RESEARCH ARTICLE

Comparison of Cultivation, Mushroom Yield, and Fruiting Body Characteristics of *Lentinula edodes* Strains according to the Inoculation Method

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ABSTRACT

The cultivation in sawdust media, mushroom productivity, and fruiting body characteristics of *Lentinula edodes* strains NIFoS 2778 and NIFoS 3363 were compared according to the inoculation conditions. The cultivation period was 5% shorter when liquid spawn was used. Fruiting bodies were induced after 113 days of incubation on media inoculated with liquid spawn, and the cultivation period was 119 days on media inoculated with solid spawn. Mushroom productivity of NIFoS 2778 was the highest (661.4 g) when 36 mL of liquid spawn was used. For NIFoS 3363, mushroom production was higher under liquid inoculation conditions when the same amount of liquid and solid spawns were used. The mushroom characteristics of the two strains were not significantly different, except for gill width and stipe diameter.

Keywords: Cultivation, Inoculum, *Lentinula edodes*, Sawdust media

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OPEN ACCESS

pISSN : 0253-651X
eISSN : 2383-5249

Kor. J. Mycol. 2021 December, 49(4): 525-530
https://doi.org/10.4489/KJM.20210051

Received: September 14, 2021
Revised: December 22, 2021
Accepted: December 22, 2021

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INTRODUCTION

Lentinula edodes, shiitake, is one of the most widely cultivated mushrooms in Korea. In 2019, 19,242 tons of fresh and 965 tons of dried mushrooms were produced and sold for 201.9 billion won [1]. Although the total shiitake production is similar every year, the number of sawdust bags used for cultivation has increased from 8,518,280 bags in 2010 to 21,057,644 bags in 2015 [2]. In Korea, three main types of sawdust media are used for cultivation: rectangular, cylindrical, and columnar with ring necks and plugs. Although sawdust media differ in shape and cultivation method, the days needed for mycelial growth and maturation after inoculation are relatively long when compared with the requirements for other cultivated mushrooms, such as oyster mushrooms. Thus, shiitake mushrooms are cultivated only twice a year.

Spawn inoculation is a very important step for successful shiitake cultivation. Most growers use sawdust spawn, but some growers use liquid spawn or grain spawn. Previous studies have shown that liquid spawn can be more productive than sawdust spawn [3-6], but the effect of liquid spawn has not been studied in detail.

In this study, we used sawdust and liquid spawns in different quantities for the cultivation of shiitake and compared their effects on mycelial growth, cultivation period, mushroom productivity, and mushroom characteristics.

MATERIALS AND METHODS

Spawn preparation

Two shiitake strains, NIFoS 2778 and NIFoS 3363, stored at the National Institute of Forest Science were used in this study. They were inoculated on potato dextrose agar (213400, Difco, Detroit, MI, USA), incubated for 7 days at 25℃, and used as inoculum. Solid and liquid media were prepared for the spawn. Solid media were prepared using a 1 L plastic bottle with 650 g of the media. The medium composition was 80% oak tree sawdust (1:1 of *Quercus mongolica* and *Quercus acutissima*) and 20% wheat bran (w/w) with 60±5% moisture content. The inocula were placed in the solid media and incubated at 24±1℃ for 30 days. The liquid media were prepared using a 100 mL Erlenmeyer flask with 40 mL of potato dextrose broth (254920, Difco). After inoculation, the flasks were incubated at 25℃ for 21 days. The strains in the liquid media were homogenized using a homogenizer (CN/MT-13K, Miulab, Hangzhou, Zhejiang, China) before use.

Cultivation using sawdust media

Cylindrical sawdust media were made using plastic bags containing 3 kg of the media. The medium composition was 85% oak tree sawdust (1:1 of *Q. mongolica* and *Q. acutissima*), 7.5% wheat bran, and 7.5% rice bran (w/w) with 55±5% moisture content. The sawdust bags were autoclaved and inoculated with the solid and liquid spawns via four holes. The amount of inoculation was as follows: 9, 12, and 15 g of the solid spawn in each hole of the sawdust bags (total, 36, 48, and 60 g, respectively) and 9, 12, and 15 mL of the liquid spawn in each hole of the sawdust bags (total, 36, 48, and 60 mL, respectively). They were incubated at 23±1℃ in the dark. During the spawn run, depending on the mycelial growth, air holes were drilled in the bags three times (about 15, 30, and 60 days after inoculation) to avoid carbon dioxide accumulation in the bags. When the substrates in the bags were fully colonized by shiitake mycelia, the bags were exposed to low levels of light (approximately 350 lx). Fruiting was induced after browning and formation of the outer protective surface.

Fruiting and fruiting body characteristics

To induce fruiting, the plastic bags were removed, and the sawdust media were exposed to low temperature (18±1℃) and high humidity (90±10%). After harvest, the sawdust blocks were induced to dormancy at 21±1℃ and low humidity (70±10%), and the subsequent flushes were performed by injection of water into the media at one-month intervals. Mushroom productivity was calculated after three flushes. The harvested fruiting bodies were measured according to the International Union for the Protection

of New Varieties of Plants (UPOV) guidelines for the examination of shiitake varieties [7]. The measured characteristics were the fresh weight of the fruiting bodies, diameter and height of the caps, gill width, and diameter and length of the stipes.

Statistical analysis

Statistical analysis was performed using SPSS software (PASW statistics 18, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Cultivation characteristics

After spawn inoculation, the mycelial growth rate was measured on the surface of the media. Initially, the growth rate in the media inoculated with the solid inoculum was faster than that in the media inoculated with the liquid inoculum. NIFoS 2778 and NIFoS 3363 strains took about 42 days to cover the surface of the media with white mycelia when the solid inoculum was used, and they took about 56 days when the liquid inoculum was used (Fig. 1). However, the growth rates of the two strains were not statistically different. Because the liquid inoculum could penetrate into the media and grow from the inside of the media, browning and formation of the outer protective surface was faster in the media with the liquid inoculum. Fruiting was induced after 113 days of incubation when the liquid inoculum was used, and it took 119 days when the solid inoculum was used. However, Lee et al. [6] reported that the amount of liquid spawn affected the mycelial growth of *Lentinula edodes*. Liquid spawn (45-60 mL) was suitable for the inoculation of 2 kg of sawdust media, but mycelial growth in the media with 10-40 mL of liquid spawn was slow. Lee et al. [3] also mentioned that the media used for liquid culture and amount of liquid spawn affected the mycelial growth of *Lentinula edodes*. It seems that a certain amount of inoculum is required for the liquid spawn to shorten the incubation period, and the liquid inoculum used in this study was sufficient for mycelial growth in the sawdust media.

Fruiting body production differed in terms of the strain and inoculation conditions (Fig. 2.). The amount of fruiting body production by NIFoS 2778 decreased as the amount of liquid spawn increased, and it increased as the amount of solid spawn increased. The amount of fruiting body production by NIFoS 3363 was higher after liquid spawn inoculation than after solid spawn inoculation when the same amount of solid and liquid inocula were used. The highest production (661.4 g) was observed by NIFoS 2778 when 36 mL (9 $\times$ 4 mL) of the liquid spawn was used.

As shown in this study, the inoculation conditions affected the mushroom yield. Bak et al. [5] reported that the total fruiting body yield of *Lentinula edodes* was the highest when 30 g of solid spawn was used in 2 kg of medium, and the yield was not significantly different when 20 mL and 30 mL of liquid spawn were used in the same medium. Lee et al. [4] reported that the yield of *L. edodes* was higher when 30 mL of liquid spawn was used in 2 kg of medium than when 10 g of solid spawn was used. In addition, substrate

composition influences mushroom yield [8]. Öztürk and Atila [9] reported that *Hypsizygus ulmarius* yield is related to cellulosic substrate degradation, and the amount of lignin negatively affects mushroom yield. The solid and liquid inocula used in this study contained fungal mycelia and the growth media contained sawdust or potato dextrose, and different amounts of growth media may have affected the spawn run, maturation, and mushroom yield.

The fresh weight of the fruiting bodies, diameter and height of the caps, and length of the stipes were not significantly different between the two strains and under the three inoculation conditions (Table 1). Only the gill width and diameter of the stipes were significantly different. The gill width of NIFoS 3363 was thicker than that of NIFoS 2778. The inoculation conditions did not affect the gill width of NIFoS 2778; however, the gill width of NIFoS 3363 differed according to the inoculation method, and the gill width was the thickest when 60 mL of liquid spawn (15×4 mL) was used. The stipe diameter of NIFoS 3363 was also larger than that of NIFoS 2778. The stipe diameter of NIFoS 3363 was larger under the solid spawn inoculation conditions, but no significant differences in NIFoS 2778 were found under the different inoculation conditions.

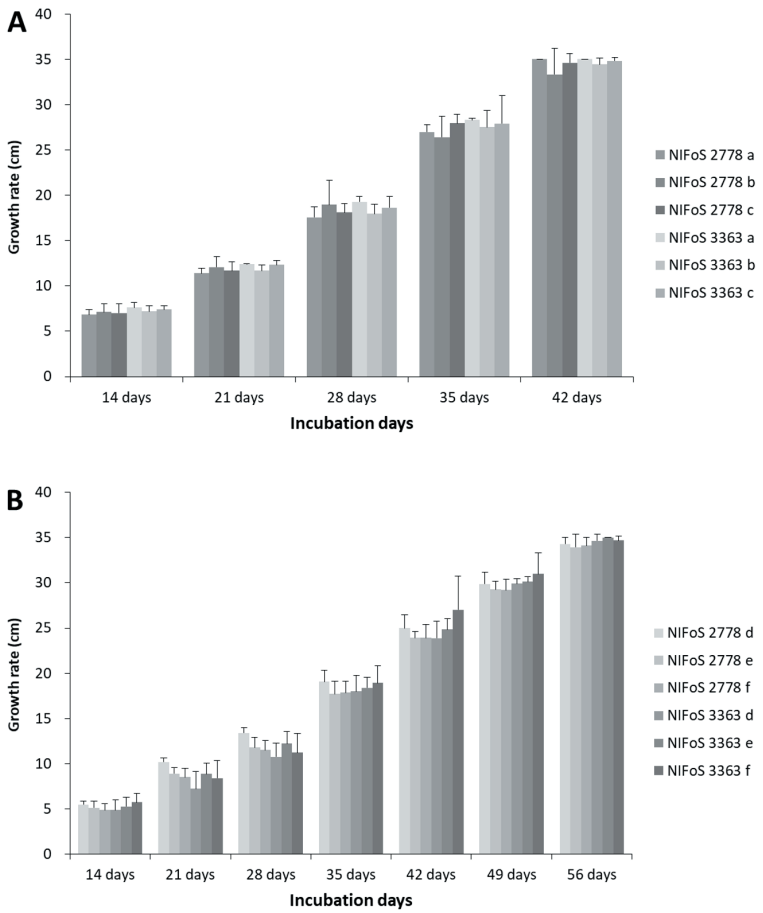

Fig. 1. Initial mycelial growth rate of *Lentinula edodes* strains NIFoS 2778 and NIFoS 3363 in sawdust media. A: solid spawn. B: liquid spawn. a: 9×4 g; b: 12×4 g; c: 15×4 g; d: 9×4 mL; e: 12×4 mL; f: 15×4 mL.

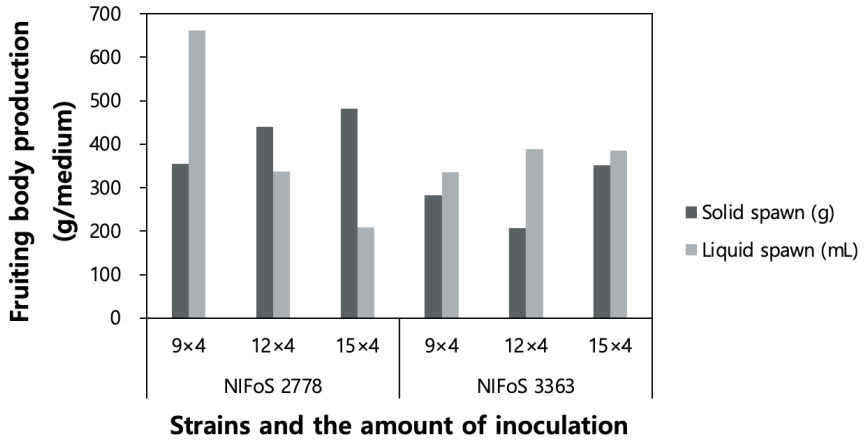

Fig. 2. Comparison of fruiting body production of *Lentinula edodes* on sawdust media under different strain and inoculation conditions.

Table 1. Comparison of production and morphological characteristics of fruiting bodies of *Lentinula edodes* strains.

Comparative characteristics	Solid spawn						Liquid spawn					
	NIFoS 2778			NIFoS 3363			NIFoS 2778			NIFoS 3363		
	9×4 g	12×4 g	15×4 g	9×4 g	12×4 g	15×4 g	9×4 mL	12×4 mL	15×4 mL	9×4 mL	12×4 mL	15×4 mL
Fresh weight (g)	60.8±32.9a	61.7±23.6a	58.1±35.7a	68.3±22.1a	75.9±18.6a	63.6±32.0a	46.7±17.9a	75.9±34.2a	52.3±36.6a	42.9±21.7a	55.6±24.1a	49.3±28.3a
Cap diameter (mm)	79.9±20.2a	78.5±14.3a	75.1±23.2a	69.9±12.1a	74.7±10.1a	75.4±22.3a	72.7±14.6a	80.0±18.2a	74.6±18.5a	71.5±17.3a	74.3±13.7a	72.5±17.0a
Cap height (mm)	15.2±3.6a	14.3±4.2a	14.5±4.3a	18.2±2.9a	18.6±4.9a	15.6±4.5a	13.9±2.3a	16.1±3.0a	14.5±2.3a	11.7±3.9a	14.6±2.4a	15.4±13.9a
Gill width (mm)	2.1±0.8c	1.8±0.7c	1.8±1.2c	2.5±1.0bc	2.9±0.8abc	3.9±1.7ab	1.8±0.7c	2.4±1.3bc	1.9±0.9c	3.0±1.6abc	4.1±1.7ab	4.6±1.5a
Stipe length (mm)	55.8±11.0a	57.8±10.0a	58.7±11.8a	54.8±6.5a	60.9±11.4a	54.0±12.2a	51.7±12.4a	57.8±13.2a	47.5±16.1a	45.3±12.4a	48.3±11.5a	44.9±11.5a
Stipe diameter (mm)	15.6±3.7bc	16.2±4.0bc	15.7±4.2bc	26.4±3.8a	24.0±3.4a	22.5±6.2ab	14.6±4.2c	16.2±3.2bc	13.4±5.5c	16.5±4.6bc	22.0±5.5ab	19.4±6.8abc

a-c: The value indicates a significant difference (Scheffe test, $n \geq 10$, $p < 0.05$).

In this study, we compared the cultivation period, mushroom productivity, and fruiting body characteristics when different amounts of solid and liquid spawns were used. The liquid inoculum has advantages in terms of the cultivation period and mushroom productivity when NIFoS 2778 and NIFoS 3363 strains were used. Further studies are needed to fully understand the relationship between inoculation conditions, mycelial growth in sawdust media, and mushroom yield.

ACKNOWLEDGEMENTS

This study was supported by a grant from the General Project (FP0800-2020-01) of the National Institute of Forest Science, Republic of Korea.

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