Yield Stimulation of Shiitake by Millet Supplementation of Wood Chip Substrate

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ABSTRACT:

Three experiments were performed to determine the effect of millet supplementation on mushroom yield and basidiome size of shiitake (Lentinula edodes) when grown on a synthetic substrate. Substrate formulations of sawdust, wheat bran, rye and $CaCO_3$ were amended with white millet (17, 23, 29, and 34% [dry weight]). Addition of 34% millet to the substrate stimulated mushroom yield by 68% compared to a millet addition of only 17%. Biological efficiencies averaged (3 crops) 59.1%, 67.1%, 81.7% and 99.6% for millet additions of 17, 23, 29 and 34%, respectively. Thus, biological efficiencies increased an average of 13.5% for each additional 6% increase in the amount of millet supplementation. Alternatively, as millet levels increased, basidiome size decreased. Average basidiome size decreased from 23 g at 17% millet to 13 g per mushroom at 34% millet supplementation.

1 INTRODUCTION

Production of shiitake in the United States has increased an average of 24.2% annually since 1987 (USDA, 1995). This increase in production is in response to increasing consumer demand for the product. According to the United States Department of Agriculture (1995), farmers produced 2,499 t (=5,498,000 lbs) of fresh shiitake in the 1994 to 1995 growing season.

In the United States, most commercial production of shiitake is on synthetic logs. The amount of controlled-environment production surface devoted to growing shiitake on synthetic logs has increased 243% from 1987 to 1995 (54,000 m^2 to 130,000 m^2, respectively). At the same time, the number of natural logs maintained under cultivation (both outdoor and indoor) has fallen from a high of 1,028,000 in 1990 to 451,000 in 1995. Thus, it seems clear that most future production of shiitake will be on synthetic logs. This trend is not surprising because consistent market supply is more predictable and more sustainable from synthetic logs as opposed to natural logs. In addition, increased yields are possible and decreased time is required to complete a crop cycle on synthetic logs as opposed to natural logs. These advantages far outweigh the major disadvantage of a relatively high initial investment cost to start a synthetic log manufacturing and production facility.

2 MATERIALS AND METHODS

2.1 Substrates and preparation

Mixed hardwood sawdust, collected from a local sawmill in Centre County, Pennsylvania, was used as the main substrate ingredient. The exact composition of hardwood species comprising the sawdust could not be determined, but the predominant species was Northern Red Oak (*Quercus*

rubra L.). The sawdust was collected in fall, 1993, and stored in an enclosed building until used. The moisture content was approximately 35% by weight when used. All substrate formulations (designated 1, 2, 3, and 4) contained basal ingredients as follows: hardwood sawdust (24.12 kg, oven dry wt); red winter wheat (*Triticum aestivum* L.) bran (2.27 kg); rye (1.14 kg); precipitated CaCO3 (0.06 kg); sucrose (0.39 kg). Each formulation received white millet (*Panicum miliaceum* L.) supplementation as follows: (1) 5.68 kg, (2) 8.52 kg, (3) 11.36 kg, and (4) 14.2 kg. After mixing, substrate formulations 1, 2, 3, and 4 contained 17%, 23%, 29% and 34% millet, respectively.

Mixed substrate ingredients were pasteurized, cooled, inoculated and bagged with a 10 ft^3 paddle mixer. The substrate was pasteurized by injecting live steam into the mixer and allowing the substrate to heat to 111^oC. This temperature was maintained for 20 min with continuous agitation to insure uniform substrate heating. After pasteurization, the substrate was rapidly cooled by passing cold tap water through a jacket fitted to the mixer. Sterility of the mixture was maintained by injecting filtered air into the mixer during cool down to create a positive air flow. When the substrate had cooled to below 27^oC, the substrate was spawned with 210 g rye grain spawn contained in 500 ml Erlenmeyer flasks. When the spawn was thoroughly mixed with the substrate, the resulting mixture was bagged in unused virgin polyethylene bags (20.3 cm x 12.7 cm x 50.8 cm) and closed with a twist-tie. The amount of substrate was weighed at time of filling with a digital scale placed under the bagging port of the mixer. Each bag contained 2.5 kg spawned substrate at 59% moisture (1.03 kg oven dry weight). Dry substrate weight was determined by drying 100 g of the processed substrates in an oven for 24 h at 105^oC. Dry weight subsequently was used to determine the % biological efficiency (ratio of fresh mushrooms harvested per dry substrate weight and expressed as a percentage).

2.2 Experimental design

The experiments were a completely randomized design with 25 replicates per treatment. The general linear models procedure was used to perform an analysis of variance (SAS Institute 1995). The Waller-Duncan K-ratio T test was used to separate treatment means (Steel and Torrie 1980). The experiments were repeated three times and designated Crops I, II, and III.

2.3 Isolate and spawn

Isolate R26 was selected because it is a commercially used cultivar. The isolate was maintained on potato-dextrose yeast-extract agar as outlined previously by Jodon and Royse (1979). Spawn of R26 was prepared as outlined previously by Royse and Bahler(1986).

2.4 Spawn run, log browning and soaking

After a spawn run of 7 days, 20 slits (5 mm each) were made in the top of each bag with a sharp scalpel to provide gas exchange. At the end of 22 days incubation at 22±1^oC, the plastic bags were removed and the synthetic logs moved to a ¡°browning room¡±. In the browning room (93-98% relative humidity, 18±1^oC air temperature), synthetic logs were hand-watered lightly with a 600-hole roseface nozzle each day. At the end of 14 days in the browning room all logs were rotated 180^o to provide a more uniform browning of the logs' surface. Three h of light were provided daily by cool-white fluorescent bulbs. Sufficient air changes were maintained to hold CO_2 levels below 1,900 ppm (1,200 µl/liter). At the end of 28 days in the browning room environment, the logs were soaked in cool water (13± 2^oC) until each weighed approximately 2.3 kg. Care was taken not to over soak logs, i.e., more than 2.4 kg final weight. After each flush of mushrooms

was harvested, logs were resoaked to increase log weight to 2.3 kg.

2.5 Harvesting and determination of BE and basidiome size

Mushrooms were harvested from the substrate at the same time each day, when the veil had broken and the gills were fully exposed. The mushrooms were then counted and weighted. At the end of the harvest period (63 days), the accumulated data were used to calculate the BE. The substrate dry weights were used to calculate the percentage of BE ([weight of fresh mushrooms harvested/substrate dry matter content] x 100). Basidiome size was determined as follows: total weight of fresh mushrooms harvested/total number of mushrooms harvested.

3 RESULTS

3.1 Biological efficiency and yield

In general, as millet levels in the substrate increased, BE increased. For crop I, biological efficiencies ranged from a low of 31.3% for 17% millet addition to a high of 77.8% BE for 34% millet supplementation (Table 1). There was no significant difference observed in BE between 17% millet addition and 23% millet addition. However, this was the only crop where BE's did not significantly increase for each 6% increment in millet addition (Table 1).For crop II, overall yields were the highest observed for any of the crops. Biological efficiencies ranged from 81% for 17% millet addition to 119% for a 34% millet supplementation rate. An approximate 12% increase in BE was observed for each 6% increase in millet addition. For crop II, yields per log were 885 g, 1013 g, 1150 g, and 1294 g for 17, 23, 29 and 34% millet supplementation, respectively. Crop III BE's were intermediate between those of crop I and crop II. BE's ranged from 65% to 102% for 17 and 34% millet additions, respectively. Incremental increases in BE's were not as uniform as increases observed for crop II (Table 1).

Table 1. Effect of millet supplementation of oak wood chips on biological efficiency (BE) and size of shiitake produced from synthetic logs for 63 days (Crops I, II and III).

Millet	Crop I		Crop II		Crop III	
Supplementation	BE	Size	BE	Size	BE	Size
----% dry wt---	-%-	-g-	-%-	-g-	-%-	-g-
17	31.3a[1]	31.4a	81.4a	19.0a	64.6a	17.1a
23	34.5a	27.7b	93.1b	16.1b	73.6b	16.6ab
29	52.4b	22.3c	105.8c	13.6c	87.0c	14.0c
34	77.8c	15.4d	119.1d	11.6d	101.9d	12.1d
CV(%)[2]	31.1	16.5	15.7	14.8	20.1	23.0

[1]Means followed by the same number in the same column are not significantly different according to Waller-Duncan K-ratio T test at P=0.05.

[2]Coefficient of variation.

The data for mean yield per log and mean BE revealed that increases were approximately 19% for each 6% increment in millet supplementation (Table 2). This increase was not linear as the response tended to be greater at the higher levels of millet supplementation (Table 2).

Table 2. Mean yield (kg and lbs per 2.5 kg log), percent biological efficiency (%BE) and mushroom size (g/mushroom) for three crops (Crops I, II and III) of mushrooms produced for 63 days on synthetic logs supplemented with four levels of millet.

Millet	Mean for Crops I, II and III			
Supplementation	Yield per log		BE	Size
---% dry wt--	-kg-	-lbs-	-%-	-g-
17	0.64	1.42	59.1	22.5
23	0.73	1.61	67.1	20.1
29	0.89	1.96	81.7	16.6
34	1.08	2.38	99.6	13.0

3.2 Basidiome size

In nearly all cases, mushroom size decreased significantly as millet levels in the substrate increased (Table 1). For crop I, mushroom size decrease by 50% as millet levels were doubled. For crop II, basidiome size decreased from 19 g per mushroom to 11.6 g per mushroom for 17 and 34% millet addition, respectively. Basidiome size decreases were not as dramatic for crop III as that observed for crops I & II.

4 DISCUSSION

Millet addition to shiitake substrate first was used commercially several years ago. Mee (1978) reported formulas advocating grains such as milo and sorghum in ratios of 1 to 15% of the substrate dry weight. Other workers (Royse 1985, Royse and Bahler 1986, Royse and Schisler 1986, Przybylowicz and Donoghue 1988, Royse et al. 1990) subsequently have reported formulas incorporating millet as a significant ingredient in shiitake substrates. Millet widely is used in the United States as a substrate supplement for commercial cultivation of shiitake.

Royse (1985) reported on the effect of spawn run time on BE and basidiome size using two formulas containing millet and one formula containing wheat bran. It was found that a combination of wheat bran (10%) and millet (10%) gave a higher production rate than either millet (20%) or wheat bran (20%) used alone. However, this study did not examine the effect of increasing levels of millet on yield.

Our data show that an addition of 34% millet to a supplemented (wheat bran; rye grain; sucrose) sawdust substrate stimulated mushroom yield by 68% compared to a millet addition of only 17%. Biological efficiencies significantly increased (ca 13.5%) for each additional 6% increase in the amount of millet supplementation. Alternatively, as millet levels increased, basidiome size decreased. Average mushroom size decreased from 23 g at 17% millet to 13 g per mushroom at 34% millet supplementation. In some markets, a smaller basidiome size would result in a decrease in the value of the mushroom crop. In other markets, reduced size may not be of much concern.

Use of higher levels of millet supplementation appears to be a trend at several shiitake production plants in the United States and other western countries. Research is needed to determine the factor(s) in millet responsible for yield stimulation in shiitake. This knowledge might then be used to design a higher yielding substrate and further reduce the cost of production.

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