Growing Shiitake Mushroom on Hardwood Sawdust in the Greenhouse

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Summary. Seven strains of shiitake mushroom [*Lentinula edodes* (Berk.) Pegler] produced spawn in culture vessels containing hardwood sawdust amended with YVMBS (yeast extract, multigrain oatmeal, brown sugar) broth within 35 to 45 days after mycelia inoculations. Under greenhouse conditions, shiitake basidiocarps (mushroom fruit) appeared from 1 to 3 months after spawn inoculations of the hardwood sawdust amended with YVMBS broth. The shiitake mushroom strains LE2, LE1, LE6, and LE5 had 10.4, 7.3, 2.5, and 1.6 times more fresh harvested basidiocarps, respectively, on the amended hardwood sawdust compared to the controls. The amount of basidiocarps produced by the shiitake strains LE3, LE4, and LE7 was the same on both hardwood sawdust treatments. The basidiocarps of LE1, LE2, LE3, LE4, and LE5 were averaged 5 inches (12.7 cm) in diameter, however, the basidiocarps of LE6 and LE7 were averaging only 2 inches (5.1 cm) in diameter. The only pests of the shiitake basidiocarps in the greenhouse were slugs, but they were easily controlled by applying table salt. The start up cost of inoculating 100 shiitake spawn blocks on hardwood sawdust on one bench in the greenhouse was $77. The start up cost of inoculating shiitake spawn on 100 logs was $1,329.75. In 1 year, shiitake strains LE1, LE2, LE5, and LE6 produced 19.5, 20.2, 7.9, and 4.5 lb (8.8, 9.2, 3.6, and 2.0 kg), respectively, of harvested fresh basidiocarps on amended hardwood sawdust in the greenhouse. The mushrooms retail for $3.20 to $4.20/lb ($7.05 to $9.26/kg). The use of the hardwood sawdust amended with YVMBS broth for shiitake production in the greenhouse has considerable economic potential for shiitake mushroom growers.

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The shiitake mushroom (*Lentinula edodes*) is a wood-rotting, but potentially useful fungus that belongs to the class basidiomycetes. The basidiocarp (caps and stipe) is edible. Compared to the common button mushroom [*Agaricus bisporus* (Lange) Sing.], shiitake is considered an exotic mushroom. It is becoming popular in the United States for its food and medicinal value. In fact, some shiitake researchers believed that this mushroom may become equal to the soybean [*Glycine max* (L.) Merrill] crop in terms of commercial benefits (Packer, 1984).

The world production of the shiitake mushroom was ≈700 million lb (318 million kg) in 1986 (Chang, 1987). The United States imports ≈90% of the world's dried shiitake mushrooms worth about $1 million (Turner, 1988). Production of the fresh shiitake mushroom crop in the United States was 4.8 million lb (2.2 million kg) of shiitake mushroom in 1991 worth about $16.3 million (USDA, 1991); and 6.2 million lb (2.8 million kg) worth about $19.8 million in 1995–96 (USDA, 1996).

Shiitake mushrooms are traditionally produced by inoculating spawn into holes on logs, which usually bear basidiocarps in ≈1 to 2 years depending on the shiitake strain used for the inoculation (Donoghue, 1994; Sabota, 1992, 1994). Also, it takes ≈4 to 6 months to grow shiitake mycelia in any substrate to attain spawn texture (Sabota, 1992, 1994). Since, there have been no previous reports of attempts to grow shiitake mushroom on hardwood sawdust in the greenhouse; the purpose of the present study was to determine if the seven-strain collection of *L. edodes* could produce basidiocarps on hardwood sawdust in the greenhouse.

Materials and methods

The strains of *L. edodes* (LE) used in this study were LE1 [isolate #48860 from American Type Culture Collection (ATCC), Rockville, Md.], a summer fruiting commercial mushroom; LE2 (isolate #48861 from ATCC, Rockville, Md.), a warm-weather mushroom strain; LE3 (WW 44, Lot 34A Unit #8 of Field and Forest Products, Inc., Peshtigo, Wis.), a warm weather mushroom strain; LE4 (WW 70, Lot 915B, Unit #4 of Field and Forest Products, Inc., Peshtigo, Wis.), a warm weather mushroom strain; LE5 (CS-125, Batch 754, Unit #8 of Field and Forest Products, Inc., Peshtigo, Wis.), a cold weather mushroom strain; LE6 (isolate #48855 from ATCC, Rockville, Md.), a summer fruiting commercial mushroom; and LE7 (isolate #48856 from ATCC, Rockville, Md.), a winter fruiting mushroom. The shiitake strains were maintained in the laboratory in sterile YVMBSA medium (Pacumbaba and Pacumbaba, 1999) in petri dishes. YVMBSA medium consists of 0.60 g of yeast extract, 60 mL V-8 vegetable juice, 52 g of multigrain oatmeal (grounded to floury texture), 10 g brown sugar (semipure sugar condiment), 10 g bacto-agar (Difco Co., Detroit, Mich.), and 1.0 L distilled water.

Spawn growth in the laboratory. The sawdust was a combination or individual components of oaks (red and white), maple, hickory, tulip poplar, white willow, sycamore, cherry, sweetgum, hophornbeam, american hornbeam/ironwood, ash, osage orange, hackberry, birch, etc. from sawdust of hardwood lumbers, obtained from Moss Lumber Industries, Gurley, Ala., used for the production of spawn in this study. The hardwood sawdust was either fresh or aged (2 to 6 months) and passed through a sieve [#H 10/64 inch × 3/4-inch (1.91-cm) slotted; Seedburo Equipment Co., Chicago, Ill] to obtain uniform sawdust texture, before placing it in P4928 culture containers. The size of the culture container was 3.5 inches bottom diameter × 4.25 inches high × 4.5 inches top diameter (8.89 × 10.80 × 11.43 cm) with lid, (Phytacon vessels, Sigma Chemical Co.,

Fig. 1. (A) P4928 vessels (Phytacon, Sigma Chemical Co., St. Louis, Mo.) where the shiitake mycelia were grown, turning into spawn texture in 35 to 45 d. (B) Basidiocarps of shiitake mushroom on the halved spawn block and on the amended hardwood sawdust inside the greenhouse.

St. Louis, Mo.). The carbon to nitrogen (C:N) ratio of the hardwood sawdust was not determined. To each of the P4928 culture vessel filled with hardwood sawdust, 4.06 fl oz (120 mL) of YVMBS broth was added and autoclaved at 15 psi (6.82 kg·cm^{-2}) for 20 min. YVMBS broth consists of 0.6 g yeast extract, 60 mL V-8 vegetable juice, 52 g multigrain oatmeal (grounded to floury texture), 10 g brown sugar (semipure sugar condiment), and 1.0 L distilled water. The cooled autoclaved vessels were then inoculated with axenic portions of agar block containing shiitake mycelia, which had been growing on YVMBSA medium and were allowed to develop to spawn texture for 35 to 45 d (Fig. 1A). The spawn blocks consisting of profusely growing mycelia were separated from the inside walls by applying pressure to the side of the culture vessels, which induced production of basidiocarp initials or pins 3 to 5 d later.

GREENHOUSE MUSHROOM PRODUCTION. The greenhouse bench was supported from the ground with a 24 × 2-inch (60.96 × 5.08-cm) diameter metal pipes and the bottom was made of masonite measuring 248 inches long × 48 inches wide × 8 inches high (6.30 × 1.22 × 0.21 m). The bottom of the bench was variegated masonite, which allowed water to drain. The bench was divided into two equal sections laterally, filled with the hardwood sawdust, and inoculated with one strain of shiitake mushroom. Hardwood sawdust (amended treatment) was placed in 14 × 19-inch (35.56 × 48.26-cm) polypropylene autoclave bag, two-thirds full and 1.06 quarts (1.0 L) of YVMBS broth was added. Control bag (nonamended treatment) was added with 1.06 quarts (1.0 L) distilled water. All the bags were autoclaved at 15 psi (6.82 kg·cm^{-2}) for 20 min. Twenty bags containing amended hardwood sawdust were placed on the half section of the bench, and another 20 bags containing the nonamended sawdust were placed on the other half section of the bench.

The spawn blocks with basidiocarp initials or pins were divided into halves lengthwise and laid on the amended or the nonamended hardwood sawdust treatments on the bench. The spacing was 1.36 inches (3.45 cm) to 1.60 inches (4.06 cm) between and within the rows of these spawn blocks, then partly covered with the amended or nonamended hardwood sawdust. Two hundred spawn block halves were required to inoculate one bench. Divided spawn blocks of LE1 and LE2 were inoculated on amended and nonamended hardwood sawdust treatments in the greenhouse in July 1995 (Pacumbaba, 1996) and divided spawn blocks of LE3, LE4, LE5, LE6, and LE7 were inoculated on amended and nonamended hardwood sawdust treatments in the greenhouse in July 1996. The inoculated benches were watered by automated mist spray for 40 s every 4 h for 12 h each day. Harvesting of the shiitake mushroom basidiocarps was done by cutting the stipe attached close to the sawdust by scalpel every 2 d during the full flush, and every 3 to 5 d when the shiitake mushrooms were less abundant. Slugs liked to feed on the basidiocarps of shiitake mushroom and were the major pest problem in the greenhouse, but were easily controlled by applying table salt. The experimental design was randomized block with no replication.

The greenhouse was made of glass, and cooled automatically by PDR-4 water distribution and return system mounted on one side of the greenhouse with two, 42-inch (106.7-cm)

fans mounted on the opposite sides of the greenhouse, which pulled the air from the inside to the outside. The siding of the greenhouse was whitewashed. The light intensity was then reduced to ≈63% by black polypropylene shade fabric placed on the roof of the greenhouse. During late fall, winter, and early spring, the inside temperature of the greenhouse was maintained from 65 to 75 °F (18.3 to 23.9 °C) by two built-in heaters. At late spring, summer and early fall, the temperatures inside the greenhouse during the day would vary from 75 to 95 °F (23.9 to 35 °C) and 65 to 75 °F (18.3 to 23.9 °C) during the night.

Results and discussion

The basidiocarps of *L. edodes* appeared on the halved spawn blocks and the inoculated hardwood sawdust amended treatment with YVMBS broth (Pacumbaba, 1995, 1996; Pacumbaba and Pacumbaba, 1999) in the greenhouse 1 and 3 months, respectively, after inoculation (Fig. 1B). Strains LE2, LE1, LE6, and LE5 had 10.4, 7.3, 2.5, and 1.6 times more harvested basidiocarps on amended hardwood sawdust compared the control treatment (Table 1). However, the strains LE3, LE4, and LE7 on the amended hardwood sawdust treatment had almost equal amounts of harvested fresh basidiocarps compared to the control (Table 1). The effects of months, strains, and broth on yield of *L. edodes* grown on hardwood sawdust in the greenhouse were highly significant (P = 0.01 or 0.001) (Table 2). The interaction of strain × broth on yield of *L. edodes* grown on hardwood sawdust was also highly significant (P = 0.001) (Table 2). The amount of basidiocarps obtained in this study greatly depended on the strains of shiitake mushroom used. Also, strains LE1, LE2, LE3, LE4, and LE5 had an average basidiocarp of 5 inches (12.7 cm) in diameter, as compared to the basidiocarps of strains LE6 and LE7, which only had a 2 inch (5.1 cm) diameter. No attempt was made to analyze the hardwood sawdust for C:N ratio, hydrogen ion concentration (pH), and electrical conductivity (EC) during the shiitake cropping program.

The estimated cost of producing one spawn block in a tissue culture container was $0.52. The estimated cost of producing 100 spawn blocks to inoculate one bench was $52. The estimated cost of one bag containing amended hardwood sawdust was $0.80. The estimated cost of 40 bags containing amended and nonamended hardwood sawdust was $25. The estimated start up cost of inoculating amended and nonamended hardwood sawdust on a 248 × 48 × 8-inch (6.30 × 1.22 × 0.20-m) bench with 100 shiitake spawn blocks in the greenhouse was $77. These estimates do not include the labor cost, electricity, watering system, etc. The estimated start up cost of inoculating 100 logs, 3 to 8 inches (7.62 to 20.32 cm) in diameter × 4 ft (1.22 m) long, with shiitake spawn was $1,329.75 (Sabota and Nall, 1993). In only 1 year, the shiitake mushroom strains LE1, LE2, LE5, and LE6 produced 19.5, 20.2, 7.9 and 4.5 lb (8.8, 9.2, 3.6, and 2.0 kg), respectively, of harvested fresh basidiocarp on amended hardwood sawdust in the greenhouse. Shiitake mushrooms retail for $3.20 to $4.20/lb ($7.05 to $9.26/kg) indicating that using hardwood sawdust amended with YVMBS broth for shiitake production in the greenhouse has significant economic potential to shiitake mushroom growers.

Conclusion

This is the first time that the shiitake mushroom has been induced to produce basidiocarps on hardwood sawdust amended with YVMBS broth in the greenhouse in 1 to 3 months after spawn block inoculation. The amount of basidiocarps obtained greatly depends on the shiitake strains used. The results indicated that using hardwood sawdust amended with YVMBS broth considerably speeds up fructification of shiitake mushroom compared to log inoculation. The start up cost of 100-spawn block for inoculation of one bench with hardwood sawdust amended with YVMBS broth was $77 while the start up cost of inoculating 100 logs was $1,329.75.

Table 1. Production of basidiocarps by the seven strains of *Lentinula edodes* (LE) on hardwood sawdust amended with yeast extract, multigrain oatmeal, and brown sugar (YVMBS) broth in the greenhouse for 1 year.

Shiitake strain	YVMBS treatment[y]	Wt of fresh basidiocarps[z] (lb) Harvest month July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	Annual yield
LE1[x]	A	0.00	0.00	2.68	3.89	1.64	0.64	0.14	0.52	0.92	2.92	4.04	2.08	19.50
C[w]	N	0.00	0.00	0.21	0.50	0.15	0.16	0.02	0.22	0.23	0.44	0.58	0.38	2.69
LE2	A	0.00	0.00	7.19	6.76	1.71	0.30	0.12	0.68	0.28	1.46	1.27	0.37	20.15
C	N	0.00	0.00	0.50	0.54	0.18	0.03	0.01	0.09	0.04	0.29	0.21	0.07	1.94
LE3	A	0.00	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
C	N	0.00	0.00	0.13	0.14	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.32
LE4	A	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
C	N	0.00	0.00	0.02	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
LE5	A	0.00	0.00	1.47	1.50	1.45	0.55	0.17	2.33	0.42	0.00	0.00	0.00	7.89
C	N	0.00	0.00	0.73	0.82	1.49	0.60	0.03	1.34	0.10	0.00	0.00	0.00	5.10
LE6	A	0.00	0.00	0.00	1.20	2.06	0.73	0.04	0.29	0.17	0.00	0.00	0.00	4.49
C	N	0.00	0.00	0.00	0.02	0.84	0.56	0.04	0.28	0.08	0.00	0.00	0.00	1.82
LE7	A	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.25	0.09	0.00	0.00	0.00	0.36
C	N	0.00	0.00	0.00	0.00	0.00	0.02	0.03	0.31	0.08	0.00	0.00	0.00	0.43

[z]Marketable fresh weight of shiitake basidiocarps.
[y]A = amended, N = nonamended.
[x]Hardwood sawdust added with YVMBS broth (amended).
[w]C = control; hardwood sawdust with no broth added (nonamended).

Table 2. Analysis of variance of the effects of months, strains, and broth on yield of shiitake mushroom grown on hardwood sawdust in the greenhouse.[z]

Source	df	Mean square	*P* > F
Month (M)	11	1.80032316	0.0170**
Strain (S)	6	4.07415655	0.0002***
M × S (error A)	66	0.77041218	0.0687
Broth (B)	1	9.60015238	0.0001***
S × B	6	2.75400933	0.0002***
Error	77	0.54207652	

[z]The statistical analysis used for this particular data is ANOVA for the cumulative yield of each shiitake mushroom strain. No replication of each shiitake strain was made because of limited space inside the greenhouse. Daily harvest of fresh basidiocarps from a plot is not independent variable.
,*Significant at $P = 0.01$ or 0.001, respectively.

In 1 year, the shiitake mushroom strains LE1, LE2, LE5, and LE6 produced 19.5, 20.2, 7.9, and 4.5 pounds, respectively, of harvested fresh basidiocarps on amended hardwood sawdust. Shiitake mushrooms retail for $3.20 to $4.20/lb ($7.05 to $9.26/kg). The use of amended hardwood sawdust for shiitake mushroom production inside the greenhouse has a significant economic potential for shiitake mushroom growers.

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