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Effects of different cultivation material formulas on the growth and quality of *Morchella* spp.

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ABSTRACT

To study the effects of different cultivation material formulas on the growth and quality of *Morchella* spp. With the cultivated species strains extracted from wild *Morchella* spp. in Diebu County, Gannan Prefecture as experimental materials, an experiment was designed and the data then obtained was analyzed using the single factor variable method. By measuring the pileus length, pileus perimeter, stipe length, stipe perimeter and yield as well as the ash content, total sugar content, crude protein content and crude fiber content of wild *Morchella* spp., the effects of four different cultivation material formulas on the growth and quality of *Morchella* spp. were studied. The result showed that the *Morchella* spp. cultivated using Formula 1, i.e., the formula to which *Morchella* spp. footing soil was added, grew best, and had the highest yield and the best quality; and the qualities of *Morchella* spps cultivated using other formulas decreased in a row. Formula 1 to which *Morchella* spp. footing soil was added had the optimal effect on promoting the growth and quality of *Morchella* spp.

1. Introduction

Morchella spp., belonging to *Aseomycotina, Discomycetes*, *Pezizales, Morchellaceae* and *Morchella* (Guo et al., 2010), enjoys a reputation as "the world's most precious rare edible fungi" and is high nutritional. With a unique flavor and a variety of functions, it is rich in essential amino acids, vitamins, carbohydrates and proteins (Razali and Said, 2017; Halim and Phang, 2017). It is given this name because its pileus looks like lamb tripe (Che et al., 2010). With refreshing and kidney reinforcing functions, it also plays an important role in health care (Du et al., 2014). In addition, *Morchella* spp. is a very good traditional Chinese medicine that is mild and sweet., and can be used to promote digestion, benefit intestinal tract and tonify spleen and stomach (Liu, 2013), having a great development and utilization value in the food, medicine, and cosmetics industries (Guan, 2012; Gao et al., 2017). At present, there have been many studies of *Morchella* spp. and most of them have focused on the fields of mycelium and sclerotium, health care, pharmacology, biological characteristics, and polysaccharide extraction (Wang, 2012; Zhang et al., 2002; Ren and An, 2010; Lei et al., 2013; Quan and Zhang, 2012; Liu, 2014; Xue, 2011). However, reports on the effects of cultivation material formulas on promoting the growth and quality of *Morchella* spp. are rarely seen (Liu et al., 2013; Dai, 2013; Zhao et al., 2010; Zhao et al., 2009; Shamsudin et al., 2017). Therefore, a comprehensive study on the effect of different three-level strain cultivation material formulas on promoting the growth and quality of *Morchella* spp. was conducted, which has provided a theoretical basis for further efforts to determine the optimum cultivation material formula for the growth of *Morchella* spp., and a scientific basis for improving the yield and quality of *Morchella* spp.

2. Materials and methods

2.1. Materials

2.1.1. Main drugs and test materials

Gypsum, sawdust, lime, wheat bran, sucrose, plant ash, Ca $(H_2PO_4)_2$, mushroom promoter, KH_2PO_4, *Morchella* spp. footing soil, $MgSO_4$, 75% alcohol, one -level strain of *Morchella* spp. (extracted from wild *Morchella* spp. in Diebu County, Gannan Prefecture), etc.

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2.1.2. Main apparatuses

DF205 type electric blast drying box, vernier caliper,Ceramic crucible, analytical balance, drying box, electrothermal furnace, mortar, Soxhlet extractor, automatic nitrogen analyzer, etc.

2.1.3. Preparation of three-level strain cultivation material

(1) Cultivation material formula

Formula 1: sawdust 74%, wheat bran 20%, KH_2PO_4 1%, $MgSO_4$ 1%, *Morchella* spp. footing soil 1%, gypsum 1%, lime 1% and $Ca(H_2PO_4)_2$ 1%.

Formula 2: sawdust 74%, wheat bran 20%, KH_2PO_4 1%, $MgSO_4$ 1%, mushroom promoter 1%, gypsum 1%, lime 1% and $Ca(H_2PO_4)_2$ 1%.

Formula 3: sawdust 74%, wheat bran 20%, KH_2PO_4 1%, $MgSO_4$ 1%, plant ash 1%, gypsum 1%, lime 1% and $Ca(H_2PO_4)_2$ 1%.

Formula 4: sawdust 74%, wheat bran 20%, KH_2PO_4 1%, $MgSO_4$ 1%, sucrose 1%, gypsum 1%, lime 1% and $Ca(H_2PO_4)_2$ 1%.

(2) Sealing material formula

Morchella spp. footing soil 75%, wheat bran 15% and sawdust 10%.

2.2. Preparation of strains

After raw materials were weighed according to the different formula proportions, the sawdust, wheat bran, *Morchella* spp. footing soil, lime and gypsum were mixed evenly. Then, a small amount of water was added to dissolve sucrose, KH_2PO_4, $MgSO_4$ and $Ca(H_2PO_4)_2$, and the mixed solution was sprinkled evenly on a mixed material pile. A proper amount of water was then added to the material pile and the pile was stirred to a certain degree where, the cultivation material did not discharge water or break into pieces when held in hand. The sealing material was mixed evenly with water until it did not discharge water or breake into pieces when held in hand. The mixed cultivation material was put into a 750-ml mushroom dedicated bottle, and the bottle mouth was sealed with sealing material. The cultivation material was sterilized in a high temperature and high-pressure sterilization pot at 0.12 MPa and 120°Cfor 60 min. After the temperature of the sterilized cultivation material decreased, the cultivation materials, apparatuses for inoculation and test strains were placed onto an ultra-clean bench, and inoculation started after ultraviolet sterilization continued for 30 mins. The bean-size two-level strains were inoculated to the cultivation material with an inoculating shovel, and 15–20 bottles of each formula were inoculated. Finally, the inoculated cultivation materials were placed into a constant temperature incubator at 23 °C for darkness culture, until the sclerotium grew big enough to cover the materials.

2.3. Experimental methods

The experiment was using the randomized block method in field. The strains were sown in the greenhouse on September 25, 2016, and the area of a small plot was $3 \times 4\ m^2$. In each small plot, 5 bottles of cultivation materials with the same formula were sown, and the experiment for each cultivation material formula was repeated three times.

2.4. Determination indexes and methods

(1) Pileus length (cm): The pileus lengths of 10 marked *Morchella* spps were measured with a vernier caliper, and their average was taken.
(2) Pileus diameter (cm): The pileus diameters of 10 marked *Morchella* spps were measured with a vernier caliper, with which the pileus perimeters were calculated, and their average was taken.
(3) Stipe length (cm): The stipe lengths of 10 marked *Morchella* spps were measured with a vernier caliper, and their average was taken.
(4) Stipe perimeter (cm): The stipe perimeters of 10 marked *Morchella* spps were measured with a vernier caliper, with which the pileus perimeters were calculated, and their average was taken.
(5) Fresh weight per marked *Morchella* spp. (g) = Total fresh weight of the third tide *Morchella* spps/total number of the third tide *Morchella* spps picked.
(6) Dry weight per marked *Morchella* spp. (g) = Total dry weight of the third tide *Morchella* spps/total number of the third tide *Morchella* spps picked.
(7) Fresh yield of small plot (kg) = Fresh weight per marked *Morchella* spp. × total number of the third tide *Morchella* spps picked in average small plot/1000.
(8) Dry yield of small plot (kg) = Dry weight per marked *Morchella* spp. × *total number of* the third tide *Morchella* spps picked in average small plot/1000.
(9) Ash content (%) = $(W_2 - W_0)/(W_1 - W_0) \times 100\%$

Where, W_2-mass of ceramic crucible and ash content after carbonization (g), W_0-mass of constant weight ceramic crucible (g), W_1-mass of ceramic crucible and test sample (g) (Chen, 2011).

(10) Crude fiber (%) = $(m_1 - m_0)/m \times 100\%$

Where, m_0-mass of ceramic crucible (g), m_1-mass of ceramic crucible and crude fiber (g), m-mass of test sample (g)[17].

(11) Crude protein content, amino acid content and total sugar content were measured (Chen, 2011; Wang et al., 2010).

2.5. Data analysis

A variance analysis of the experimental data was conducted using the DPS7.05 statistical software.

3. RESULTS AND ANALYSIS

3.1. Effects of different cultivation material formulas on increasing the pileus of Morchella spp.

The data in Table 1 showed that the pileus of *Morchella* spp. gradually became larger as the *Morchella* spp. continued to grow; the pileus of *Morchella* spp. in Formula 1 to which *Morchella* spp. footing soil was added grew best during the growth and development stages of *Morchella* spp., and as of December 17, 2015, the pileus length and pileus diameter were 5.1 cm and 15.7 cm respectively, an increase of 42.10% and 46.73% respectively compared with those in Formula 4, the worst formula, during the same growth period; Formula 2 to which mushroom promoter was added was the second best formula. The four cultivation material formulas in terms of the effect on increasing the pileus length and pileus perimeter of *Morchella* spp. in descending order was Formula 1 > Formula 2 > Formula 3 > Formula 4.

3.2. Effects of the different cultivation material formulas on increasing the stipe of Morchella spp.

As shown in Table 2, the data obtained on December 9, 2015 showed that the stipe lengths of *Morchella* spps in the first three

Table 1
Effects of the four cultivation material formulas in increasing the pileus of *Morchella* spp.

Treatment	Index					
	2015-12-09		2015-12-13		2015-12-17	
	Pileus length/cm	Pileus perimer/cm	Pileus length/cm	Pileus perimer/cm	Pileus length/cm	Pileus perimer/cm
Formula 1	4.7	15.5	4.8	15.6	5.1	15.7
Formula 2	3.7	11.0	4.0	13.7	4.9	14.1
Formula 3	3.3	10.6	3.7	12.0	3.8	13.0
Formula 4	1.5	4.5	1.7	5.2	2.7	10.7

Table 2
Effects of the four cultivation material formulas on increasing the stipe of *Morchella* spp.

Treatment	Index					
	2015-12-09		2015-12-13		2015-12-17	
	Pileus length/cm	Stipe perimer/cm	Stipe length/cm	Stipe perimer/cm	Stipe length/cm	Stipe perimer/cm
Formula 1	4.4	7.5	5.2	7.6	5.7	8.1
Formula 2	4.0	6.4	5.0	7.3	5.1	7.7
Formula 3	3.9	6.4	4.5	6.9	4.6	7.3
Formula 4	2.9	4.3	3.1	5.0	4.0	6.2

cultivation material formulas were all 4.4 cm, but in Formula 4 the length was 2.9 cm; the stipe in Formula 1 was the thickest, and its perimeter was 7.5 cm; and the stipe perimeter in Formula 4 was the smallest, which was 4.3 cm. The data obtained on December 13, 2015 showed that the stipe lengths in the four cultivation material formulas decreased in a row, and so did the stipe perimeters. The data obtained on December 17, 2015 suggested that the same as those obtained on December 13, 2015.

3.3. Effects of different cultivation material formulas on increasing the fresh weight of fruiting body of Morchella spp.

The data in Table 3 showed that the *Morchella* spp. kept growing and its fresh weight also gradually increased as the growth of *Morchella* spp. continued. During the same growth period, the fresh weight of the fruiting body of *Morchella* spp. in Formula 1 was the heaviest, and that in Formula 4 was the lightest. The four cultivation material formulas in terms of the effect on increasing the average fresh weight per piece in descending order was Formula 1 > Formula 2 > Formula 3 > Formula 4. On December 17, 2015, the fruiting bodies of *Morchella* spps met the picking standard, and all the fresh weights per piece in the four cultivation material formulas reached their maximum levels. The heaviest fresh weight per piece was 28.28 g in Formula 1, and the lightest was 9.74 g in Formula 4, with an extremely significant difference of 65.56%. The difference between Formula 1 and Formula 4 was extremely significant, and the difference between other formulas was significant.

Table 3
Effects of the four cultivation material formulas on increasing the fresh weight of fruiting body of *Morchella* spp.

Treatment	Index Average fresh weight per piece (g)		
	2015-12-09	2015-12-13	2015-12-17
Formula 1	21.86 ± 2.89aA	24.97 ± 6.33aA	28.28 ± 8.75aA
Formula 2	13.65 ± 4.07abA	14.28 ± 2.75bAB	18.03 ± 8.29abA
Formula 3	13.46 ± 4.16abA	13.94 ± 5.03bAB	14.99 ± 3.38bA
Formula 4	12.11 ± 0.156bB	5.86 ± 6.123bB	9.74 ± 2.84bA

Note: Lowercase letters indicate significance at P < .05 Uppercase letters indicate significance at P < .01, the same below.

3.4. Effects of the different cultivation material formulas on increasing the dry weight of fruiting body of Morchella spp.

The data in Table 4 showed that the *Morchella* spp. kept growing and its dry weight also gradually increased as its growth continued. During the same growth period, the dry weight of the fruiting body of *Morchella* spp. in Formula 1 *was the* heaviest and that in Formula 4 was the lightest. On December 17, 2015, the fruiting bodies of *Morchella* spps met the picking standard, and all the dry weights per piece in the four cultivation material formulas reached their maximum levels just like the fresh weights, which was 3.67 g in Formula 1, the heaviest, and 1.39 g in Formula 4, the lightest, with an extremely significant difference of 62.12%. The difference between Formula 1 and Formula 4 was extremely significant, the difference between Formula 2 and Formula 3 was not significant, and the difference between Formula 3 and Formula 4 was significant.

3.5. Effects of the different cultivation material formulas in increasing the yield of Morchella spp.

The data in Table 5 showed that the total number of *Morchella* spps picked in the small plot of Formula 1 was the largest among the 4 formulas, which was 77.09 pcs. Formula 1 also surpassed other formulas in terms of fresh weight and dry weight per piece and yield, and the equivalent yields of fresh and dry *Morchella* spps of Formula 1 were 1,366.87 kg/hm^2 and 177.38 kg/hm^2 respectively, the highest among the four cultivation material formulas. The total number of *Morchella* spps picked in the small plot, the equivalent yields of fresh *Morchella* spps and the equivalent yields

Table 4
Effects of the four cultivation material formulas in increasing the dry weight of fruiting body of *Morchella* spp.

Treatment	Index Average dry weight per piece (g)		
	2015-12-09-	2015-12-13	2015-12-17
Formula 1	2.85 ± 0.88aA	3.31 ± 0.60aA	3.67 ± 1.16aA
Formula 2	2.04 ± 1.17aA	2.64 ± 0.37abA	2.73 ± 0.88abAB
Formula 3	1.97 ± 0.37aAB	2.12 ± 1.19abA	2.47 ± 0.12abAB
Formula 4	0.53 ± 0.19bB	1.01 ± 0.98bA	1.39 ± 0.38bB

Table 5
Effects of the four cultivation material formulas in increasing the yield of *Morchella* spp.

Treatment	Index			
	Formula 1	Formula 2	Formula 3	Formula 4
Fresh weight per piece/g	28.28	18.03	14.99	9.74
Dry weight per piece/g	3.67	2.73	2.47	1.39
Yield of fresh *Morchella* spps picked in the small plot/kg	4.47	2.95	2.41	1.73
Equivalent yield of fresh *Morchella* spps (kg/hm^2)	1366.87 ± 15.15aA	961.60 ± 9.09bB	762.00 ± 10.00cC	624.98 ± 11.07dD
Yield of dry *Morchella* spps picked in the small plot/kg	0.71	0.48	0.39	0.31
Equivalent yield of dried *Morchella* spps (kg/hm^2)	177.38 ± 18.00aA	145.60 ± 10.95bB	125.58 ± 7.57cBC	89.19 ± 10.57cdCD
Total number of *Morchella* spps picked in the small plot/pcs	77.09 ± 6.21 aA	64.33 ± 5.25bB	61.57 ± 9.52bcB	58.41 ± 3.60 cBC

Table 6
Effects of the four cultivation material formulas on the quality of *Morchella* spp.

Composition	Part	Formula 1	Formula 2	Formula 3	Formula 4
Ash content/%	Pileus	6.07 ± 0.05aA	6.11 ± 0.04abAB	6.13 ± 0.04abB	6.16 ± 0.02bB
	Stipe	7.40 ± 0.02aA	7.59 ± 0.06abA	7.73 ± 0.06bB	7.81 ± 0.02cC
Total sugar content/%	Pileus	48.09 ± 0.06aA	47.82 ± 0.18abB	47.56 ± 0.03bcC	47.39 ± 0.15cC
	Stipe	21.12 ± 0.03aA	21.06 ± 0.06aA	20.53 ± 0.05bB	20.29 ± 0.14cC
Crude protein content/%	Pileus	28.06 ± 0.10aA	27.82 ± 0.20abAB	27.61 ± 0.10bB	27.08 ± 0.20
	Stipe	18.56 ± 0.66aA	15.06 ± 0.05aA	15.53 ± 0.05aA	15.04 ± 39bA
Crude fiber content	Pileus	12.19 ± 0.12aA	12.82 ± 0.18aA	13.04 ± 0.09aA	12.84 ± 0.08bB
	Stipe	15.27 ± 0.27aA	16.06 ± 0.05aA	16.17 ± 0.06aA	15.91 ± 0.12bB
Total amino acid content/%	Pileus	25.24 ± 0.29aA	24.82 ± 0.18abB	24.24 ± 0.29bcC	23.97 ± 0.24cC
	Stipe	7.87 ± 0.16aA	7.69 ± 0.29abA	7.31 ± 0.26bcA	7.16 ± 0.30cA

of dry *Morchella* spps in Formula 4 were 58.41, 624.9 kg/hm^2 and 889.19 kg/hm^2 respectively, a decrease of 24.23%, 54.28% and 49.72% respectively compared with those in Formula 1.

3.6. Effects of the different cultivation material formulas on the quality of Morchella spp.

The data in Table 6 showed that the parts of *Morchella* spp. in terms of ash content and crude fiber content in the four cultivation material formulas in ascending order was pileus < stipe. The ash content and the crude fiber content of pileus and those of stipe in Formula 1 were 6.07%, 12.19%, 7.40% and 15.27%, respectively, the lowest among the four cultivation material formulas; the ash content and the crude fiber content of pileus and those stipe in Formula 4 were 6.16%, 25.24%, 7.81% and 15.91%, respectively, the highest among the four cultivation material formulas. The four cultivation material formulas in terms of ash content and crude fiber content in ascending order was Formula 1 < Formula 2 < Formula 3 < Formula 4, and there was an extremely significant difference between Formula 1 and Formula 4. In contrast, the parts of *Morchella* spp. in terms of total sugar content, crude protein content and total amino acid content in the four cultivation material formulas in descending order was pileus > stipe, and the four cultivation material formulas in terms of total sugar content, crude protein content and total amino acid content in descending order was Formula 1 > Formula 2 > Formula 3 > Formula 4. The total sugar content, the crude protein content and the total amino acid content of pileus and those of stipe in Formula 1 were 48.09%, 28.06%, 23.97%, 21.12%, 18.56% and 7.87%, respectively, and there was an extremely significant difference between Formula 1 and Formula 4.

4. Discussion

Based on the above analysis, among the four cultivation material formulas provided in this paper, Formula 1 had the greatest effect on promoting the growth of *Morchella* spp., in which the pileus and stipe of *Morchella* spp. were the longest and thickest, and Formula 1 surpassed the other three formulas in total number of *Morchella* spps picked in the small plot, fresh weight per piece, dry weight per piece and yield of small plot. The four cultivation material formulas in terms of the above indexes in descending order was Formula 1 > Formula 2 > Formula 3 > Formula 4. In terms of quality, the ash content and the crude fiber content of pileus and those of stipe in Formula 1 were the lowest among the four cultivation material formulas, and the four cultivation material formulas in terms of ash content and crude fiber content in ascending order was Formula 1 < Formula 2 < Formula 3 < For mula 4; in contrast, the total sugar content, the crude protein content and the total amino acid content in Formula 1 were the highest, and the four cultivation material formulas in terms of total sugar content, crude protein content and total amino acid content in descending order was Formula 1 > Formula 2 > Formula 3 > For mula 4. Therefore, the quality of *Morchella* spp. in Formula 1 was the best, and the ash content, the total sugar content, the crude protein content and the total amino acid content in Formula 4 were the lowest, so the quality of *Morchella* spp. in Formula 4 was the worst. The four three-level strain cultivation material formulas in terms of the effect on promoting the growth and quality of *Morchella* spp. in descending order was Formula 1 > Formula 2 > Formula 3 > Formula 4. The study by Cheng Yuanhui et al. (2009) showed the maximum dry weight per hectare of *Morchella* spp. as 135 kg. This conflict with the result of this study may be due to a different management of fruiting period and the lack of inorganic salts in the formula. The study by Shen showed that the soil in which *Morchella* spp. has been planted produces higher yield than the soil in which *Morchella* spp. has not been planted (Shicai et al·· 2011). This finding can be used to explain why the fruiting body of *Morchella* spp. grew better and more vigorously when the cultivation material formula to which *Morchella* spp. footing soil was added (Ong et al., 2017; Daya and Pantt, 2017).

5. Conclusion

In summary, in this study, the cultivation material formula to which *Morchella* spp. footing soil was added had the greatest effect on promoting the growth of *Morchella* spp. Cultivated using Formula 1, the *Morchella* spp. had the longest pileus and the thickest stipe and it also surpassed those cultivated using other formulas

in terms of fresh weight per piece, dry weight per piece, equivalent dry yield per hectare of *Morchella* spp. and quality. Therefore, the cultivation material formula to which *Morchella* spp. footing soil was added was the optimum formula for the growth of *Morchella* spp.. This study has provided a practical basis for future research on the continuous cropping of *Morchella* spp. and the physiological mechanism why the cultivation material formula added with *Morchella* spp. footing soil may improve the yield and quality of *Morchella* spp. will be the content and direction of further research in this field.

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