American-Eurasian J. Agric. & Environ. Sci., 7 (4): 433-439, 2010
ISSN 1818-6769

Development of Improved Technology for Commercial Production and Preservation of Shiitak Mushroom (*Lentinus edodes*)

L. Ramkumar, P. Thirunavukkarasu and T. Ramanathan

Centre of Advanced Study in Marine Biology, Annamalai University,
Parangipettai - 608 502, Tamil Nadu, India

Abstract: The present study was an attempt on commercialization of shiitake mushroom (*Lentinus edodes*). For spawn production, the additive $CaCo_3$ significantly enhanced the mycelial growth and basidiocarp formation. The substrate Ill-filled paddy with additive $CaCO_3$ enhanced the browning of mycelia (16.40 days), basidiocarp formation (51.80 days), yield (360.70 g/kg) and biological efficiency (36.07 per cent), which were better than other substrates and additives. Shiitake mushroom could be stored up to 10 and 30 days in perforated polybag under natural condition and refrigerated conditions respectively. Dehydrated mushroom could be stored for > 8 months in airtight containers. Shiitake mushroom was most suitable for canning and stored > 8 months under brine solution.

Key words: *Lentinus edodes* · Ill-filled paddy · Polybag · Canning and Dehydrated mushroom

INTRODUCTION

The black oak mushroom commonly known as shiitake, is the second most important edible mushroom in the world [1]. This mushroom has been cultivated in Japan and China for about 2000 years [2,3] as well in Thailand, Korea and Brazil. Among the countries, Japan is the major world producer of this mushroom, reaching production of 7.5 million tones [3,1]. Shiitake cultivation is widely practiced in Southeast Asia, also in North America, Europe, Australia and New Zealand [4,5]. The great interest in shiitake's commercialization is due to its unique flavour / taste, nutritive value and medicinal properties [6,7]. Its fungal mycelia has high content of proteins, fibers, vitamins, minerals and low content of lipid specifically cholesterol [8]. The production of shiitake mushroom has increased at an accelerated rate during last five years [2]. This increase in production is in response to increasing consumer demand and to the relatively high prices that farmers receive for the products. Thus, production of shiitake is potentially a lucarative commercial enterprise. However, the growers must be able to provide the specialized management of this mushroom. It is cultivated on logs of broad leaved trees especially tree trunk of Shia trees (*Quercus* sp.) or Oak. It has also been successful on the substrate enriched with sawdust [2]. Shiitake is globally a well known cultivated species, yet to find a place in Indian market. Lack of cultivation technology on locally available substrates and suitable high temperature strains are the reason for its non-availability in India. Despite sporadic research efforts to standardize its cultivation technology in India [9-11], it could not reach the commercial level, as available technology is not viable. A few growers in the State of Manipur and Mizoram initiated the cultivation of shiitake mushroom based on Japanese log system with limited success. However, the scenario is rapidly changing now. The two major research centers *viz.,* NRCM (Solan) and IIHR (Bangalore) are trying to develop cultivation technology based on locally available substrates [11,12]. The present study was carried out to finds suitable simple technologies for commercial production of shiitake mushroom.

MATERIALS AND METHODS

Isolation: Shiitake mushroom fungus was isolated on potato-dextrose agar (PDA) medium from freshly harvested sporophores, collected from the mushroom house, Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India. The sporophores of individual mushroom fungi were first

Corresponding Author: Mr. L. Ramkumar, Research Scholar, CAS in Marine Biology, Annamalai University, Parangipettai - 608 502, Tamil Nadu, India. Tel: +91 04144 243223

swabbed with 80 per cent ethyl alcohol to remove external microbial contaminants. At the junction of pielus and stripe, tissue bits taken separately, using a sterile foreceps, were surface disinfected with hypochlorite (commercial) for 5 min and rinsed with three changes of sterile distilled water. The surface sterilized tissue bits were placed on PDA in Petri dishes and incubated at laboratory temperature (25 ± 3°C) for 10 days.

Purification: Mushroom fungal cultures were purified by hyphal tip method [13]. A tip of actively grown mycelium from the individual culture transferred to PDA slant and maintained at 4°C with periodic transfer was used as stock cultures.

Effect of Substrates with Additives on Spawn Production of L. Edodes: Instead of sorghum grain, ill-filled paddy and sawdust were used as a substrate for fungal culture preparation. The ill-filled paddy substrate was collected from the local rice mill was presoaked in 0.1 per cent Carbendazim (BASF@, Germany) solution overnight [14]. The solution was drained completely and dried under shade. At 50 per cent moisture level [15], the substrate was mixed thoroughly with $CaCO_3$ / sawdust + corn flour @ 20g/kg and filled in wide mouth glass bottles to 3/4 capacity. Then the mouth was tightly plugged with non-absorbent cotton and sterilized at 15 lbs pressure for 2 h. The substrate was allowed to cool after autoclave sterilization and inoculated with fungal culture. A 10-mm mycelial disc from the fungal stock culture was transferred into the bottles aseptically and incubated at laboratory temperature (25 ± 3°C) for 15 days. Same procedure was followed for the preparation of sawdust spawn. However, sorghum grain spawn was prepared following different method [16]. For each and every treatment ten replicate bottles were maintained. Observation on fungal growth and basidiocarp formation was recorded daily.

Treatments

- T_1 – Sorghum grain + sawdust (20g/kg) + corn flour (20g/kg)
- T_2 – Sorghum grain + $CaCO_3$ (20g/kg)
- T_3 – Ill-filled paddy + sawdust (20g/kg) + corn flour (20g/kg)
- T_4 – Ill-filled paddy + $CaCO_3$ (20g/kg)
- T_5 – Sawdust + corn flour (20g/kg)
- T_6 – Sawdust + $CaCO_3$ (20g/kg)
- T_7 – Sorghum grain alone
- T_8 – Ill-filled paddy alone
- T_9 – Sawdust alone

Effect of Various Substrates with Additives on the Growth and Yield of L. Edodes: Shiitake mushroom was cultivated by using the synthetic or poly bag method of cultivation. The substrates *viz.,* silver oak sawdust, ill filled paddy, paddy straw and sugarcane trash, earhead with organic (corn flour) inorganic (gypsum and $CaCO_3$) additives were used separately @ 20g/kg of substrates. After browning, the top portion of polypropylene bag was removed and moistened the beds frequently to avoid drying of substrates.

Effect of Silver Oak Sawdust with Various Additives on the Growth and Yield of L. Edodes: Bed preparation [1,17,18,19,] was made. The silver oak sawdust substrates were initially steam sterilized (15 lbs pressure for 30 min) and used for the preparation of bags similar to that of other cultivable mushroom. At the time of bag preparation the moisture level of the saw dust was maintained at 50 per cent. The organic (corn flour) and inorganic (gypsum and $CaCO_3$) additives were mixed with sawdust @ 20g/kg. Then the spawn was inoculated separately @ 1 spawn / bag and gently plugged with non absorbent cotton and incubated in mushroom shed, where sufficient temperature (22-25°C) and humidity (> 85 per cent) were maintained.

Treatments

- T_1 – Silver oak sawdust + corn flour (20g/kg)
- T_2 – Silver oak sawdust + gypsum (20g/kg)
- T_3 – Silver oak sawdust + $CaCO_3$ (20g/kg)
- T_4 – Silver oak sawdust + $CaCO_3$ + corn flour + gypsum (20g/kg)
- T_5 – Silver oak sawdust + $CaCO_3$ + gypsum (20g/kg)
- T_6 – Silver oak sawdust + $CaCO_3$ + corn flour (20g/kg)
- T_7 – Silver oak sawdust + gypsum + corn flour (20g/kg)
- T_8 – Control (silver oak sawdust alone)

Observations on mycelial coverage, days for browning, basidiocarp formation and total yield were recorded. For each treatment the percentage of biological efficiency (BE) was calculated as per the formula.

$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of mushroom harvested/bag}}{\text{Dry weight of the substrate used/bag}} \times 100$$

Effect of Ill-filled Paddy with Various Additives on the Growth and Yield of L. Edodes: Ill-filled paddy substrate was pre treated as described earlier [14]. Addition of additives and bed preparation were followed as described

earlier. For each treatment ten replications were maintained. Observations on growth and yield parameters were recorded. Per cent of biological efficiency was calculated as described earlier.

Treatments

T_1 – Ill-filled paddy + corn flour (20g/kg)
T_2 – Ill-filled paddy + gypsum (20g/kg)
T_3 – Ill-filled paddy + $CaCO_3$ (20g/kg)
T_4 – Ill-filled paddy + $CaCO_3$ + corn flour + gypsum (20g/kg)
T_5 – Ill-filled paddy + $CaCO_3$ + gypsum (20g/kg)
T_6 – Ill-filled paddy + $CaCO_3$ + corn flour (20g/kg)
T_7 – Ill-filled paddy + gypsum + corn flour (20g/kg)
T_8 – Control (ill-filled paddy alone)

Effect of Paddy Straw with Various Additives on the Growth and Yield of L. Edodes: Well dried chopped paddy straw (5 cm length) @ 1 kg / bed, previously soaked in water for 12 h, followed by steam sterilization (15 lbs pressure for 30 min.) was used for the preparation of cylindrical (polybag 2×1") beds similar to that of oyster mushroom bed preparation. At the time of bed preparation the moisture level of the straw was maintained at 50 per cent. First, the straw was placed inside the polybag and pressed gently upto 10 cm height. This was sprinkled with organic/inorganic additives @ 20g/kg of substrate. Then one segment of ill-filled paddy spawn (one spawn = 4 segments) was sprinkled over the treated straw. Like wise, 4 layers of seed + additives were prepared. Then, finally it was filled with straw at 10 cm height [14].

Treatments

T_1 – Paddy straw + corn flour (20g/kg)
T_2 – Paddy straw + gypsum (20g/kg)
T_3 – Paddy straw + $CaCO_3$ (20g/kg)
T_4 – Paddy straw + $CaCO_3$ + corn flour + gypsum (20g/kg)
T_5 – Paddy straw + $CaCO_3$ + gypsum (20g/kg)
T_6 – Paddy straw + $CaCO_3$ + corn flour (20g/kg)
T_7 – Paddy straw + gypsum + corn flour (20g/kg)
T_8 – Control (paddy straw alone)

Effect of Sugarcane Trash with Various Additives on the Growth and Yield of L. Edodes: Same methodology described in paddy straw was followed for the preparation of cylindrical bed. Similar observations were also recorded as in previous experiment.

Treatments

T_1 – Sugarcane trash + corn flour (20g/kg)
T_2 – Sugarcane trash + gypsum (20g/kg)
T_3 – Sugarcane trash + $CaCO_3$ (20g/kg)
T_4 – Sugarcane trash + $CaCO_3$ + corn flour + gypsum (20g/kg)
T_5 – Sugarcane trash + $CaCO_3$ + gypsum (20g/kg)
T_6 – Sugarcane trash + $CaCO_3$ + corn flour (20g/kg)
T_7 – Sugarcane trash + gypsum + corn flour (20g/kg)
T_8 – Control (sugarcane trash alone)

Preservation and Canning of Mushrooms:

Short Term Preservation: Harvested mushrooms were cleaned and packed separately in perforated polythene bags (about 20 holes) and placed under natural condition. Under refrigerated condition, they were placed in non-perforated polythene bags. Observation on keeping quality was recorded daily. Each treatment replicated five times.

Long Term Preservation: The harvested mushroom was washed separately in clean cool water to remove the dirt. The cleaned mushrooms were blanched separately in hot water for 3 min.

Then, they were sun dried for 8 h. After drying the mushrooms were powdered using pulverizer. After the powdered mushroom was kept in an air tight container for storage at room temperature (30 ± 2°C) [20].

Canning of Mushrooms: Fresh shiitake mushroom was selected and washed with cleaned water, presoaked for 30 min in 2 per cent salt solution containing 0.1 per cent citric acid and 100 mg ascorbic acid. Drained the solution and filled into cans, covered with hot 2 per cent brine containing 0.1 per cent citric acid and 100 mg ascorbic acid and exhausted at 85°C for 5-7 min. sealed immediately and sterilized at 116°C for 35 min. Cooled and stored at ambient temperature [21]. Observation on keeping qualities was recorded after canning.

Statistical Analysis: All experiments were done in three replicates except first experiment (Ten replicates) and mean values are presented. Statistical analysis was performed on the data by Dunccan's Multiple Range Test (DMRT) with means followed by a common letter are not significantly different at the 5% level by DMRT.

RESULTS AND DISCUSSION

The results of the present experiment spawn production, clearly indicated that ill-filled paddy overnight soaking in carbendazim 0.1 per cent and $CaCO_3$ (20g/kg) significantly increased the mycelial growth and covered the container within 12.30 days. Basidiocarp formation inside the container was also early (53.20 days) by this treatment (Table 1). This was followed by ill-filled paddy + sawdust + corn flour (17 and 61 days), sorghum grain + sawdust + corn flour (18 and 66 days), sorghum grain + $CaCO_3$ (19 and 60 days), sawdust + $CaCO_3$ (21 and 76 days) and sawdust + corn flour (23 and 74 days). The suitability of ill-filled paddy + $CaCO_3$ for spawn production has also been reported by Kalaiselvan [22]. The fast mycelial growth and basidiocarp formation due to incorporation of additive $CaCO_3$ might be due to the neutral pH of the substrate and prevention of substrate aggregation. Moreover, this additive provides sulphur and calcium which are essential mineral nutrients for the growth of mushroom [23]. Good growth of the fungus without contamination might be due to incorporation of fungicide carbendazim 0.1 per cent. Ill-filled paddy spawn has been reported to prevent rat damage in mushroom production. Non preference of ill-filled paddy to rot might be due poor grain filling and throat irritation [14].

In the present study, various substrates with various additives on the growth and yield of *L. edodes,* silver oak sawdust + $CaCO_3$ treatments was significantly superior in reducing the days for mycelial coverage (25.7 days), browning (41.4 days), basidiocarp formation (71.6 days) and increased the yield (55.04 per cent) and biological efficiency (31.08 per cent) (Table 2). The treatment of ill-filled paddy + $CaCO_3$ encouraged the mycelial growth (16.4 days), browning (35.7 days) and basidiocarp formation (51.8 days) with highest yield of 360.70g/kg and the highest biological efficiency of 36.07 per cent (Table 3). The paddy straw treatments, highest biological efficiency (9.01 per cent) was recorded in paddy straw + $CaCO_3$ (Table 4). In general, delayed mycelial coverage, browning and basidiocarp formation were recorded in sugarcane trash substrate with various additives. Among the treatments, maximum yield of 35.2g/kg (BE: 3.52 per cent) was recorded in sugarcane trash + $CaCO_3$ (Table 5).

Moreover, this substrates is pre-soaked in 0.1 per cent carbendazim solution (over night), the chance of substrate contamination was fully eliminated. Similar result was recorded in spawn production, where ill-filled paddy was presoaked in carbendazim 0.1 per cent [14]. Though various agro wastes such as oak, horn bean, sweet gum, poplar, alder, ironwood, willow, pine, maple

Table 1: Influence of various substrates with additives on spawn production

Additives (2 %)	Mycelial growth (days)	Days for basidiocarp production
Sorghum grain + sawdust + corn flour	17.80^{c}	103
Sorghum grain + $CaCO_3$	19.20^{d}	214
Ill- filled paddy + sawdust + corn flour	16.50^{b}	120
Ill- filled paddy + $CaCO_3$	12.30^{a}	121
Sawdust + corn flour	22.90^{f}	210
Sawdust + $CaCO_3$	20.50^{e}	20
Sorghum grain alone	23.40^{g}	53
Ill -filled paddy alone	28.60^{i}	11
Pine College	26.90^{h}	4
Sawdust alone	17.80^{c}	52

Mean of ten replicates

Means followed by a common letter are not significantly different at the 5% level by DMRT (Dunccan's Multiple Range Test)

Table 2: Effect of silver oak sawdust with various additives on the growth and yield of *L. edodes*

Additives (20g / kg)	Mycelial coverage (days)	Browning (days)	Basidiocarp production (days)	Total yield (g/kg)	BE %	% increase yield
Silver oak Sawdust + corn flour	27.30^{b}	45.60^{b}	73.60^{ab}	280.45^{c}	28.04^{bc}	(31.97) 39.88^{d}
Silver oak Sawdust + gypsum	37.40^{h}	46.30^{c}	78.80^{c}	240.49^{f}	24.04^{e}	(29.36) 19.95^{g}
Silver oak Sawdust + $CaCO_3$	25.70^{a}	41.40^{a}	71.60^{a}	310.84^{a}	31.08^{a}	(33.88) 55.04^{a}
Silver oak Sawdust + $CaCO_3$ + corn flour + gypsum	28.20^{c}	47.60^{e}	75.80^{b}	290.64^{b}	29.06^{b}	(32.62) 44.96^{b}
Silver oak Sawdust + $CaCO_3$ + gypsum	31.10^{g}	49.40^{g}	80.50^{cd}	270.56^{d}	27.05^{cd}	(31.33) 34.94^{e}
Silver oak Sawdust + $CaCO_3$ + corn flour	29.60^{d}	47.30^{d}	74.60^{b}	280.54^{c}	28.05^{bc}	(31.98) 39.92^{c}
Silver oak Sawdust + gypsum + corn flour	30.20^{f}	50.50^{h}	80.70^{cd}	260.72^{e}	26.07^{d}	(30.70) 30.04^{f}
Control (silver oak sawdust alone)	29.80^{e}	48.80^{f}	78.60^{c}	200.49^{g}	20.04^{f}	(26.59) -

Mean of ten replicates

Figures in parentheses are arcsine transformed values

Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 3: Effect of ill filled paddy with various additives on the growth and yield of *L. edodes*

Additives (20g / kg)	Mycelial coverage (days)	Browning (days)	Basidiocarp production (days)	Total yield (g/kg)	BE %	% increase yield
Ill-filled paddy + corn flour	17.30[b]	39.40[e]	56.20[c]	290.50[d]	29.05[d]	(32.61) 31.99[d]
Ill-filled paddy + gypsum	22.50[g]	40.30[f]	62.50[h]	260.35[g]	26.03[g]	(30.67) 18.26[g]
Ill-filled paddy + $CaCO_3$	16.40[a]	35.70[b]	51.80[a]	360.70[a]	36.07[a]	(36.91) 63.84[a]
Ill-filled paddy + $CaCO_3$+ corn flour + gypsum	21.70[f]	37.20[c]	58.30[d]	300.15[c]	30.01[c]	(33.21) 36.33[c]
Ill-filled paddy + $CaCO_3$ + gypsum	20.20[e]	41.40[g]	61.40[g]	280.20[e]	28.02[e]	(31.96) 27.27[e]
Ill-filled paddy + $CaCO_3$+ corn flour	18.40[c]	35.30[a]	53.70[b]	320.10[b]	32.01[b]	(34.45) 45.40[b]
Ill-filled paddy + gypsum + corn flour	19.30[d]	41.60[h]	60.30[f]	270.45[f]	27.04[f]	(31.33) 22.84[f]
Control (ill-filled paddy alone)	21.80[f]	38.70[d]	59.40[e]	220.15[h]	22.01[h]	(27.97) -

Mean of ten replicates
Figures in parentheses are arcsine transformed values
Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 4: Effect of paddy straw with various additives on the growth and yield of *L. edodes*

Additives (20g / kg)	Mycelial coverage (days)	Browning (days)	Basidiocarp production (days)	Total yield (g/kg)	BE %	% increase yield
Paddy straw + corn flour	37.80[g]	42.20[a]	88.20[f]	60.20[f]	6.02[f]	(14.02) 47.73[f]
Paddy straw + gypsum	35.30[f]	43.30[b]	87.40[e]	70.15[d]	7.01[d]	(15.35) 72.14[d]
Paddy straw + $CaCO_3$	32.90[a]	43.50[b]	84.70[a]	90.10[a]	9.01[a]	(17.46) 121.10[a]
Paddy straw + $CaCO_3$+ corn flour + gypsum	34.50[d]	41.90[a]	85.40[b]	85.25[b]	8.52[b]	(16.97) 109.20[b]
Paddy straw + $CaCO_3$+ gypsum	33.70[c]	43.90[b]	86.70[d]	75.30[c]	7.53[c]	(15.92) 84.78[c]
Paddy straw + $CaCO_3$+ corn flour	33.30[b]	43.70[b]	86.50[c]	65.45[e]	6.54[e]	(14.81) 60.61[e]
Paddy straw + corn flour + gypsum	34.80[e]	44.20[c]	87.30[e]	55.30[g]	5.53[g]	(13.60) 35.70[g]
Control (paddy straw alone)	38.70[h]	41.30[a]	89.60[g]	40.75[h]	4.07[h]	(11.63) -

Mean of ten replicates
Figures in parentheses are arcsine transformed values
Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 5: Effect of Sugarcane trash with various additives on the growth and yield of *L. edodes*

Additives (20g / kg)	Mycelial coverage (days)	Browning (days)	Basidiocarp production (days)	Total yield (g/kg)	BE %	% increase yield
Sugarcane trash + corn flour	48.50[e]	61.20[b]	99.40[g]	25.05[e]	2.50[c]	(9.09) 65.89[e]
Sugarcane trash + gypsum	48.90[f]	61.30[b]	97.50[d]	20.15[f]	2.01[d]	(8.15) 33.44[f]
Sugarcane trash + $CaCO_3$	44.70[a]	60.90[a]	95.30[b]	35.20_a	3.52[a]	(10.81) 133.11[a]
Sugarcane trash + $CaCO_3$+ corn flour + gypsum	45.40[b]	61.50[c]	96.20[c]	30.15[c]	3.01[b]	(9.99) 99.66[c]
Sugarcane trash + $CaCO_3$+ gypsum	46.90[c]	62.20[e]	98.30[e]	30.25[b]	3.02[b]	(10.00) 100.33[b]
Sugarcane trash + $CaCO_3$+ corn flour	47.70[d]	61.90[d]	98.70[f]	25.15[d]	2.51[c]	(9.11) 66.55[d]
Sugarcane trash + corn flour + gypsum	50.30[g]	61.30[b]	98.40[e]	20.05[g]	2.00[d]	(8.13) 32.78[g]
Control (sugarcane trash alone)	52.50[h]	62.80[f]	94.60[a]	15.10[h]	1.51[e]	(7.05) -

Mean of ten replicates
Figures in parentheses are arcsine transformed values
Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 6: Preservation and canning of Shiitake mushroom

Method of preservation	Shelf life
Short term preservation	
Natural condition	10 days
Refrigerator condition	30 days
Long term preservation	
Powder (dehydration)	> 8 months
Canning (brine solution)	> 8 months

and birch sawdust, cereal straw, corn cobs, sugarcane bagasse, tea waste, sunflower seed hulls, peanut shells, coffee straw and seed hulls can be used alone or in combination with other wastes in shiitake cultivation [23-26], the substrate ill-filled paddy with additive $CaCO_3$ (2 per cent) for successful cultivation of shiitake has not been reported so far. However, a widely used standard substrate formula is 80 per cent hardwood sawdust and 20 per cent supplements on a dry weight basis [27]. Some formulation, with all ingredients based on oven dry substrate weight, consisting of 80 per cent sawdust and 20 per cent bran in Asia [28], 80 per cent sawdust, 10 per cent bran and 10 per cent wheat or millet in USA [27] and 84 per cent sawdust, 5 per cent rice bran, 5 per cent wheat bran, 3 per cent soybean and 3 per cent lime in Taiwan [29], are commonly used for *L. edodes* cultivation as standard substrates. Swiss researchers have reported that the mixture of 75 per cent spruce sawdust, 24 per cent wheat bran and 1 per cent lime can be used for the successful cultivation of *L. edodes*.

The storage life of shiitake mushroom is similar to that of white button mushroom *Agaricus* spp [30]. The results of the present study showed that shiitake

mushroom could be stored up to 10 days in perforated polythene bags under natural condition. They could be stored up to 30 days under refrigerated condition (polybags without perforation) (Table 6). Among the various methods employed for the preservation of mushroom, canning is the most frequently adopted method on commercial scale both for domestic consumption as well as for exports [31, 21].

REFERENCES

1. Royse, D.J., 2005. Cultivation of shiitake on natural and synthetic logs. http://www.cas.psu.edu/ FreePubs/pdfs/ul203.pdf, Penn State's College of Agricultural Science.
2. Silva, E.M., 2003. Analyses of crescimento mycelial e das atividades lignocelulolíticas produzidas Durante o cultivable *Lentinula edodes* emresiduo de *Eucalyptus saligna*. Dissertacao de Mestrado, FAENQUIL. Lorena, pp: 274-276.
3. Smith, J.E., N.J. Rowan and R. Sullivan, 2002. Medicinal mushrooms: Their therapeutic properties and current medical usage with special emphasis on cancer treatments. In Cancer Research, EUA. University of Strathelyde, pp: 200-202.
4. Oei, P., 1996. Mushroom cultivation. Tool Publicatons, Leiden. The Netherlands, pp: 126-137 and 93-204.
5. Romanens, P., 2001. Shiitake, the European reality and cultivation on wood-chips logs in Switzerland. 15th North American Mushroom Conference, Las Vagas. U.S.A, pp: 140-142.
6. Sugui, M.M., P.L.A. Lima, R.D. Delmanto, A.F. Eira, D.M.F. Salvadori and L.R. Ribeiro, 2003. Antimutagenic effect of *Lentinula edodes* (Berk.) Pegler mushroom and possible variation among lineages. Food and Chemical Toxicol., 41: 555-560.
7. Silva, E.S., J.R.P. Cavallazzi., G. Muller and J.V.B. Souza, 2007. Biotechnological applications of *Lentinus edodes*. J. Food Agriculture & Environment, 5(3& 4): 403-407.
8. Yang, B.K., D.H. Kim, S.C. Jeong, S. Das, Y.S. Choi, J.S. Shin, S.C. Lee and C.H. Song, 2002a. Hypoglycemic effect of a *Lentinus edodes* exo-polymer produced from a submerged mycelial culture. Bioscience, Biotechnology and Biochemistry, 52: 89-91.
9. Thakur, K. and S.R. Sharma, 1992. Substrate and supplementation for the cultivation of shiitake, *Lentinus edodes* (Berk.) Sing. Mush Information, 9(7/8): 7-10.
10. Shukla, A.N., 1995. Effect of hormones on the production of shiitake, *Lentinus edodes* (Berk.) Sing. Mushroom Res., 4: 39-42.
11. Sharma, S.R., S. Kumar and V.P. Sharma, 2006. Physiological Requirement for Cultivation of Malaysian Strain of Shiitake mushroom. J. Mycology and Plant Pathol., 36(2):149-152.
12. Pandey, M., 2005. Mushroom Research and Development of India. http://www. mushworld.com/ oversea/view.asp?_id=5140&vid=7165.
13. Rangaswamy, G., T.K. Kandaswamy and K. Ramasamy, 1975. *Pleurotus sajor-caju* (Fr.) Singer- protein rich nitrogen fixing mushroom fungus. Current Sci., 44: 403-444.
14. Lakshmanan, P., 2004. New crop Varieties, Farm Implements and Management Technologies, Tamil Nadu Agricultural University. Coimbatore, pp: 74-77.
15. Piper, C.S., 1966. Soil and Plant Analysis, Bombay. Hanos Publishers.
16. Alice, D., M. Muthusamy and I. Yesuraja, 1999. History and mycetismus of mushrooms. Mushroom culture. Tamil Nadu Agricultural University, Madurai, pp: 87.
17. Chen, A.W., N. Arrold and P. Stamets, 2000. Shiitake cultivation systems. Griensven (Eds.). Science and cultivation of Edible Fungi, Balkema, Rotterdam, the Netherlands, pp: 125.
18. Thiribhuvanamala, G., A.S. Krishnamoorthy, K. Shanthi and T. Marimuthu, 2005a. Development of suitable substrates for spawn and mushroom production of *Lentinula edodes* and *Auricularia polytricha*. Madras Agriculture J., 92(4-6): 344-348.
19. Thiribhuvanamala, G., A.S. Krishnamoorthy, K. Shanthi and T. Marimuthu, 2005b. Scope for commercialization of medicinal mushrooms in India. World J. Microbiology and Biotechnol., 7(2): 263-269.
20. Kozak, M. and J. Krawczyk, 1989. Growing shiitake mushrooms in a continental climate. Field and Forest Products, 10: 312-315.
21. Sandhu, K.S. and P. Agarwal, 2001. Studies on various methods for the preservation of mushroom. J. Research. Punjab Agricultural University, 38: 53-57.
22. Kalaiselvan, B., 2007. Studies on modern Techniques for cultivation of paddy straw mushroom (*Volvariella volvacea* (Bull. ex Fr.)) Sing., on commercial scale. M.Sc. (Ag.) Thesis. Tamil Nadu Agricultural University, Coimbatore, pp: 89-95.
23. Curvetto, N., D. Figlas and S. Delmastro, 2002. Sunflower seed hulls as substrate for the cultivation of shiitake mushrooms. Horticulture Technol., 12: 652-655.

24. Morais, M.H., A.C. Ramos, N. Matos and E.J. Santos- Olivera, 2000. Production of shiitake mushroom (*Lentinula edodes*) on lignocellulosic residues. International J. Food Science and Technol., 6: 123-128.
25. Rossi, I.H., A.C. Monteiro, J.O. Machado, J.L. Andrioli and J.C. Barbosa, 2003. Shiitake *Lentinula edodes* production on a sterilized bagasse substrate enriched with rice bran and sugarcane molasses. Brazilian J. Microbiol., 34: 66-71.
26. Salmones, D., G. Mata., L.M. Ramos and K.N. Waliszewski, 1999. Cultivation of shiitake mushroom *Lentinula edodes* in several lignocellulosic materials originating from subtropics. Agronomie, 19(1):13-19.
27. Miller, M.W. and S.C. Jong, 1987. Commercial cultivation of shiitake in sawdust filled plastic bags. In: P.J. Wuest, D.J. Royse and R.B. Beelman, (eds.): Development in Crop Science, Cultivating Edible Fungi. Elsevier Publishers. The Netherlands, pp: 421-426.
28. Lizuka, C. and M. Takeuchi, 1978. Method of Artificially Growing Edible Fungi. US Patent, 4: 971- 973.
29. Kalberer, P.P., 1987. Experiments on the cultivation of shiitake (*Lentinus edodes*) on sawdust. Mushroom Sci., 12: 231- 235.
30. Przybylowicz, P. and J. Donoghue, 1988. Shiitake Grower's Handbook. Kendall/Hunt Publishing Co., Dubuque. Iowa, pp: 457-459.
31. Coskumar, Y. and Y. Ozdemir, 2000. The possibilities of using EDTA as browning inhibitor as well as control spoilage during storage and canning operations. J. the Science of Food and Agriculture, 80: 2074-2076.