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Short Communication

Influence of precipitated calcium carbonate ($CaCO_3$) on shiitake (*Lentinula edodes*) yield and mushroom size

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Abstract

Synthetic substrate consisting of oak sawdust (50%), white millet (28%), winter rye (11%) and soft red wheat bran (11%) was non-supplemented or supplemented with 0.2%, 0.4% or 0.6% (dry weight basis) precipitated calcium carbonate ($CaCO_3$). Shiitake (*Lentinula edodes*) was grown in two crops to determine the effect of three $CaCO_3$ levels on mushroom yield and size. Yields and biological efficiencies (averages for two crops) from substrates non-supplemented with $CaCO_3$ were lower by 14.1%, 18.4% and 24.9% compared to treatments supplemented with 0.2%, 0.4% and 0.6% $CaCO_3$, respectively. Mushroom size (weight) was larger with non-supplemented substrate (16.8 g) compared to substrate supplemented with 0.6% $CaCO_3$ (15.1 g). However, mushroom production was more consistent from crop to crop when 0.6% $CaCO_3$ was added to substrate.

Keywords: *Lentinula edodes*; Shiitake; Edible mushrooms

1. Introduction

Production of shiitake [*Lentinula edodes* (Berk.) Pegler] worldwide increased more than seven-fold in the 14-year period from 1983 (207,000 t) to 1997 (1,573,000 t; Chang, 1999, 2002). China has emerged as a major producer of this mushroom where more than ten million part and full-time farmers cultivate shiitake (Luo, 1998; Wang, 1998). In the United States, production of shiitake is a relatively new enterprise, having begun only in the late 1970s. Farmers have learned to provide specialized management for this crop, thereby reducing production costs (Royse, 2001; Royse et al., 2002). The average output per grower has nearly doubled in the last four years (from 306 kg/week in 1998 to 587 kg/week in 2002; USDA, 2002).

Sawdust is the most popular basal ingredient used in synthetic formulations of substrate for producing shiitake in the United States (Miller and Jong, 1987; Royse, 1997, 2001), but other basal ingredients may include straw, corncobs, or both. Starch-based supplements (20–60% dry weight) such as wheat bran, rice bran, millet, rye, and maize may be added to the mix. These supplements serve as major nutrients to provide a more optimum growth medium (Royse, 1996, 1997, 2001).

Other supplements, such as minerals may be added to the mix depending on the preferred formulas of each grower. Some growers add chalk ($CaCO_3$) to their substrates while others do not. Research work by Ishikawa (1967) demonstrated an increase in the number and dry weight of mushrooms formed on synthetic medium containing 0.2% $CaCO_3$. However, the work of Ishikawa (1967) only assessed the effect of $CaCO_3$ added to synthetic medium on first flush production of shiitake and, overall supplement levels were low (only 18% rice bran). The purpose of this work, therefore, was to determine the effect of various levels of precipitated $CaCO_3$ added to nutrient-rich synthetic medium on mushroom yield and size over a three-break production period.

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2. Methods

2.1. Isolate and spawn

Isolate R26 of *L. edodes* 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).

2.2. Precipitated calcium carbonate

Precipitated calcium carbonate ($CaCO_3$) was obtained from Pfizer Minerals Division, Easton, Pennsylvania. Grade extra light 5971 was used (properties as follows): specific gravity = 2.71, tapped density 400.5 kg/m^3, dry brightness = 97, oil absorption = 48, pH = 9.5, surface area 7 m^2/g, and average particle size = 1.8 μm.

2.3. Substrates and preparation

Four substrates containing various levels of $CaCO_3$ (0, 0.2%, 0.4% and 0.6% dry substrate weight) were prepared from mixed hardwood sawdust collected from a commercial sawmill in Centre County, Pennsylvania. The predominant species found in the sawdust was the Northern red oak (*Quercus rubra* L.; as determined by tree morphology at the sawmill). The sawdust was collected in the fall of 2001 and was stored in an enclosed building until it was used. The moisture content of the fresh sawdust was approximately 34% by weight. The general substrate formulation (all ingredients based on oven dry substrate weight) consisted of 50% sawdust, 28% millet, 11% wheat bran and 11% rye.

Mixed substrate ingredients were pasteurized, cooled, inoculated and bagged with a 0.283 m^3 paddle mixer (Marion Mixers, Marion, IA). Injecting live steam into the mixer allowed the substrate to heat to 111 °C to pasteurize the substrate. This temperature was maintained for 20 min with continuous agitation to insure uniform substrate heating. After pasteurization, cold tap water was passed through a jacket fitted to the mixer to rapidly cool the substrate. Sterility of the mixture was maintained by injecting filtered air into the mixer during cool down to create a positive airflow. When the substrate had cooled to below 27 °C, it was spawned with 210 g rye grain/sawdust spawn contained in a 500 ml Erlenmeyer flask. When the spawn was thoroughly mixed with the substrate, the resulting mixture was bagged in unused polyethylene bags (20.3 cm × 12.7 cm × 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 48 h at 80 °C. Dry weight subsequently was used to determine the percentage biological efficiency (BE; ratio of fresh mushrooms harvested per dry substrate weight and expressed as a percentage).

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 °C, the plastic bags were removed and the synthetic logs moved to a "browning room". In the browning room (93–98% relative humidity, 18 ± 1 °C air temperature), the synthetic logs were manually watered lightly with a 600-hole rose-face nozzle each day. At the end of 10 and 20 days in the browning room all logs were rotated 180° 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 1900 ppm (1900 μl/l). At the end of 28 days in the browning room, the logs were soaked in cool water (13 ± 2 °C) until each weighed approximately 2.3 kg. After each flush of mushrooms was harvested, logs were re-soaked to increase log weight to 2.3 kg.

2.5. Harvesting and determination of biological efficiency (BE)

Mushrooms were harvested from the substrates at the same time each day, when the veil had broken and the gills were fully exposed. The mushrooms were then counted and weighed. At the end of the harvest period (49 days; 3 flushes), 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] × 100). Mushroom size was determined by dividing the total weight of harvested fresh mushrooms (per replicate) divided by the total number of mushrooms harvested (per replicate).

2.6. Experimental design and statistical treatment

The experiments were completely randomized designs with 18 (Crop I) and 35 (Crop II) replicates per treatment. The general linear models procedure was used to perform an analysis of variance (SAS Institute, 1998). The Waller–Duncan *k*-ratio *t*-test was used to separate treatment means (Steel et al., 1997). The experiments were repeated two times and designated as Crops I and II.

3. Results

Overall yields for Crops I and II were similar (see overall means for BE and yield, Table 1). For Crop I, mushroom yields were highest from substrate containing 0.4% and 0.6% $CaCO_3$. Mushroom yields were lowest from substrates containing no $CaCO_3$. BEs ranged from 62.3% for the substrates non-supplemented with $CaCO_3$ to 90.6% for the 0.4% supplemented substrate. For Crop I, there was no significant difference in yields from substrates containing 0.4% and 0.6% $CaCO_3$.

Results for Crop II were similar to Crop I where the trend for mushroom yields for the substrate containing higher levels of $CaCO_3$ gave higher yields. However, yields for the non-supplemented control treatment and the treatments containing 0.2% or 0.4% $CaCO_3$ were not significantly different ($P = 0.05$) from each other.

Mean BEs of the two crops ranged from 64.9% for the treatment non-supplemented with $CaCO_3$ to 86.4% for the treatment supplemented with 0.6% $CaCO_3$. Mean mushroom size ranged from 16.8 g/mushroom for substrate non-supplemented with $CaCO_3$ to 14.7g/mushroom for substrate supplemented with 0.4% $CaCO_3$.

4. Discussion

The trend toward greater use of synthetic media compared to natural logs (USDA, 1992, 2002) to produce shiitake in the United States has been attributed to greater yield potential and reduced time required to produce a crop on synthetic media (Royse and Bahler, 1986; Royse et al., 1990; Royse, 1996). Most synthetic media used commercially are composed of approximately 50% wood chips and 50% nutrient supplements such as millet, wheat bran and rye. In the present study, yield and mushroom size were measured in relation to supplemented sawdust further supplemented with calcium carbonate.

Growers throughout the world use various formulas to produce shiitake on supplemented wood chips. Some formulas include the addition of $CaCO_3$ alone or in combination with $CaSO_4$ (gypsum, Oei, 1996). In Gutien County, Fujian Province, China, for example, growers may supplement their substrates with $CaCO_3$ at rates ranging from 0.04% to 1.6% (Oei, 1996). Conversely, growers using the JUNCAO method (use of wild and artificially cultured grasses used for the main substrate ingredient; Zhanxi and Zhanhua, 2001) of shiitake production in Fujian province may use only $CaSO_4$. The preferential use by growers of one form of calcium over another appears to have evolved empirically and thus, experimental data is not available to determine optimum levels of either $CaCO_3$ or $CaSO_4$. We have found that the addition of $CaSO_4$ (various levels up to 2% of the dry weight) to the same medium reported here did not affect yield or mushroom size (D.J. Royse and J.E. Sanchez, unpublished).

Minerals such as magnesium, calcium, iron, copper, manganese, zinc and, often molybdenum, are required by fungi for growth (Jennings, 1995). Requirements of calcium in the growth medium are among the highest of the aforementioned minerals. It is known that calcium concentrations found in the apical regions of growing hyphae are higher than those found in the distal portions of hyphae. Calcium ions are believed to enter hyphal tips through passive mechanisms and are expelled by energy-dependent mechanisms at subapical zones (Ruiz-Herrera, 1992). Gradients of H^+, pH, electrical and ionic are thus created. Calcium ions, therefore, play important roles in the regulation of the growth of hyphal apices and the formation of branches (Gadd, 1995). Calcium also could have a role as second messenger (Gadd, 1995). Calcium is believed to transduce stimuli at the cell surface that may include chemical, electrical or physical signals into specific intracellular effects. The variation in calcium concentration in the medium and inside the cells has induced diverse effects on growth, differentiation and sporulation. The stimulatory effect of $CaCO_3$ for mushroom yield, therefore, may be related to the various gradients and stimuli created at the hyphal tips that ultimately influence growth and development of

Table 1
Yield (g/log), percentage BE, size (g/mushroom) and means for two crops of *Lentinula edodes* grown on synthetic medium non-supplemented or supplemented with three levels of precipitated $CaCO_3$ (% of substrate dry weight)

Treat no.	Precipitated $CaCO_3$ (%)	Crop I			Crop II			Means (I,II)		
		BE	Yield	Size	BE	Yield	Size	BE	Yield	Size
1	0	62.3	677c	15.8a	67.5	733b	17.7a	64.9	705	16.8
2	0.2	77.7	844b	15.3a	73.4	797b	16.3ab	75.6	821	15.8
3	0.4	90.6	984a	13.2b	68.5	744b	16.2ab	79.6	864	14.7
4	0.6	84.4	917ab	14.8ab	88.3	960a	15.4b	86.4	939	15.1
Overall mean		78.8	856	14.8	74.4	809	16.4	76.6	832	15.6

$$\%BE = \frac{\text{kg fresh mushrooms}}{\text{kg dry substrate}} \times 100.$$

Means followed by the same letter in the same column are not significantly different according to the Waller–Duncan k-ratio t-test ($P = 0.05$).

the mushroom. It is known that $CaCO_3$ has a profound effect on pH, while $CaSO_4$ has no or only minimal effect on substrate pH. In addition, the release of Ca^{2+} ions from $CaCO_3$ in the substrate may be facilitated more than $CaSO_4$ by the mycelium of *L. edodes*.

This study demonstrated that substrates containing up to 0.6% precipitated calcium carbonate were more productive than substrates containing no additional calcium. Further study is needed to determine the effects of other forms and concentrations of calcium on mushroom yield and BE. In addition, it may be important to assay the amount of calcium (and other minerals) present in fruitbodies to determine if various calcium sources and concentrations affect mineral concentrations in harvested mushrooms. Increased mineral concentrations would enhance nutritional value of mushrooms and, therefore, may help to increase demand from consumers. As shiitake production becomes more competitive and profit margins are squeezed, growers willing to optimize their production media may have an advantage in the marketplace. Ultimately, consumers will benefit from increased mushroom yields by having shiitake available at a lower price.

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