African Journal of Biotechnology Vol. 8 (13), pp. 2999-3006, 6 July, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Substrate affects growth and yield of shiitake mushroom

M. Ashrafuzzaman[1,2]*, A. K. M. Kamruzzaman[1], M. Razi ismail[2], S. M. Shahidullah[2] and S. A. Fakir[1]

[1]Department of Crop Botany, Bangladesh Agricultural University, Mymensingh 2202, Bangladesh.
[2]Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

Accepted 26 May, 2009

Lentinus edodes **(Berk.), the shiitake mushroom, is worldwide one of the most widely cultivated mushrooms. Sawdust is the most popular basal ingredient used in synthetic substrate formulations for producing shiitake spawn. However, the best sawdust for this uses needs to be determined. Shiitake mushroom was cultivated on sawdust from the woody plants Babla (*Acacia nilotica* L.), Champa (*Michelia champaca* L.), Garzon (*Dipterocarpus alatus* Roxb.), Ipil-ipil [*Leucaena glauca* (Linn) Benth], Jackfruit (*Artocarpus heterophyllus* Lam), Mango (*Mangifera indica* L.), Raintree [*Albizia saman* (Jacq.) F Müll], Segun (*Tectona grandis* L), Shimul (*Bombax ceiba* L), Shisoo (*Dalbergia sissoo* Roxb) or mixtures of sawdust from all of the trees with equal ratio or rice straw to determine growth and fruiting characteristics. Cultivation on Jackfruit resulted in significantly faster mycelial growth compared to other substrates. With respect to fructification, culture on Jackfruit produced the first pinhead (primordium) earlier compared to other substrates. Numbers of primordial and effective fruiting bodies was highest on Jackfruit sawdust. Rice straw, surprisingly, did not produce any fruiting bodies as well as showing no yield attributes. Yield attributes including stalk length, stalk diameter and diameter and thickness of the pileus were significantly higher on Jackfruit. The lowest biological and economic yields were found when culture was on Champa. Biological efficiency and biological yield, economic yield and dry yield at the first and final harvests were highest with culture on Jackfruit and its use is recommended in the production of shiitake mushroom in the tropics.**

Key words: *Lentinus edodes*, growth, hardwood, sawdust, rice straw.

INTRODUCTION

Lentinus edodes (Berk.), shiitake mushroom, accounts for 17% of world production in terms of weight (Chang and Miles, 2004; Miles and Chang 1997). It can grow in winter season and also it can grow all the year in controlled condition. After the button mushroom [*Agaricus bisporus* (J.E. Lange) Imbach], shiitake is the most cultivated mushroom in the world (Chang and Miles, 2004). The fungus is saprophytic and grows on dead material (Chang and Miles, 2004). Various species of trees have been used for its cultivation, but most production is on species of oak (*Quercus* spp.), (Harris, 1986; Stamets and Chilton, 1982; Przybylowicz and Donoghue, 1988). Shii- take mushroom has a long shelf-life because fruiting bodies are dried before sale; most other mushrooms are sold fresh. Shiitake mushroom has been reported to boost the immune system, lower cholesterol, function as an anticoagulant and may have use in treatment of some cancers (Tokuda et al., 1974; Fujii et al., 1978; Suzuki et al., 1979; Mizuno, 1995; Wasser, 2002).

Mushrooms depend on substrates for nutrition and the substrate is normally a source of lignocellulose material which supports growth, development and fruiting of mushroom (Chang and Miles, 2004). Sawdust is the most popular basal ingredient used in substrates to produce shiitake (Miller and Jong, 1987; Palomo et al., 1998; Grodzinskaya et al., 2003). Other basal ingredients can include straw and corn cobs, or their mixes. Regardless of the main ingredient used, starch-based supplements such as wheat bran, rice bran, millet, rye or corn, can be added at 10 to 40% of dry weight to the main ingredient (Ivan et al., 2003; Royse et al., 1990; Royse, 1996).

Yield of mushroom might vary due to use of hetero-

*Corresponding author. E-mail: drashraf2007@yahoo.com.

Table 1. Plant sources of sawdust for substrate base.

Bengali name	English name	Scientific name	Family
Babla	Babul tree	*Acacia nilotica* L.	Fabaceae
Champa	Teak chambul	*Michelia champaca* L.	Verbenaceae
Garzan	Garjan	*Dipterocarpus alatus* Roxb.	Dipterocarpaceae
Ipil-ipil	Ipil-ipil	*Leucaena glauca* (L.)Benth.	Leguminosae
Jackfruit	Jackfruit	*Artocarpus heterophyllus* Lam	Moraceae
Mango	Mango	*Mangifera indica* L.	Anacardiaceae
Raintree	Raintree	*Albizia saman* (Jacq.) F Müll	Leguminosae
Segun	Teak	*Tectona grandis* L.	Verbenaceae
Shimul	Silk cotton	*Bombax ceiba* L.	Bombacaceae
Shisu	Blackwood-tree	*Dalbergia sissoo* Roxb.	Fabaceae

geneous mixes of sawdust. Shiitake mushroom is traditionally cultivated on the shi tree [*Castanopsis cuspidata* (Thunb.) Schott] or wood logs in Japan. Scarcity of the shi tree has necessitated a search for alternative substrates for shiitake cultivation. Large amounts of freely available sawdust from different trees offer a potential alternative substrate source for mushroom cultivation in the tropics. This study was undertaken to evaluate growth and yield of shiitake mushroom on locally available sawdust of different trees.

MATERIALS AND METHODS

Experimental materials

The experiment was carried out at the mushroom center development project, Savar, Dhaka, Bangladesh, between July and December, 2005. Sawdust from Babla, Champa, Garzon, Ipil-ipil, Jackfruit, mango, Segun, Shimul, Shisoo, or Raintree, a mix of sawdusts from all trees in equal proportions, collected from the different sawmills, or rice straw, were used as substrates. The substrates were dried for 4 to 5 days fully exposed outdoors and then stored for 15 days. The experiment was laid out in a completely randomized design with 12 treatments and replicated 5 times. The list of plants used as substrate is shown in Table 1.

Preparation of pure culture

Potato dextrose agar (PDA) medium was prepared using 200 g peeled and sliced potato, 20 g dextrose and 26 g agar in a litre of water. Clean sliced potato (200 g) was boiled in a litre of water until the tissues became soft. The boiled potato slices were mashed and filtered and the filtrate made up to 1 litre with distilled water. The potato extract obtained was subsequently heated and stirred after adding 20 g dextrose and 26 g agar. Heating was stopped when the agar was dissolved in the solution. The medium was allowed to cool before 10 ml each of the PDA preparation was dispensed into test tubes, corked and sterilized at 121^{o}C and 1.5 kg/cm^{2} pressure.

The test tube containing the sterilised PDA was kept in a slanted position. These slants were maintained to inoculate fungus. The agar and tubes were allowed to cool in upright position for plating and isolation. To obtain pure culture, PDA culture or tissue culture method was used. A small piece of tissue was collected from the fruiting body of shiitake mushroom and placed on the sterilized PDA medium under aseptic conditions. This was incubated at 25^{o}C for 7-10 days for sufficient mycelial growth. Pure cultures were obtained by subculturing.

Preparation of mother culture

The mother culture was prepared by mixing sawdust and wheat bran 2:1 (v:v). Calcium carbonate was applied at 0.2% of the mixture. The moisture level of the mixture was maintained at 65% with tap water. Polypropylene bags (18 × 25 cm) were filled with 200 g of the mix and the air pressed out. The bags were sterilized in an autoclave for 1 h at 121°C under 1.5 kg/cm^{2} pressures and allowed to cooled down for 24 h and transferred to a clean bench. A piece of pure culture medium containing shiitake mushroom mycelium was placed aseptically in the opening of the mother culture bags.

Substrates preparation

Each sawdust (30% of volume) was supplemented with wheat bran (15%), rice husk (5%), calcium carbonate (0.02%) and water (50%) and thoroughly mixed by hand in bowls. Rice straw, cut into 2 - 3 cm pieces, was used without any supplementation. Polyprophylene bags (22.5 × 30 cm) were filled with 1 kg of each substrate, sterilized once at 121°C and 1.5 kg/cm^{2} pressure for 1 h and for several hours allowed to cool down to the desired inoculation temperature in a dark room. Each bag was stood up, the mouth was opened and the spawn (3% w/w) was placed into each bag at the approximate center using a sterilized metal implement. Bags were closed.

Cultivation conditions

The bags were subsequently placed, longside down, into a spawn running room at 20 - 23°C in the dark and 65 - 70% relative humidity until completion of spawn running. After completion of spawn running the temperature and relative humidity was changed to 19 to 20°C and 80 - 90% RH, respectively. The bags were slit and the cut portions folded back. Water was sprayed for maintaining moisture up to the desired level in the form of fine mist from a nozzle.

Fructification

To obtain a homogenous production it is necessary to undertake a thermal shock which consists in subjecting the substrate to a change in temperature namely lowering the temperature of the substrate to 4 - 10^{0}C. This was done by placing the bags in a refrigerator for 24 h at a temperature of 4 to 10^{0}C. Then the bags were

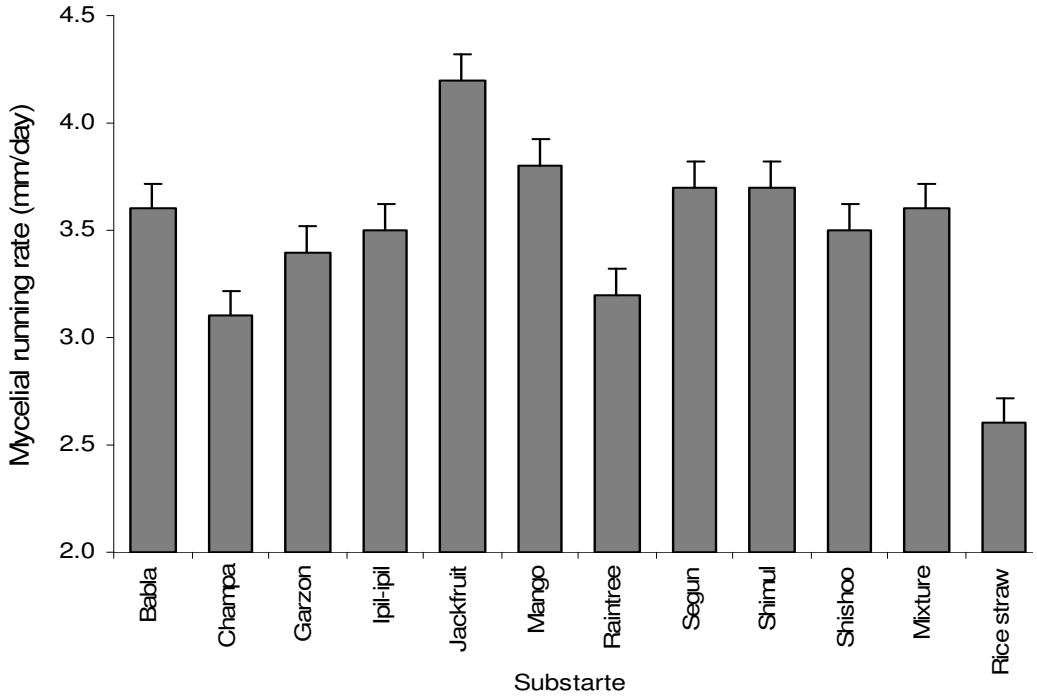

Figure 1. Effect of different substrates on mycelial running rate of shiitake mushroom. Vertical bars represent $LSD_{(0.05)}$.

taken to the fructification room and the plastic bags were taken off. During the fruiting process, temperatures of 15 to 20°C, humidity of 80 - 93% were maintained in almost total darkness. The water used in humidification must contain 2 ml/L sodium hypochlorite at 5%. Production in each bag will gradually decrease until there is no more fructification because of degradation in the mycelium.

Data collection

Mycelial running rate (MRR) on each substrate was estimated on the basis of the ratio between the total distance covered by the mycelium and the time needed for growth to occur:

$$MRR = \frac{L}{N} \text{ (cm·day}^{-1}\text{)}$$

where L = average length (cm) of mycelium running measured at 4 different places, and N = number of days.

Time required (in days) from inoculation to completion of mycelium running, days required from opening of the packet to primordial initiation and days from opening to harvesting were recorded. Numbers of well-developed fruiting bodies were recorded. Dry and pinheaded fruiting bodies were culled, but twisted fruiting bodies were acceptable. Pileus thickness of 4 randomly selected fruiting bodies was determined with a slide calliper. Pileus and stalk diameters were recorded. Biological yield (g/1000 g) was determined by weighing the whole cluster of fruiting bodies without removal of base of stalks and the substrate. Economic yield (g/1000 g) was determined by weighing all the fruiting bodies in a packet after removal of the base of stalks and the substrate. Biological efficiency was calculated with the following:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g)}}{\text{Total substrates used (g)}} \text{ X 100}$$

Dry yield was determined by collecting about 50 g of randomly selected mushroom tissue which was then placed in a paper envelope, weighted and placed in an oven and dried at 72°C for 24 h and weighed again. The weight of the envelope was subtracted from both weights.

Statistical analysis

The data from the study were statistically analyzed. Analysis of variance was performed and means were separated using Duncan's multiple range test (DMRT) at 5% level of probability (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Mycelial running rate (MRR) in spawn packet

Remarkable differences were observed in mycelial running rate on spawn packets of the different substrates used. It ranged from 2.6 to 4.2 mm/day. The highest MRR was observed in Jackfruit sawdust (4.2 mm/day) followed by mango sawdust. MRR did not show any significant difference when Shimul, Babla, mixture and Segun sawdust were used as substrates. The lowest MRR was recorded on rice straw (2.6 mm) (Figure 1). The presence of the right proportion of alpha-cellulose, hemi-cellulose, pectin and lignin was the probable cause of higher rate of mycelium running in Jackfruit sawdust. Suitable C:N ratio might be responsible for the higher mycelial growth. The capacity of mushroom to grow on lingo-cellulosic substrates is related to the vigor of its

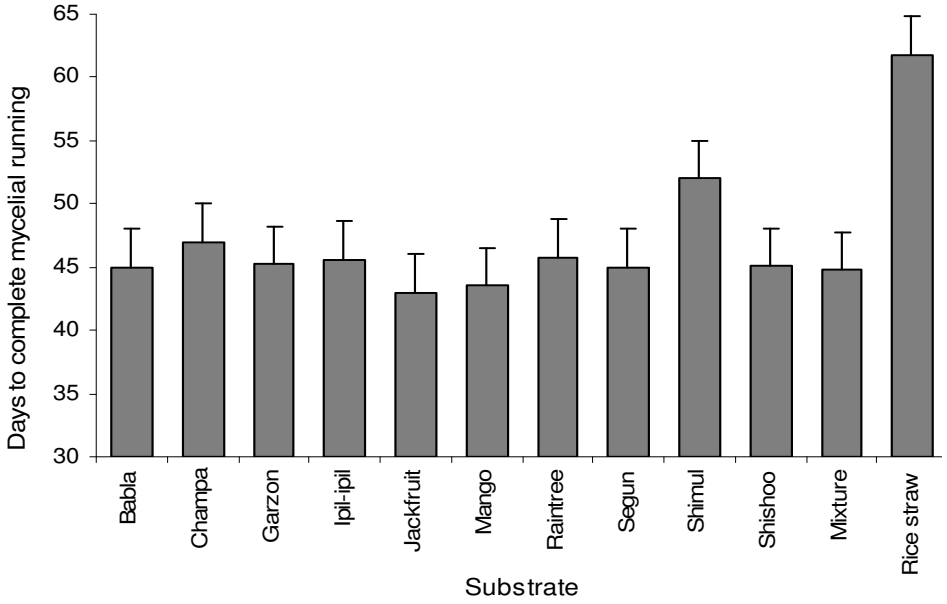

Figure 2. Variation in the duration to complete mycelial running of shiitake mushroom on different substrates. Vertical bars represent $LSD_{(0.05)}$.

mycelium (Permana et al., 2004).

Days required from opening to primordial initiation

Rice straw did not produce any primordia and there was no data for yield. Substrate affected the duration from opening of spawn packet to primordia initiation (Figure 1). The shortest time to primordial initiation was on the Jackfruit substrate. On the mango, mixture of all sawdusts, Ipil-ipil, Raintree, Segun, Babla, Shishoo, Champa substrates time to primordial initiation were similar. The longest time to primordial initiation was on the Garzon substrate followed by the Shimul substrate.

Days to complete mycelium running

Substrate affected days required to complete mycelium running (Figure 2). The longest time for completion of MRR was on rice straw followed by Shimul; the others were shorter and similar. Variation in MRR on different substrates might be due to variations in the chemical composition and C:N ratio of substrates as reported by Bhatti et al. (1987).

Days required from opening to first harvest

The duration from opening of spawn packet to first harvest on the substrates of Jackfruit, Mango, mixture, Raintree, Babla, Segun and Shishoo was 12.2, 14.0, 14.0, 14.0, 14.4, 14.6 and 15.2 days, respectively. The maximum number of days required from opening to first harvest was recorded with Garzon (15.6) and Ipil-ipil (15.6). Time duration for first harvest on the sawdust of Shimul, Champa did not differ significantly ($p < 0.05$) (Figure 3).

Number of primordia/packet

Substrate affected number of primordia/packet (Table 2). The highest number of primordia was for culture on the Jackfruit substrate. Numbers of primordia on Ipil-ipil, Raintree, Garzon, Segun, Shishoo, Champa and Babla were similar. The lowest number of primordia was on Mango, Shimul and the mixture of all sawdusts. Kitamoto et al. (1975) reported that substrates containing glucose, fructose and trehalose produced the highest number of primordia while those containing glycerol, xylose, sucrose and fructose produced abnormal fruiting bodies. The best fruiting body production was on glucose and fructose containing substrates.

Number of effective fruiting bodies

Number of well-developed fruiting body was recorded and presented in Table 2. Dry and pin headed fruiting body was discarded but twisted and tiny fruiting body was included during counting. The percentage of effective fruiting body varied significantly ($p < 0.05$) in different substrates. Substrate affected percentage of effective fruiting bodies formed. The highest number of effective fruiting bodies was from culture on the Jackfruit substrate; the lowest was from culture on Shimul. Numbers

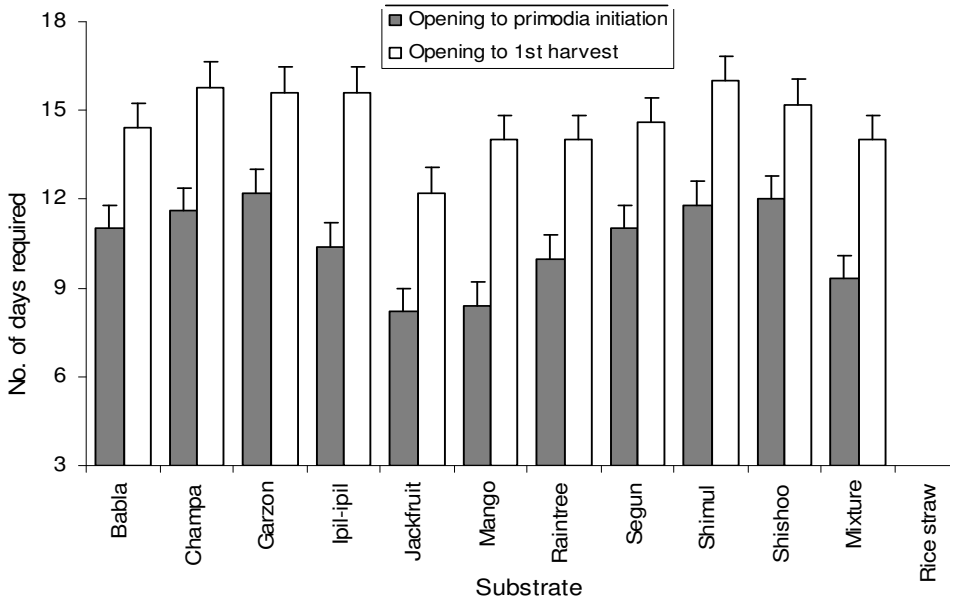

Figure 3. Time required for fruiting body formation of shiitake mushroom on different substrates. Vertical bars represent $LSD_{(0.05}$

Table 2. Yield attributes of shiitake mushroom grown on different substrates.

Substrate	No. of Primordia/ Packet	No. of EFB/ packet	Stalk length (mm)	Stalk Diameter (mm)	Pileus diameter (mm)	Pileus thickness (mm)
Babla	11.8b	8.2c	45.5d	12.0bc	66.7ab	10.9cd
Champa	12.2b	9.6b	40.5e	11.4c	58.4c	10.1e
Garzon	12.6b	9.4b	49.4a	13.0ab	69.2ab	12.0b
Ipil-ipil	12.8b	9.6b	45.7cd	11.2c	62.1bc	10.5de
Jackfruit	16.4a	13.6a	49.1a	13.5a	66.1ab	12.8a
Mango	9.8c	7.6c	49.6a	13.0ab	71.1a	11.1cd
Raintree	12.8b	9.6b	46.5bcd	11.3c	66.3ab	10.5de
Segun	12.4b	9.6b	49.8a	12.4abc	70.9a	11.6bc
Shimul	9.6c	7.0c	48.1abc	11.8c	63.5abc	10.7de
Shishoo	12.2b	9.8b	48.1abc	12.3bc	69.2ab	11.1cd
Mixture	10.4c	7.8c	48.0ab	11.8c	69.3ab	11.1cd
Rice straw	0.0d	0.0d	0.0f	0.0d	0.0d	0.0f
CV (%)	10.43	14.31	4.07	7.27	8.99	5.71

EFB = Effective fruiting body.

of effective fruiting bodies from the Shisoo, Segun, Ipil-ipil, Champa, Raintree, Garzon and Babla substrates were similar.

Stalk length

The different substrates used in shiitake mushroom production had significant influence on stalk length. The longest stalks were from the Segun substrate. Stalk length from Mango, Garzon, Jackfruit, mixture of all sawdusts, Shimul, Shishoo, Raintree, Ipil-ipil and Babla substrates were similar ($p < 0.05$) (Table 2).

Stalk diameter

Stalk diameter was affected by substrates. The highest diameter was found in Jackfruit sawdust (13.5 mm), while the lowest value was recorded on Ipil-ipil (11.2 mm). Dia-

Table 3. Biological efficiency and yield of shiitake mushroom grown on different substrates.

Substrate	Biological yield (g/packet)	Economic yield (g/packet)	Biological efficiency (%)	Dry yield (g/packet)
Babla	491.40b	477.80b	98.28b	115.82b
Champa	397.40c	383.40c	79.48c	91.17c
Garzon	481.00b	466.00b	96.12b	112.46b
Ipil-ipil	476.20b	465.00b	95.94b	111.32b
Jackfruit	509.20a	494.20a	101.84a	123.55a
Mango	491.00b	475.20b	98.20b	114.96b
Raintree	480.40b	466.60b	96.08b	110.49b
Segun	485.60b	471.80b	97.12b	113.08b
Shimul	363.00b	349.60d	72.40d	75.51d
Shishoo	479.80b	465.60b	95.96b	112.49b
Mixture	481.00b	467.60b	96.24b	111.33b
Rice straw	0.00e	0.00e	0.00c	0.00e
CV (%)	2.74	2.69	2.74	3.62

meter of stalk on the different substrates of Garzon, Mango, Segun, Shisoo, Babla, mixture of all sawdusts, Shimul and Champa did not vary significantly (Table 2).

Diameter of pileus

Substrate affected pileus diameter (Table 2). The maximum pileus diameter was with culture on Mango sawdust; the lowest value was with culture on Champa sawdust. Pileus diameter due to culture on Segun, mixture of all sawdusts, Garzon, Shisoo, Babla, Raintree, Jackfruit, Shimul and Ipil-ipil were similar. It is likely that Mango sawdust has a high C:N ratio which resulted in the enhanced growth of the pileus (Veena et al., 1998).

Thickness of pileus

Substrate affected pileus thickness (Table 2). The thickest pileus was produced with culture on the Jackfruit substrate, while the thinnest pileus was due to culture on the Champa substrate. The thickness of pileus on the substrates of Garzon, Segun, mixture of all sawdusts, Mango, shisoo, Babla and Shimul did not vary significantly.

Biological efficiency (BE)

The biological efficiency was affected by substrate. The highest biological efficiency was for the Jackfruit substrate. The lowest biological efficiency was on the Shimul and Champa substrates. The biological efficiency on the Babla, Mango, Segun, mixture of all sawdusts, Garzon, Raintree, Shisoo and Ipil-ipil substrates were similar (Table 3).

Yield at first harvest

Significant yield variations were recorded on different substrates at first harvest. The highest biological yield and economical yield was recorded with Jackfruit sawdust (343 and 332 g). The BY and EY on Babla, Raintree, Shishoo, Ipil-ipil, Mango, Segun, Garzon and mixture did not vary significantly (Figure 4). The lowest biological yield and economic yield was observed in Shimul (220.4 and 212.2 g).

Yield per packet

Significant variation was found in yield of shiitake mushroom grown on different substrates used. The maximum biological yield was recorded with sawdust of Jackfruit (509.2 g) followed by sawdust of Babla which was statistically similar with those of Mango, Segun, mixture, Garzon, Raintree, Shishoo and Ipil-ipil (Table 3). The lowest biological yield was observed in Shimul sawdust (363 g). The highest economic yield was observed in Jackfruit sawdust (494.2 g) which was statistically similar with those of Babla, Mango, Segun, mixture, Raintree, Garzon, Shishoo and Ipil-ipil. The lowest economic yield was found in Shimul (349.6 g). Chaudhary et al. (1985) explained the process of break-down of lignin. There is an apparent correlation between the ability to degrade lignin and the production of phenolases, which oxidize phenolic compounds to simple aromatic compounds that can be absorbed by mushroom mycelium and is used for it growth. The product of cellulolytic action in simple and soluble carbohydrates and the end products being glucose was absorbed by the fungal mycelium for growth and energy. Therefore, cellulose rich organic substrates are good for the cultivation of mushroom (Gerrits and

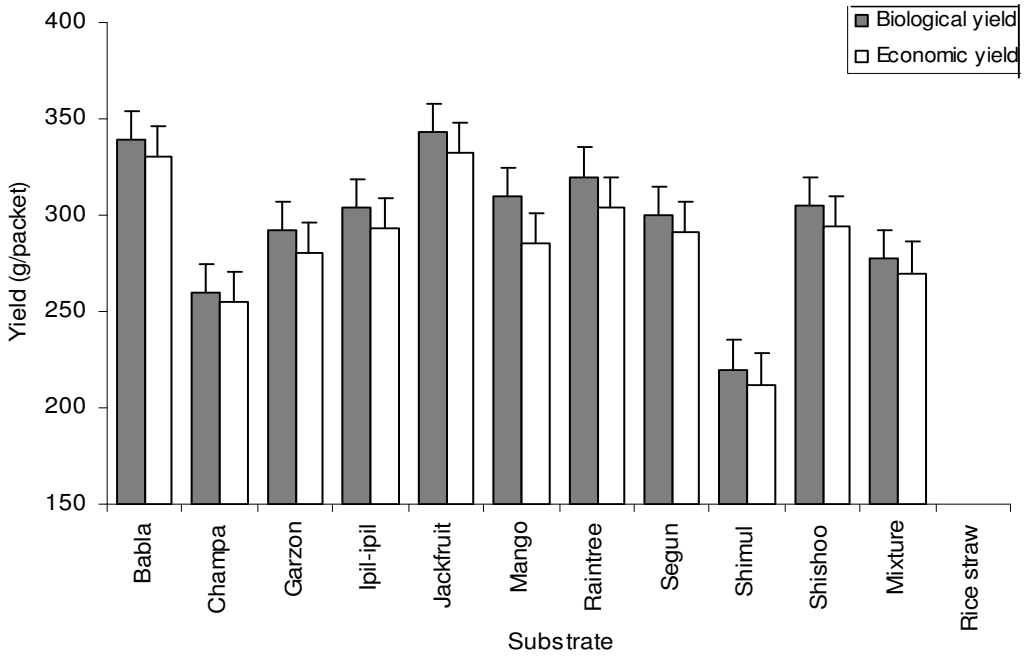

Figure 4. Biological yield and economic yield at first harvest of mushrooms grown on different substrates. Vertical bars represent $LSD_{(0.05)}$.

Muller, 1965; Quimio, 1987). High cellulose content in wood results in enhanced cellulose enzyme production and increased yield of mushroom (Ramasamy and Kandaswary, 1976). The substrates with high lignin and phenolic content decreased the activity of the enzyme, hence slow growth and low yield.

Dry yield per packet

Dry yield of shiitake mushroom grown on different substrates varied from 75.51 to 123.55 g. The highest dry yield was recorded on Jackfruit sawdust (123.55 g), which was statistically similar with those of Babla, Mango, Segun, Shishoo, Garzon, mixture, Ipil-ipil, Raintree (Table 3). The lowest dry yield was found on Shimul (75.51 g). Dry yield varied possibly due to the variation of chemical composition of different substrates.

Conclusion

Use of Jackfruit sawdust as a substrate appears to be most suitable due to improved growth and sporophore production and may be useful for large scale production of shiitake mushroom. Rice straw as substrate for mushroom production is not recommended since it did not support sporophore production. The production of mushrooms worldwide is increasing at an annual rate of about 10%. Mushroom cultivation has a special relevance to Bangladesh, because sawdust and other materials are abundantly available to our farmers. The findings made in the present study may be useful for large scale production of shiitake mushroom.

REFERENCES

Bhatti MA, Mir FA, Siddiq M (1987). Effect of different bedding materials on relative yield of oyster mushroom in the successive flushes. Pak. J. Agril. Res., 8(3): 256-259.

Chaudhary K, Mittal SL, Tauro P (1985). Control of cellulose hydrolysis by fungi. Biotechnol. Lett., 7: 455-456.

Chang ST, Miles, PG (2004). Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact, 2nd ed. CRC Press, Boca Raton, FL.

Fujii T, Maeda H, Suzuki F, Ishida N (1978). Isolation and characterization of a new antitumor polysaccharide, KS-2, extracted from culture mycelia of *Lentinus edodes*. J. Antibiot. 31: 1079-1090.

Gerrits JPG, Muller EM (1965). Changes in compost constituents during composting, pasteurization and cropping mushroom. Science, 6: 225.

Gomez KA, Gomez AA (1984). Statistical procedures for agricultural research, John Wiley & Sons, Inc. New York.

Grodzinskaya AA, Infante HD, Piven NM (2003). Cultivation of edible mushrooms using agricultural and industrial wastes. Agronomia-Tropical-Maracay, 52(4): 427-447.

Harris B (1986). Growing shiitake commercially. Science Technology, Madison, WI.

Ivan HR, Antonio CM, Jose OM, Jose CB (2003). Supplementation of sugarcane bagasse with rice bran and sugarcane molasses for shiitake (*Lentinula edudes*) spawn production. Brazil J. Microbiol., 34: 61-65.

Kitamoto Y, Horkoshi T, Hosio N, Ichikawa Y (1975). Nutritional study of fruiting body formation in psilocybe panacoliformis. Trans. Mycol. Sco. Japan, 16(3): p. 268.

Miles PG, Chang ST (1997). Mushroom biology, concise basics and current developments. World scientific Publ., London.

Miller MW, Jong SC (1987). Commercial cultivation of shiitake in sawdust filled plastic bags. Dev-Crop-Sci. Amsterdam: Elsevier Scientific Pub. Co. 10: 421-426.

Mizuno T (1995). Shiitake, *Lentinus edodes*: functional properties for medicinal and food purposes. Food Rev. Int. 11(1): 111-128.
Palomo A, Door C, Mattos L (1998). Comparative study of different substrates for the growth and production of *Lentinus edodes* Berk ("Shiitake"). Fitopatologia, 33: 71-75.
Permana IG, Meulen ter U, Zadrazil F (2004). Cultivation of *Pleurotus ostreatus* and *Lentinus edodes* on lignocellolosic substrates for human food and animal feed production. J. Agric. Rural Dev. Tropics Subtrop. 80: 137-143.
Przybylowicz P, Donoghue J (1988). Shiitake growers handbook. Kendall/Hunt Publ. Co. Dubuque, IA.
Quimio TH (1987). Introducing *Pleurotus flabellatus* for your dinner table. Mushrooms J., 69: 282-283.
Royse DJ (1996). Yield stimulation of shiitake by millet supplementation of wood chip substrate. Mushroom Biol. Mushroom Prod. 2: 277-283.
Ramasamy K, Kandaswamy TK (1976). Effect of certain amendments on cellulose(s) and yield of straw mushroom. Indian J. Mushroom, 2(1): 8-12.
Royse DJ, Bahler BD, Bahler CC (1990). Enhanced yield of shiitake by saccharide amendment of the synthetic substrate. Appl. Environ. Microbiol. 56: 479-482.
Stamets P, Chilton JS (1982). The mushroom cultivator: A practical guide to growing mushrooms at home. Agarikon Press, Olumpia WA.
Suzuki F, Suzuki C, Shimomura E, Maeda H, Fujii T, Ishida N (1979). Antiviral and interferon-inducing activities of a new peptidomannan, KS-2, extracted from culture mycelia of *Lentinus edodes*. J. Antibiot. 32: 1336-1345.
Tokuda S, Tagiri A, Kano E, Sugawara Y, Suzuki S, Sato H, Kaneda T (1974). Reducing mechanism of plasma cholesterol by shiitake. Mushroom Sci. 9(1): 445-462.
Veena S, Vijaykumar S, Kulkarni, JH, Savalgi V (1998). Cultivation of oyster mushroom on common weed in combination with bagasse. Karnotaka J. Agril. Sci., 11(3): 699-695
Wasser SP (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl. Microbiol. Biotechnol. 60: 258-274.