Technology & Product Reports

Does Amendment of Soak Solution with Sucrose and Urea Increase Production of Shiitake Mushrooms on Sawdust Blocks?

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ADDITIONAL INDEX WORDS. biological efficiency, *Lentinula edodes*

SUMMARY. This study evaluated whether adding either sucrose or urea to the soak water could enhance production of shiitake mushrooms (*Lentinula edodes*) on sawdust blocks. For both sucrose and urea experiments, sawdust blocks inoculated with “QR” and “26” strains of *L. edodes* were placed in the soak water amended with either sucrose or urea at the first soaking only, at the second soaking only, or at all six soakings. Control blocks were soaked in tap water. In Experiment I, blocks were soaked in water containing 0, 20,000, or 40,000 ppm ($\text{mg}\cdot\text{L}^{-1}$) sucrose. Strain 26 produced significantly more mushrooms and greater mushroom weight than QR. Addition of sucrose to the soak water resulted in fewer mushrooms harvested and lower yields than controls. There was a significant interaction

between the sucrose rate and strain for both mushroom number and biological efficiency (BE). Both strains produced fewer mushrooms and less BE as the concentration of sucrose in the soak water increased; however, QR was less affected by the increasing concentration of sucrose. In Experiment II, sawdust blocks inoculated with QR and 26 strains of shiitake were soaked in water containing 0, 2400, or 3600 ppm ($\text{mg}\cdot\text{L}^{-1}$) urea. Strain 26 produced significantly more mushrooms and greater BE than QR. The addition of 2400 ppm of urea to the soak water resulted in more mushrooms per block harvested and a 12% increase in BE over the control. The 2400 ppm rate added at each soak produced more mushrooms and mushroom weight than the control and also produced more mushrooms than any of the blocks in the higher rate of urea (3600 ppm) treatments. Adding 16.9 oz (480 g) of urea per tank to obtain 2400 ppm urea in the soak water results in the minimal increase in cost of about \$0.20 per soak (52 sawdust blocks), but potentially increases the value of the mushrooms harvested from each block by \$0.75. In an average-sized shiitake mushroom block production facility containing 500 blocks, continuous addition of 2400 ppm urea to the soak water would provide an increased return of about \$375 over the entire season.

Shiitake mushrooms contributed \$25.2 million to the \$889 million U.S.-produced mushroom crop in 2002–03. Production of shiitake mushrooms in 2002–03 was 8.2 million lb (3.72 million kg) (U.S. Department of Agriculture, 2003). Since 1992, shiitake production has tripled while the wholesale price has gone from \$3.88 to \$3.06 per pound (\$8.55

to \$6.75 per kg). Shiitake mushroom prices increased 4% in 2003, defying the general downward trend that has occurred since 1992 (U.S. Department of Agriculture, 1995). The overall decline in price is minimal considering the increase in production. During this same period, producer numbers and log production have declined while sawdust block production and total U.S. production increased to a peak in 2000–01. It appears that producers are optimizing production by increasing sawdust substrate production. Therefore, production innovations that will continue to decrease input costs and improve producer returns are necessary to counter overall declining prices.

Integration of shiitake mushrooms into local farming systems serves to utilize forest resources and to capitalize on a crop that is primarily imported, but can be grown domestically. Commercial shiitake mushroom production in the United States started on hardwood logs. Because shiitake production on natural logs is so labor intensive, many producers are shifting to sawdust block production. Sawdust blocks are made from hardwood sawdust mixed with grains, nutrients, and lime. However, the actual ingredients and amounts vary with the producer. The ingredients are mixed, placed in a heat-resistant bag, and are then autoclaved. After the ingredients cool, shiitake spawn is added to the bag mixture and the bag is sealed. Each bag has a small breathing patch for air exchange. As mycelia grow, the sawdust mixture turns white, then brown. When the block is completely brown, it can be removed from the bag, and will usually fruit without soaking. To be profitable, sawdust blocks should be from 50% to 100% BE, which is a measure of production efficiency (Donoghue, 1994). Basal efficiency is calculated by dividing the fresh weight of mushrooms harvested by the dry weight of the block (substrate) and multiplying by 100. The sawdust block production method is more costly than production on natural logs, but can result in higher yields from a much smaller space with a shorter crop cycle.

The content of the sawdust block is also critical to the level of production of a specific strain. Royse et al. (1990) determined that adding low levels (0.6% to 1.2%) of sucrose to the substrate resulted in significant increases in the BE (22%) compared to the sub-

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Contributed by the Agricultural Experiment Station, Alabama A&M University, Journal paper no. 282. Research supported by CSREES/USDA.

strate without the addition of sucrose. Most of the increase in BE occurred during the first mushroom break, and it was observed that the 0.6% and 1.2% sucrose-supplemented blocks tended to have less scattered pick cycles. In another experiment, three saccharides (sucrose, fructose, and glucose) at three rates (0%, 0.6%, and 1.2%) were compared. Boyle (1998) determined that most nitrogen-containing supplements increased growth, while other elements, vitamins, or carbohydrates had little effect. In general, sucrose had a more favorable influence on yield than fructose or glucose. The literature cited above has shown that there is potential for increasing yields by adding supplements to the substrate. In this research, the soak water was targeted as the vehicle for delivery of the amendment because most producers do not make their own synthetic blocks. The soak water amendments were also used because, as the block ages, substrate and nutrients become depleted, and we speculated that some of this loss could be reintroduced via soaking to prolong or increase the production life of the block. The objectives of this research were to: 1) compare two sawdust block strains of shiitake; 2) evaluate sucrose and urea supplements to the soak water; 3) determine most productive levels of urea and sucrose supplementation; and 4) optimize the timing of supplementation of sucrose or urea into the soak water.

Materials and methods

Hardwood sawdust blocks inoculated with strains QR or 26 were obtained from Field and Forest Products (Peshtigo, Wis.) in 1997. These strains were developed for sawdust and do not necessarily perform well in hardwood logs. Sawdust blocks were made sequentially, 64 blocks at a time. Each 64-block batch consisted of 84 gal (318.0 L) by volume of red oak (*Quercus rubra*) sawdust, 35 lb (15.9 kg) millet, 22 lb (10.0 kg) bran, 8.8 fl oz (260.3 mL) gypsum, 8 lb (3.6 kg) winter rye, and 17 fl oz (502.8 mL) sucrose. The average dry weight of the blocks used in this experiment was 2 lb (907.2 g). Blocks remained in the bags for 6 weeks until the "skins," or outer surface of the block, had turned a light to medium brown. On 20 June 1997, the sawdust blocks were freed of the bags and mushrooms that had developed while the block was inside the

bag were removed before soaking.

For both the sucrose and urea experiments, blocks in the control treatments were soaked in tap water on 20 June and 14 July, respectively, for 24 h. The blocks in the 20,000 ppm sucrose treatment were first soaked on 23 June, and blocks in the 40,000 ppm treatment were soaked 24 June. The blocks were placed in a 150-gal (567.8 L) aluminum soak tank with a board over the top to keep the blocks from floating and soaked for 24 h. To determine the amount of sucrose or urea to be added, the empty tank was filled with 52 sawdust blocks. The amount of water needed to fill the tank containing the blocks was 53 gal (200.6 L). Since the same number of blocks was put in the tank each time and the amount of water added at each soaking was approximately the same, this volume was always used as the basis for the addition of sucrose or urea. To reduce the amount of cross contamination of blocks, new soak water was used for each treatment. In addition to the three rates of sucrose (0, 20,000, and 40,000 ppm) and two strains (QR and 26), there were four application treatments. The control was soaked in tap water each of the six times. The "first soak only" blocks were immersed in urea/sucrose-amended tap water the first time the blocks were soaked, and tap water without urea/sucrose the remaining five times. The "second soak only" blocks were soaked in tap water the first time, urea/sucrose-amended water the second time, and tap water the last four times. The "continuous soak" blocks were soaked in urea/sucrose-amended water each of the six times.

For the urea experiment, the blocks were removed from the bags on 14 July 1997. The control blocks were placed in tanks containing tap water on the same day and the 2400 and 3600 ppm urea treatments were soaked 15 and 16 July, respectively, for 24 h. The 2400 and 3600 ppm rates required the addition of 16.9 oz (480 g) and 25.4 oz (720 g) of urea, respectively, to 53 gal of water in the soak tank at each amended soak. Nitrogen in the form of urea at three rates (0, 2400, and 3600 ppm) was evaluated with three times of application (first soak only, second soak only, all soaks) plus a control, and two strains (QR and 26). Sawdust blocks were soaked at 3-week intervals after the initial soaking. Some

blocks decomposed prior to the final soaking and were disposed of as was appropriate.

Blocks were placed on racks in an insulated heated/cooled 12 × 24 ft (3.7 × 7.3 m) building. The air temperature was maintained at 68 to 72 °F (20.0 to 22.2 °C) during the day and 62 to 66 °F (16.7 to 18.9 °C) at night. Flora-MIST nozzles (Reed S. Kofford, Walnut Creek, Calif.) misted the interior of the building three times per day for 5 min to maintain humidity in the house. The nozzles hung from the ceiling and were positioned in the aisles between the racks and above the logs, spaced 3 ft (0.9 m) apart going down the aisle. The two rows of sprinklers were 4 ft (1.2 m) apart. The nozzles distributed 4 gal (15.1 L) of water per hour as a 3 to 4-ft circle of fog-like mist spray. There was minimal overlap between nozzles, as the purpose was to provide humidity, not irrigate the blocks. The three racks were located one on each sidewall and one in the middle. Each rack had four shelves, and the blocks were randomly placed on the shelves throughout the experiment.

Mushrooms were harvested once per day. A mushroom was considered "mature" when the veil covering the gills had broken or split. Mushrooms from each block were counted and weighed. Mushrooms that were less than 1 inch (2.5 cm) in diameter or badly misshapen are not considered marketable and therefore were not included in the results of this research.

All treatments were replicated three times with three subsamples. All treatments were completely randomized. Number and weight of mushrooms were recorded and BE was calculated for treatments. Incidence of disease was not specifically recorded. However, blocks that became consumed with contaminant fungi were discarded and block numbers were recorded along with the date of disposal. Data were analyzed with the statistical package StatView (SAS, Cary, N.C.) and SuperAnova (Abacus Concepts, Berkeley, Calif.). Mean separations were calculated using Duncan's new multiple range test ($P \leq 0.5$)

Yield was reported as BE and number of mushrooms.

Results and discussion

SUCROSE EXPERIMENT. Strain 26 produced an average of 52% BE and 51 mushrooms per block compared

Fig. 1. Both biological efficiency and number of shiitake mushrooms produced per block were decreased significantly when sucrose at 20,000 ppm (mg·L⁻¹) was added in the soak water, with an even greater decrease occurring with the 40,000 ppm rate of sucrose. Means are separated using Duncan's new multiple range test ($P \leq 0.05$), $n = 54$.

Fig. 2. Biological efficiency was influenced by both strain of shiitake mushroom and rate of sucrose with increasing concentrations of sucrose in the soak water having a greater negative effect on strain 26 than QR. Points represent treatment means, $n = 81$.

QR = $35.2 - (0.00028 \cdot \text{rate})$, $P = 0.006$, $r^2 = 0.092$.

26 = $67.1 - (0.001 \cdot \text{rate})$, $P = 0.0001$, $r^2 = 0.340$.

to QR, which produced only 30% BE and 19 mushrooms per block.

The addition of 20,000 and 40,000 ppm of sucrose to the soak water resulted in significantly fewer mushrooms harvested. The 40,000 ppm rate produced 24 mushrooms/block, which was about half that of the control (Fig. 1). The sucrose amendments also resulted in lower yields (39% and 31% BE, respectively) than the control (52% BE) (Fig. 1). There was a significant interaction between strain and concentration of sucrose in the soak water. Strain QR was less affected by the increased concentration

of sucrose (36.1% BE control to 24.8% BE 40,000 ppm) than strain 26 (67.7% BE for the control compared to 37.6% for 40,000 ppm) (Fig. 2).

The BE of strain 26 was reduced significantly when sucrose was added at the first and the second soak only. When sucrose was added each time the blocks were soaked, reduction in BE was nonsignificant relative to the control (Fig. 3).

Fig. 3. Biological efficiency was reduced by sucrose additions at the first and second soaks only for shiitake mushroom strain 26 but the negative impact of sucrose as a function of soak timing was not significant for strain QR. Means are separated using Duncan's new multiple range test ($P \leq 0.05$), $n = 18$.

Fig. 4. Both biological efficiency and the number of shiitake mushrooms harvested for sucrose treated blocks increased from the first harvest, peaked at the second harvest, and declined thereafter. Means are separated using Duncan's new multiple range test ($P \leq 0.05$), $n = 162$.

Yields peaked at the second harvest irrespective of sucrose treatment, giving 18% BE, 15 mushrooms per log vs. 14% BE and 11 mushrooms per log at the first harvest (Fig. 4). When the rate of amendment was plotted against the dates of harvest, the most important feature of this interaction was the sharp decline in yields at the third harvest as sucrose rates increased (Fig. 5). These results suggest that the

Fig. 5. The negative effect on biological efficiency of adding sucrose at increasing rates to the soak water was significant only at the third harvest of shiitake mushrooms. Points represent treatment means, $n = 54$.

1st harvest = $14.3 - (0.0000135 \cdot \text{rate})$, $P = 0.7939$, $r^2 = 0.0004$.

2nd harvest = $20.0 - (0.000096 \cdot \text{rate})$, $P = 0.1292$, $r^2 = 0.014$.

3rd harvest = $14.0 - (0.000318 \cdot \text{rate})$, $P = 0.0001$, $r^2 = 0.151$.

4th harvest = $1.28 - (0.0000089 \cdot \text{rate})$, $P = 0.5910$, $r^2 = 0.002$.

Fig. 6. Urea at 2400 ppm ($\text{mg} \cdot \text{L}^{-1}$) significantly increased biological efficiency and number of shiitake mushrooms produced per block over those of the control blocks, but not at the 3600 ppm rate. Means are separated using Duncan's new multiple range test ($P \leq 0.05$), $n = 54$.

external infusion of sucrose through the soak medium did not elicit the same response as adding sucrose to the substrate (Royse et al., 1990).

NITROGEN/UREA EXPERIMENT. As in the sucrose experiment, strain 26 produced more weight and greater number of mushrooms (40% BE and 37 mushrooms per block) than QR (27% BE and 19 mushrooms per block).

The addition of 2400 ppm urea to the soak water resulted in more mushrooms (34 vs. 24) harvested and an increase of 11% in biological efficiency over the control (39% compared to 28%) (Fig. 6). Both the number of mushrooms and biological efficiency were less with the 3600 ppm rate of urea than the 2400 rate, but this was significant only in the case of number

Fig. 7. The most effective treatment in terms of increasing both biological efficiency and number of shiitake mushrooms produced per block was the 2400 ppm ($\text{mg} \cdot \text{L}^{-1}$) urea treatment administered at every soak. Means are separated using Duncan's new multiple range test ($P \leq 0.05$), $n = 18$.

of mushrooms. This could indicate a declining trend in yields due to excessive nitrogen in the blocks (Leatham, 1985).

The interaction between the timing of the addition of urea to the soak water and the rate of urea was significant. The BE of blocks with the 2400 ppm urea treatment continued to increase as soak time increased (first time only—34% BE, second time only—35% BE, continuous—47% BE) (Fig. 7). The continuous soak treatment at the 2400 ppm urea level also produced almost twice the mushrooms (47) as that of the control (24) and 3600 ppm urea continuous soaks (20).

Fig. 8. Both biological efficiency and number of shiitake mushrooms per block declined after the first harvest in the urea experiment. Means are separated using Duncan's new multiple range test ($P \leq 0.05$), $n = 162$.

Biological efficiency declined after the first harvest of blocks soaked in urea (Fig. 8). Although harvest date significantly interacted with strain, soak treatments, and rate of urea, the trends noted did not vary from the main effects; therefore they have not been shown.

There are many important variables in growing mycelium and fruiting shiitake in sawdust media. The sawdust should be composed of hardwood species, and supplemented with a carbohydrate source such as grain, corn powder, glucose, and a nitrogen-vitamin-mineral source, such as oatmeal, rice bran, or wheat bran. Sugars provide easily available carbohydrates that speed colonization and degradation of the medium, reducing the time to fruiting. If there are excess carbohydrates, weed fungi or contaminants often invade the substrate. This

can result in loss of yields and rapid deterioration of the substrate. In this study, it was found that external application of sucrose via the soak water reduced yields. While contaminants could be an issue, no specific data were recorded on contaminated blocks and their distribution throughout the treatments. Productivity in the urea amendment experiment was increased by the addition of 2400 ppm urea to the soak water at each soaking. While the added urea minimally increased the cost of production, the value of the mushrooms harvested from each block increased. The addition of 2400 ppm or 16.9 oz urea to each tank increased the cost of production about \$0.20 per soak (52 sawdust blocks) but increased the value of the mushrooms harvested from each block by \$0.75. The potential increase in return for the 52 blocks soaked five times with urea

added each time would be \$38.00. In an average-sized shiitake mushroom block production facility, the increase in returns for 500 blocks would be about \$375 over the entire season, a substantial return on the investment of urea at 2400 ppm.

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