spawn per kg of substrates needed to colonize the substrate completely in a short time at optimum temperature.

The spawned substrate bags are kept in a dark room at temperature around 25^{o}C, which is optimum for mycelial growth for most of *Pleurotus* spp. (Oei, 1996). It takes 3-5 weeks to colonize the substrate throughout by the mycelium of mushroom, depending on the substrate and species of *Pleurotus* used.

Fruiting, Harvesting and Yield

The substrate after being completely colonized by the mycelium becomes compact and white. Polyethylene bags are cut open and compact substrates are kept on benches in a mushroom house. Water is sprayed daily and in 3-4 days mushrooms start coming out along all sides of the bag. Fruiting of *Pleurotus* spp. requires a temperature range of 10-30^{o}C (depending on individual species), ventilation, light, moisture and humidity of 80–95% (Quimio *et al.*, 1990). Table 3 presents information on colonization time and fruiting temperatures of different *Pleurotus* spp.

Table 3: Complete colonization time and fruiting temperatures of different *Pleurotus* spp. in 1.2 kg bags at 25^{o}C on standard sawdust – rice bran substrate, spawned with 10g of sawdust spawn

Species	Colonization	Fruiting temperature
P. sajor-caju	3 weeks	18-30^{o}C
P. cystidiosus	5-6 weeks	25-28^{o}C
P. florida	4-5 weeks	10-20^{o}C
P. flabellatus	4-5 weeks	20-28^{o}C
P. eryngii	6-7 weeks	18-22^{o}C
P. pulmonarius	3-5 weeks	13-20^{o}C
P. cornucopiae	4-5 weeks	15-25^{o}C
P. abalonus	4-5 weeks	25-30^{o}C

Source: Oei (1996)

Mushrooms are harvested till the spawned substrate ceases to fruit. Mushrooms are generally harvested individually, by grasping the stalk with hand, and then twisting the mushroom and pulling it out. Quimio *et al.* (1990) suggested that if the harvested surface is scrapped lightly to expose a new surface, it might fruit again. Normally three to five flushes could be harvested from each bag.

The yield of *Pleurotus* mushrooms depends on the quality of spawn, substrate used, climatic factors and nutrients supplemented to the substrate before and at the time of fruiting. Jandaik and Kapoor (1976) found that addition of oatmeal with paddy straw gave better yield of *P. sajor-caju.* Supplementation of paddy straw with cottonseed powder (132g/kg dry straw) after the spawn run increased the yield of *P. flabellatus* by 85% (Bano *et al*., 1979). Quimio *et al.,* (1990) suggested spraying of urea (100g/100 l water) on the surface of the substrate before fruiting to increase the yield. The method of spawning the substrates also has an impact on the yield of *Pleurotus* mushrooms. The layer spawning proved to enhance the yield when compared to thorough spawning (Sivaprakasam and Ramaraj, 1991). The yield is usually calculated as weight of fresh mushrooms in kg per 100 kg of dry weight of the substrate. This is also expressed in terms of percentage weight of fresh mushrooms produced per kg. of the dry substrates. This is referred to as the biological efficiency (Chang and Miles, 1989).

Utilization of Spent Mushroom Compost (SMC)

The substrate from which mushrooms have been cultivated and harvested is called spent mushroom compost (SMC). Spent mushroom compost can be used to grow successive crops of mushrooms, as soil fertilizers or conditioners, as an energy source for fuel and as animal feed (Quimio *et al*., 1990). Quimio (1988) showed that spent substrate from *Volvariella* beds can be used for growing *Pleurotus* mushrooms after pasteurizing the substrates. Levanon *et al.,* (1993) used *Pleurotus* spent substrate (cotton straw) for the cultivation of *shiitake* mushroom (*Lentinus edodes*).

Spent mushroom compost made from wheat straw and other supplements comparatively gave higher yields of some vegetables like cabbage, cauliflower and beans as compared to poultry manure (Male, 1981). In Puerto Rico, *Pleurotus* spent compost from sugarcane bagasse is used by nursery growers as a good substitute for commercial fertilizers for conditioning the soil (Quimio *et al.*, 1990). Yadav *et al*., (2001) obtained higher grain yields and vigorous growth of maize when well-composted SMC was used in the soil.

Mushrooms are known to degrade the lignin components from lignocellulotic complex of the substrates. However, the cellulose component is not utilized by the mushroom but are converted to more digestible and protein-rich substances suitable for use as animal feed (Zadrazil, 1984). *Pleurotus* spp. are considered the most efficient lignin-degrading mushrooms (Platt *et al*., 1983, 1984). They can grow well on different types of lignocellulosic substrates. Bakshi *et al*. (1985) used *Pleurotus* spent wheat compost as feed material in buffalo rations. SMC from *Pleurotus* straw compost proved to be a highly digestible nutritious feed for cattle and sheep (Quimio *et al*., 1990). At 30% substitution level, SMC from cotton straw has been shown not to influence the growth of calves and sheep (Kerem (1991) while SMC is safe as animal feed (Bano *et al.,* (1986). Care should be taken to ensure that there is no contamination with *Aspergillus flavus* and other fungi, which produce highly toxic substances such as aflatoxins.

Application

Potential of *Pleurotus* Mushroom Cultivation in Kenya

In virtually every community in Kenya, wild mushrooms have been valued as part of the traditional diet. People hunt for and consume these mushrooms during the rain season. Apart from limiting the supply to the rain season, this poses the danger of collecting poisonous species that can bring illness and even death to people unaware of certain poisonous species (Hanko, 2001). As such, cultivated mushrooms remain the only safe source of this delicacy all year round. Being a familiar diet it is likely to be easily accepted in the market.

Currently, most of the mushroom-growing farms in Kenya grow the button mushroom, *Agaricus bisporus*. This species requires very elaborate cultivation procedures and controlled environment (e.g. controlled substrate decomposition, light, air flow, temperature and a sterilized casing layer). This makes its cultivation expensive and difficult for many farmers. *Pleurotus* mushrooms, being equally nutritious and delicious, are able to grow on undecomposed substrate, require no casing layer and are adapted to a wide temperature range. This makes their cultivation cheaper and simple.

In Kenya we have a variety of agricultural and industrial wastes (corn cobs, wheat and barley straw, maize stovers, coffee husks, cotton pulp, sawdust, tea leaf waste sugarcane baggasse, banana pseudostems etc.) that usually go unutilized. *Pleurotus* mushrooms can be used to convert these otherwise less useful organic matters into highly nutritious food. If farmers use these agricultural wastes available in their areas, the production cost will be reduced. In addition, the weed, water hyacinth, which is a threat to River Nairobi and Lake Victoria can be successfully used as substrates for cultivation of *Pleurotus* mushrooms. Use of these agricultural and industrial wastes and weeds as substrates in mushroom production can be a safe disposal of what may otherwise be environmental pollutants.

With the high rate of unemployment in Kenya, cultivation of *Pleurotus* mushrooms may be a viable proposition to those who wish to venture into self-employment. This is because the enterprise requires little space and capital. It is also worth noting that it offers very little competition, if any, to other farm enterprises like livestock and crop farming, and the SMC can be recycled for use as animal feed or soil conditioning.

Conclusions

Table 4 gives information on the major requirements in starting a *Pleurotus* mushroom cultivation business. Besides what is given in the appendix, it is important that farmers use the substrate that is available in their areas to reduce the cost of cultivation.

The method summarized in the appendix is slightly modified from those used by Bano *et al.* (1979), Jandaik and Kapoor (1976) and Quimio *et al.*, (1990) in terms of sterilization and amount of additives used. The authors found Rhodes grass (hay) a locally available substrate for the cultivation of *P. florida*, to achieve a bio-efficiency of 57% (Data unpublished). Further studies are in progress in the Department of Biological Sciences, Egerton University to find out a low cost method for maximum yield of *P. florida* and other *Pleurotus* mushrooms.

Table 4: The major requirements for *Pleurotus* mushroom production, their cost and sources

Item description	Approx. cost/unit (Kshs)	Source
3m x 4m mudded and wooden house	10,000.00	To be constructed.
Spawn	100.00/500g	Spawn producers, Botany Dept. of Egerton University
$CaCO_3$ and $CaSO_4$	450.00/kg	Agro-chemical shops
Bavistin	500.00/kg	Agro-chemical shops
Formalin	100.00/l	Chemist stores
Wheat (other) grains	50.00/kg	Cereal/grain stores
Hay (*Chloris gayana*) and other substrates	60.00/bale	Animal feed stores
Hand sprayer	150.00/piece	Agro-chemical shops/supermarkets.
Horse gram flour	100.00/kg	Supermarkets.
Gunny bags	30.00/piece	Agro-chemical shops
Polythene bags	150.00/50	Supermarket
Half drum	400.00/piece	Hardware shop

Acknowledgments

The senior author gratefully acknowledges the financial support from Internal Research Grant of Egerton University on the Research Project-Oyster Mushroom Cultivation in Kenya.

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Appendices

Cultivation of *Pleurotus florida* on Hay, Rhodes Grass (*Chloris gayana* L.)

Spawn Preparation

- Wheat grains are used for spawn production. The grains should be less than one year old, unbroken and free from damage by insects.
- Wash wheat grains thoroughly and then soak in water overnight. Thereafter remove dead grains or those that float on water and other debris.
- Wash the grains again the following day.
- Boil the grains with an equal amount of water on weight basis for 20-30 minutes to make them soft. Caution should be taken not to split the grains since this might lead to loss of starch.
- Take the grains from water and spread on a sloppy surface in order to drain-off excess water.
- Mix grains thoroughly with $CaCO_3$ and gypsum ($CaSO_4$) (2% each on wet weight basis).

- Fill the mixed grains loosely 2/3rd in spawn bottles (500-600g of mixed grains/bottles) or in glucose bottles or 250ml conical flasks or heat resistant polythene whatever is available.
- Plug the spawn bottles tightly with cotton wool.
- Sterilize the spawn bottles with mixed grains for two consecutive days at 15lb pressure for 45 minutes if autoclave is not available, pressure cooker can be used for sterilization.
- Allow spawn bottles with grains to cool prior to inoculation.
- Inoculate grains in each spawn bottle with two mycelial agar disks of 5-6 mm in diameter cut from the vigorously growing mycelial culture of *P. florida.*
- Incubate spawn bottles with inoculated grains at 25^{o}C. In 15-20 days grains will be completely colonized by the mycelium and ready for spawning the substrates.

Preparation of Substrates, Spawning and Harvesting of Mushrooms

- Cut dry hay/substrate into 1-2 cm pieces.
- Dissolve 100ml formalin and 10g bavistin (carbendazim) in 100 l of water.
- Soak 10 kg of the chopped hay in the above solution for 24 hours.
- Next day take out chopped hay from water and spread it on a sloppy cemented floor in order to drain excess water. It will take 1-2 hours to drain excess water from the hay.
- Mix the horse gram powder (8 g/kg hay) and 1% $CaCO_3$ with the moist substrates.
- Fill the mixture (2 kg/bag) in heat-resistant polyethylene bags (30x38cm) with holes of 1cm diameter and 5-6 cm apart all along the sides.
- Steam the substrate mixture in bags at 100^{o} C for 1-2 hours.
- Add wheat grain spawn (3% w/w) in two layers to the steamed substrate in polyethylene bags.
- Tie spawned bags on the top and keep them on benches in a mushroom house with sufficient light and ventilation.
- Cut polyethylene bags on the sides and remove them after 20 days of spawning, when the substrates become compact and whitish because of spawn run. While removing polyethylene, care should be taken not to disturb the compact hay bed.
- Spray water daily on the compact bed; maintain 80-90% humidity in the mushroom house by hanging moist gunny bags all around.
- Harvest mushrooms, which start coming up all along the sides of bag after 3-4 days from the day of removing polyethylene bags.

Harvest them by grasping the stalk by hands and then twisting and pulling it out.

– Record the number of fruiting bodies and yield in g/kg of dry substrates.