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The Effects of Cultivation Conditions on the Growth of Morchella by ANN Model

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Abstract

Morchella is favored for its distinctive taste and rich nutritional value, yet the traditional cultivation methods are often limited by the natural environment. The effect of different culture conditions on mycelial growth of Morchella was studied by comparative experiment. The results showed that the best soil for Morchella growth was soybean soil, the best soil depth was 2 cm, and the best soil humidity was 80%. The optimal culture condition after orthogonal experiment was soybean soil with soil depth of 2 cm and humidity of 70%. The results of correlation analysis showed that the mycelia of Morchella was most affected by soil type and least affected by soil humidity. The relationship between different culture conditions and mycelial growth of Morchella was studied by neural network model, and its R2 reached 0.995, so as to explore more cultivation management strategies and provide more comprehensive and scientific guidance for its artificial cultivation.

Keywords: Morchella, cultivation conditions, ANN model, orthogonal experiment

1. Introduction

Morchella is a kind of valuable edible and medicinal fungus with a ridged reticular structure on the surface of its cap and a pitted surface similar to that of a goat's tripe [1]. Due to the excellent edible, medicinal and health care effects of Morchella, the consumer demand for Morchella is gradually increasing [2]. In terms of edible value, the body of Morchella is elastic and tastes crisp and delicious. In terms of medicinal value, Morchella is rich in vitamins, a variety of mineral elements and trace elements, and contains a large amount of protein and 7 essential amino acids for the human body, far exceeding the content of ordinary edible fungi [3,4]. In addition, Morchella has significant effects on antiviral, antioxidant, anti-tumor and cardiovascular protection [5].

Morchella has a wide range of growth, and is distributed in Sichuan, Gansu, Yunnan, Xinjiang, Hebei, Inner Mongolia, Shanxi, Jilin and other places in China. Although the domestic demand for Morchella continues to increase, up to now, the main way to collect Morchella is still wild collection. Artificial large-scale cultivation of Morchella faces many difficulties. First of all, Morchella is widely distributed and grows in diverse environments, and different Morchella has different habitat requirements. Secondly, people's life cycle history of Morchella is not completely clear, and the scientific basis in the cultivation process is not sufficient [6]. In addition, artificial outdoor cultivation is greatly affected by climate change, and commercial cultivation has not been fully successful, showing a phenomenon of "short supply" and scientific research lagging behind production needs [7]. Therefore, how to improve the technical conditions of artificial cultivation of Morchella is an urgent problem to be solved.

With the continuous in-depth study of Morchella by researchers at home and abroad, there have been reports on the isolation, cultivation methods, harvesting and processing of Morchella. Wang showed that increasing a certain amount of carbon dioxide concentration was conducive to the cultivation of Morchella mycelia. Zhu reported that soluble starch and urea were the best carbon and nitrogen sources suitable for the growth. Zhang [8] reported that the optimal temperature for the growth of Morchella was 17 ~ 22℃. He showed that Morchella has a high water requirement, and 40% to 60% soil water content is suitable for mycelium growth [9]. The above studies on the cultivation conditions of morel were all single-factor analysis. Due to the variety of cultivation methods, from field to forest, and the cross-influence between various factors, it is necessary to design orthogonal experiments to optimize the cultivation methods of Morchella.

In this study, through different soil types, soil depth and soil humidity influence on the growth of Morchella mycelia, the most suitable conditions for cultivating Morchella were selected, so as to improve the ability of Morchella mycelia growth. At the same time, the main and secondary effects of soil types, soil depth and soil humidity on the mycelial growth of Morchella were analyzed. Finally, the relation between different culture conditions and mycelial growth of Morchella was studied by artificial neural network model, which provided scientific basis for artificial large-scale cultivation of Morchella.

2. Materials and Methods

2.1 Test strain

The Morchella strains tested were retained in the laboratory.

2.2 Cultivation medium ingredients

Combined potato medium (PDA): peeled potato 200 g, glucose 20 g, AGAR 18 g, adding distilled water to 1000 mL, pH 6.0.

2.3 Soil type

In this study, 5 soil plants were planted continuously for more than 5 years, and the soil categories were respectively: peach forest, pine forest, cotton field, vegetable field and soybean field. The soil collection site is located in the Midwest of Jilin Province, the west of Siping City Tiexi District (east longitude 124°15'-124°25', north latitude 43°1'-43°15'). During the collection of soil samples, the soil with a surface depth of less than 10 cm was selected and sterilized under 121℃ high temperature and pressure for 30 min, then dried for reserve use.

2.4 Experimental method

2.4.1 Strain culture

100 mL liquid PDA medium was added into 250 mL conical bottle and sterilized at high temperature for reserve use. Then the activated Morchella mycelia with the size of 0.5 cm^2 and the mass of 10μg were inoculated and cultured in a shaking table with a rotating speed of 160 $r\cdot min^{-1}$ and a temperature of 20℃ for 48 h. Pick out the well-grown mycelia on the medium for use.

2.4.2 Single factor test

Five different types of soil were selected for experiments. The sterilized soil was inoculated with 10μg Morchella mycelium, and each soil was repeated 3 times, with 3 groups per experiment. After 10 days of inoculation, the weight of mycelia growing on each soil was measured, and the influence of different soils on the mycelia growth of Morchella was compared, so as to select the soil most suitable for the growth of Morchella.

The different soil depthes were set for the experiment. The soil depth was divided into groups according to Table 1, and the sterilized soil of different depth was covered with 10μg Morchella mycelia. Each soil depth was repeated three times, and each test was repeated for three groups. After 10 days of inoculation, the weight of mycelia under each treatment was weighed, and the influence of different soil depth on the mycelia growth of Morchella was compared, and the soil depth most suitable for the growth of Morchella was selected.

The kinds of soil with different humidity were set for the experiment. The soil moisture was set to 50%, 60%, 70%, 80%, and 90% respectively. The dried and sterilized soil was adjusted to different soil humidity and placed in a dark incubator with different humidity. 10μg Morchella mycelium was inoculated on soil with different humidity, and each soil humidity was repeated 3 times, and each experiment was repeated 3 groups. After 10 days of inoculation, the weight of mycelia under each treatment was weighed to compare the influence of different soil humidity on the mycelia growth of Morchella, and the most suitable soil humidity for the growth of Morchella was selected.

Table 1 Screening for optimum soil depth grouping

Groups	1	2	3	4	5	6
Upper soil depth (cm)	0	2	4	6	8	10
Subsoil depth (cm)	10	8	6	4	2	0

2.4.3 Orthogonal test of culture conditions

The orthogonal test table was designed based on SPSS 26.0 software by selecting relatively good levels of soil type, soil depth and soil humidity.

Table 2 reflected 16 horizontal combinations of orthogonal tests to find the optimal conditions for Morchella mycelia growth. Each treatment was inoculated with Morchella mycelium 10μg, each treatment was repeated 3 times, and each experiment was repeated 3 groups. After 10 days of inoculation, the weight of mycelia under each treatment was weighed for data statistics.

Table 2 Orthogonal experimental design table

Groups	Parameters		
	Soil type	Soil depth	Soil humidity
1	Vegetable field	0 cm	90%
2	Vegetable field	4 cm	70%
3	Peach forest	6 cm	90%
4	Peach forest	2 cm	70%
5	Peach forest	4 cm	80%
6	Soya field	2 cm	70%
7	Peach forest	4 cm	70%
8	Soya field	4 cm	90%
9	Soya field	0 cm	70%
10	Peach forest	0 cm	80%
11	Peach forest	6 cm	70%
12	Peach forest	0 cm	70%
13	Peach forest	2 cm	90%
14	Vegetable field	2 cm	80%
15	Soya field	6 cm	80%
16	Vegetable field	6 cm	70%

2.4.4 ANN (artificial neural network) model development

This study utilized a feedforward neural network model, specifically a multi-layer perceptron (MLP), in conjunction with the Momentum algorithm, to predict mycelial weight across diverse soil types, depths, and humidity conditions. The artificial neural network (ANN) model featured a multi-layer architecture, consisting of interconnected neurons organized into input, hidden, and output layers (Figure 1). Each layer was interconnected by adjustable weights w and biases b.

The input layer consisted of three neurons corresponding to soil type, soil depth, and soil humidity. Hidden layers were employed within the network to execute complex non-linear transformations. To achieve the optimal R^2 value for the model, the number of hidden layers and neurons required fine-tuning. After experimentation with various architectures, one hidden layer with five neurons was selected for this study. The output layer had a single neuron corresponding to the predicted mycelial weight.

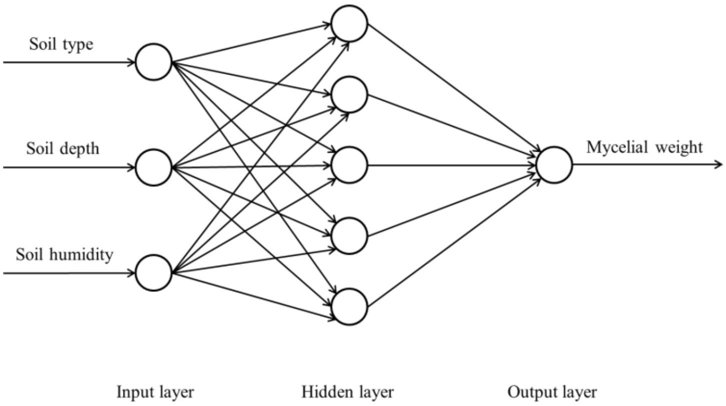

Figure 1 Optimal structure of neural network

The relationship between the different layers can be described as follows:

$$g_k = F\left[\sum_{j=1}^{M} W_{kj} f\left(\sum_{i=1}^{N} w_{ji} x_i + \theta_j\right) + b_k\right] \quad (1)$$

Here, i ranges from 1 to N, j from 1 to M and k from 1 to K. In the equations, wji represents the connection weight between the ith neuron in the input layer and the jth neuron in the hidden layer, while θj is the bias associated with the jth neuron in the hidden layer. Similarly, Wkj denotes the connection weight between the jth neuron in the hidden layer and the kth neuron in the output layer, and bk is the bias associated with the kth neuron in the output layer.

Each neuron in layers is composed of an activation functions. The output of a neuron is calculated from this activation functions. In this model, tansig was adopted for activation functions of hidden layer and output layer.

$$\tan sig(x) = \frac{e^x - e^{-x}}{e^x + e^{-x}} \quad (2)$$

The dataset utilized for training the artificial neural network (ANN) model comprised 35 normalized input/output patterns. Of these, 70% were allocated for training, 15% for validation, and the remaining 15% for testing purposes. To optimize the model, the mean square error (MSE) was minimized by fine-tuning various network parameters, such as the error goal, the maximum number of iterations, and the validation checks. As a result, the developed model can now be effectively utilized to predict mycelial weight based on the given input parameters.

2.4.5 Data analysis

The mycelia of Morchella were picked and weighed after 10 days of culture. Use Excel to record experimental data. SPSS 26.0 software was used for single factor ANOVA analysis, orthogonal experiment design, analysis of variance and other data processing. Graph data analysis using GraphPad Prism 9.0 software. Based on the above software analysis, the difference of mycelial growth of Morchella under different conditions was compared. According to the experimental data, the optimal conditions for mycelium culture were determined.

The formula for calculating mycelium growth rate (V, μg d^{-1}) is as follows:

$$V = \frac{W}{t} \quad (3)$$

where W is mycelium mass (μg), t is mycelium culture time (d).

3. Results

3.1 Mycelia growth under different soil conditions

3.1.1 Soil type

The mycelial growth of Morchella in different soils is shown in Figure 2. Morchella was inoculated in 5 different types of soil: peach forest, pine forest, cotton field, vegetable field and soybean field, the growth amount of

Morchella mycelium was also significantly different. The average mycelial growth rate was 20.33 μg d^{-1} in soybean field, which was significantly higher than that in cotton field, and the mycelial growth rate was 2.41 times higher than that in cotton field. The mycelial growth rates of vegetable field, peach forest and pine forest were 16.75 μg d^{-1}, 12.76 μg d^{-1}, 8.32 μg d^{-1}, respectively. Soybean field topped the list in Mycelium growth, which was notably bigger than peach forest and pine forest's mycelium growth, and dramatically higher than that in vegetable field. The preference of Morchella mycelium to 5 kinds of soil was: soybean field > vegetable field > peach forest > pine forest > cotton field

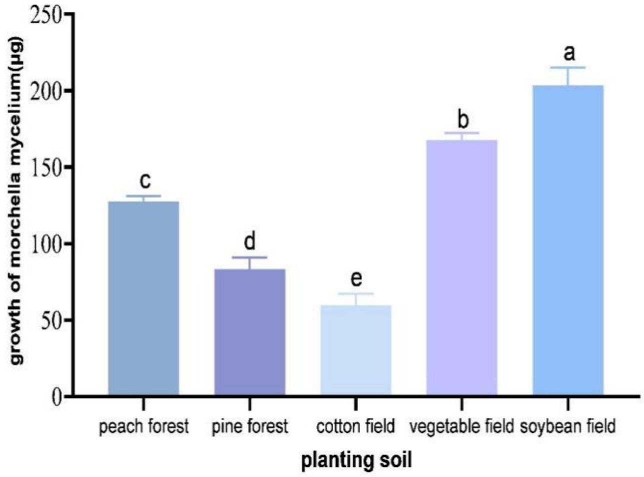

Figure 2 Results of mycelia growth of Morchella in different soils

Different lowercase letters indicate significant differences ($P < 0.05$)

3.1.2 Soil depth

The mycelial growth of Morchella in different soil depths is illustrated in Figure 3. When Morchella was inoculated at various soil depths, its mycelial growth varied accordingly. The growth rate of Morchella was highest at a soil depth of 2 cm, reaching 21.52 μg d^{-1}, which was significantly higher than the growth rate observed at a soil depth of 10 cm. At 10 cm, the mycelial growth rate was 1.29 times lower than at 2 cm. Across other soil depths (0 cm, 4 cm, 6 cm, and 8 cm), the mycelial growth rates were 19.49 μg d^{-1},16.04 μg d^{-1}, 15.81 μg d^{-1} and 12.31 μg d^{-1}, respectively. Morchella mycelium grew more rapidly at a soil depth of 2 cm compared to 4 cm and much more quickly than at 0 cm. The mycelial growth at soil depths of 4 cm and 6 cm was nearly identical, with no discernible difference in growth rates. The preference of Morchella mycelia for the six tested soil depths, ranked from highest to lowest, was: 2 cm > 0 cm > 4 cm ≈ 6 cm > 8 cm > 10 cm.

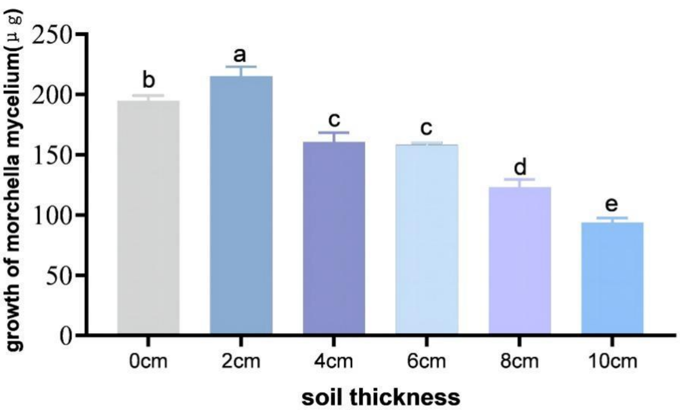

Figure 3 Results of mycelia growth of Morchella in different soil depth

Different lowercase letters indicate significant differences ($P < 0.05$).

3.1.3 Soil humidity

The mycelial growth of Morchella in different soil humidity is shown in Figure 4. The mycelial growth rate of Morchella was 20.16 μg d^{-1}, which was significantly greater than that under the conditions of 50% soil humidity. Morchella had the maximum growth rate when the soil moisture content was 80%. The mycelial growth rate was 105.30% higher than that under the condition of soil humidity of 50%. The mycelial growth rate was 16.02 μg d^{-1}, 18.49 μg d^{-1}, and 16.90 μg d^{-1}, respectively, at soil moisture levels of 60%, 70%, and 90%. In comparison to conditions when soil moisture was 60% and 90%, Morchella grew substantially more when soil moisture was 80%. The preference of Morchella mycelia to 5 soil humidity was 80% > 70% > 90% > 60% > 50%.

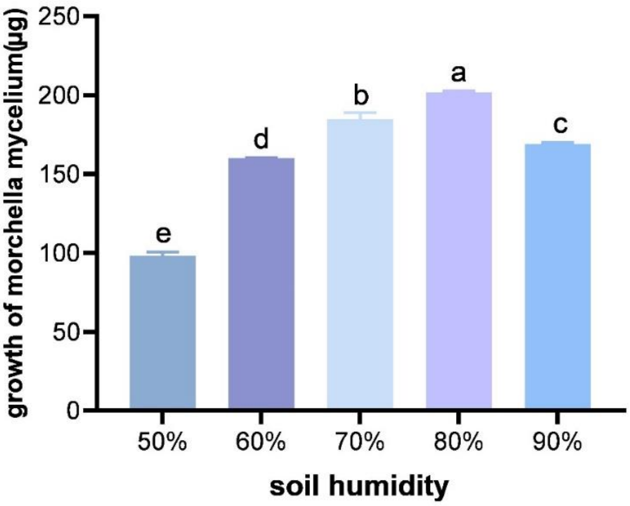

Figure 4 Results of mycelia growth of Morchella under different soil humidity

Different lowercase letters indicate significant differences ($P < 0.05$).

3.2 The optimization of Morchella mycelia growth

All factors influence each other and play a role together in the actual environment, so the single factor result cannot be taken as the final conclusion. Therefore, three factors of soil type, soil depth and soil humidity were screened. Three levels of soil types were designed, namely vegetable land, peach forest and soybean land. There was no significant difference in soil depth when Morchella was inoculated at 4 cm and 6 cm, and four levels were designed, 0 cm, 2 cm, 4 cm and 6 cm, respectively. Soil humidity was designed at three levels, 70%, 80% and 90% respectively. The statistical data of Morchella mycelium growth of 16 experimental groups in orthogonal test were shown in Figure 5.

The data in Figure 5 showed that the orthogonal test group 6 has the best growth of Morchella mycelia. The best culture condition, that was, the most suitable soil, depth and humidity for the growth of Morchella strains were soybean land, 2 cm and 70%, respectively. Table 3 displayed the findings of the orthogonal test variance analysis. Table 3 illustrates how the primary and type, soil depth, and soil humidity on the mycelial growth of Morchella may be determined by variance analysis of the orthogonal test results: kind of soil > depth of soil > humidity of soil. This aligned with the findings of the intuitive analysis. Through the comparison of F value and critical value, it can be concluded that these three factors have significant effects on the growth rate of mycelia of this strain.

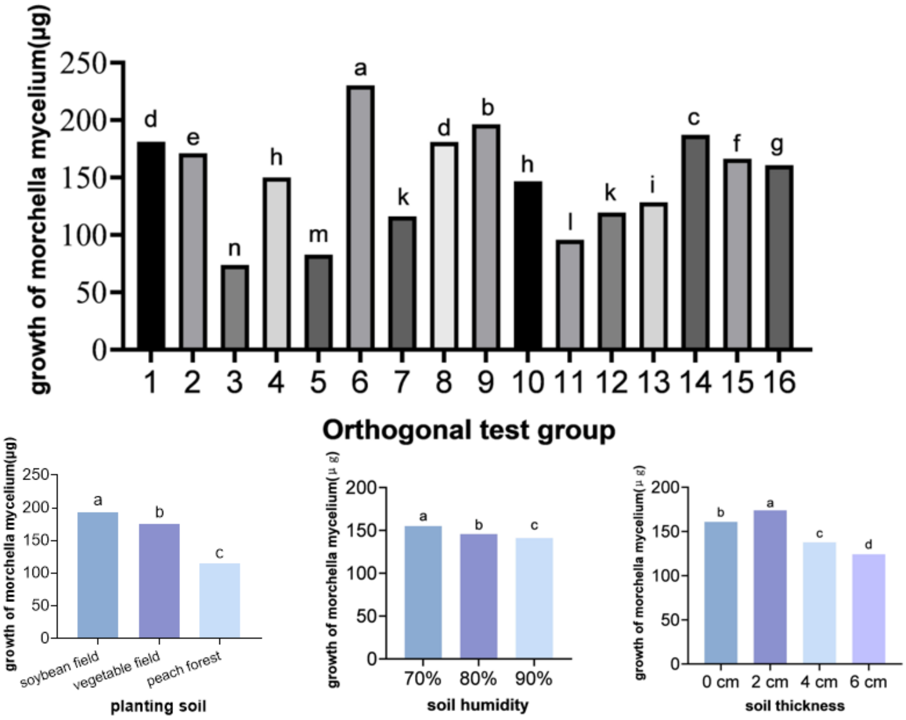

Figure 5 Statistics of mycelial growth of Morchella by orthogonal test and statistics of hyphal growth of Morchella with three factors

Table 3 Analysis of variance of the results of orthogonal test of morels

Parameters	Sum of Squares	Degrees of freedom	Mean Square	F	Significance
Soil type	61032.162	2	30516.081	249.911	＜0.001
Soil depth	18110.388	3	6036.796	49.438	＜0.001
Soil humidity	1747.130	2	873.565	7.154	＜0.001
Error	4884.320	40	122.108		＜0.001
Total	1154436.909	48			0.002
Adjusted total	85774.000	47			

In order to detect the relationship between the growth amount of Morchella mycelia, soil depth and soil humidity, the Pearson correlation coefficient analysis and processing data were used to detect the correlation (Table 4). The findings demonstrated that there was a negative correlation between the mycelial growth of Morchella and both soil depth and soil humidity.

Table 4 Pearson correlation analysis

		Soil depth	Soil humidity	Mycelial growth
Pearson correlation coefficient	Soil depth	1	0.000	-0.388**
	Soil humidity	0.000	1	-0.141
	Mycelial growth	-0.388**	-0.141	1

** At level 0.01, the correlation was significant

3.3 Performance and prediction evaluation of ANN model

Selecting appropriate model parameters is crucial in building a neural network model. Out of all the data points, 15% were selected for validating the ANN model. During the validation phase, the optimal number of hidden neurons was determined by incrementally adjusting the neuron count. It was found that the lowest mean square error (MSE) and the highest regression values for the validation dataset were achieved with 5 hidden neurons. Consequently, the final ANN structure was established with 3 input parameters (soil type, soil depth, and soil humidity), 5 hidden neurons, and 1 output parameter (mycelial weight).

The MSE and regression values for the training, validation, and testing datasets are summarized in Table 5. Notably, the R2 values for both the validation and testing datasets were close to 1, indicating the high reliability of the ANN model in predicting mycelial weight. Figure 6 presents the regression diagram of the ANN model, showing an overall R2 value of 0.9950. The error histogram depicted in Figure 7 reveals that the relative errors between experimental values and ANN-predicted values were minimal, with most errors clustering around zero. Figure 8 displays the correlation between experimental and ANN-predicted values of mycelial weight, further demonstrating the capability of the ANN model with an overall R2 value of 0.9950. The optimized parameters (weights and biases) of the ANN model are provided in Table 6. These results indicate that the ANN model developed in this study can accurately predict mycelial weight based on soil type, soil depth, and soil humidity.

Table 5 Error and regression values of the predicted model

	MSE	R2
Training	1.399E-5	0.9994
Validation	1.708E-4	0.9881
Testing	7.361E-4	0.9839

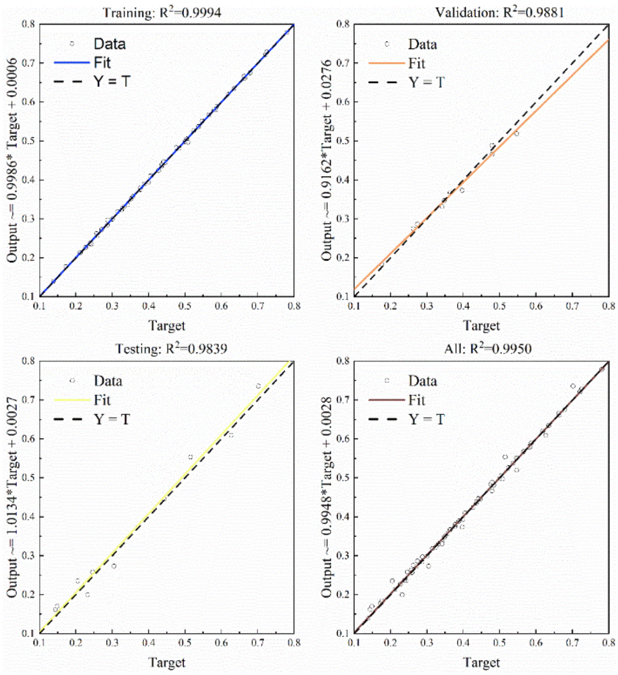

Figure 6 Regression diagram of the ANN model (Target, the experimental value; Output, the fitting value)

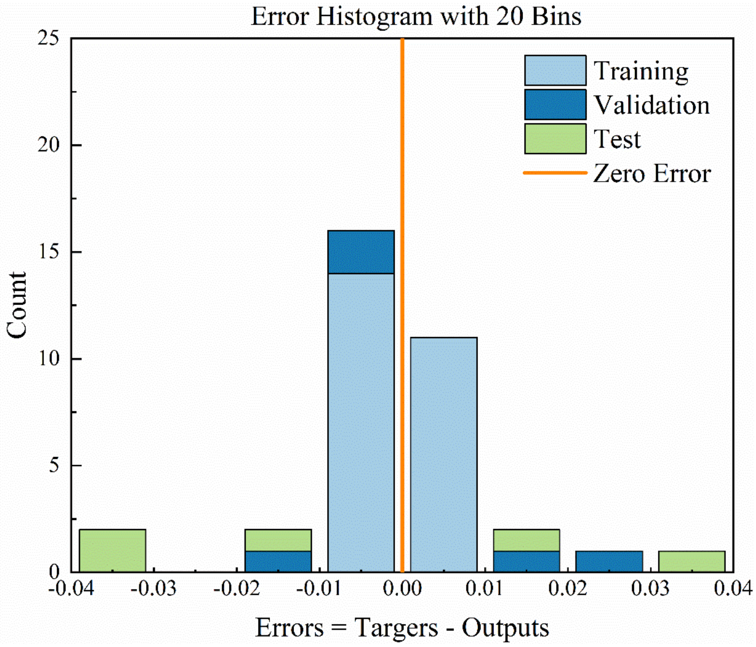

Figure 7 Error histogram of the ANN model

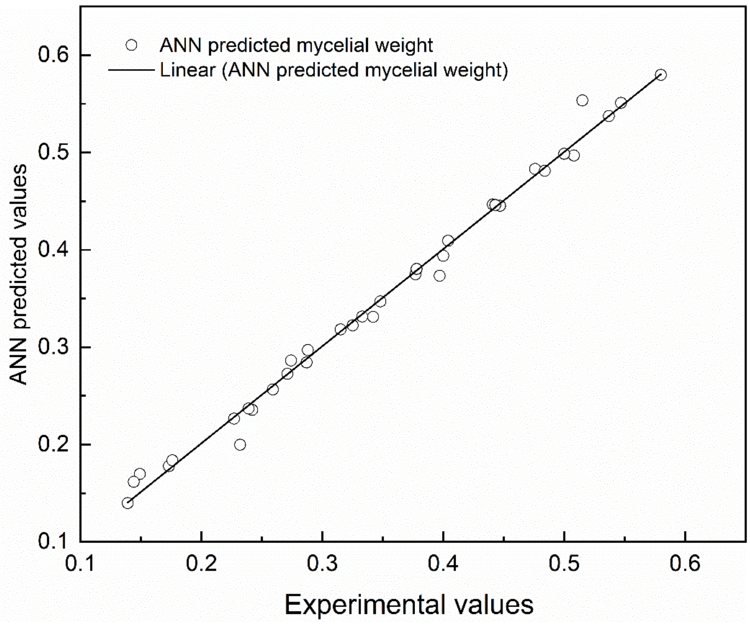

Figure 8 Experimental validation of ANN predicted mycelial weight

Table 6 Optimized parameters (weights and bias) of the ANN model

	Pih				Pho	
	wj1 (i=1)	wj2 (i=2)	wj3 (i=3)	θj	W1j (k=1)	bk (k=1)
j=1	1.23636	0.21093	0.99510	-0.88017	0.33913	1.28254
j=2	-4.65936	0.04015	-0.27420	1.52268	-0.47492	
j=3	-19.17717	-0.09925	0.18687	6.11054	-1.86969	
j=4	-0.25616	0.42812	0.21760	-0.36941	-2.52199	
j=5	0.21880	2.84718	-2.57902	-1.85810	1.03166	

P_{ih}, Parameters connecting the input and hidden neurons

P_{ho}, Parameters connecting the hidden and output neurons

4. Discussion

Considering the artificial cultivation of Morchella, there’s no doubt that environmental factors play an essential restricting role. These factors include, but are not limited to, the type of soil, the level of temperature, the suitability of humidity, the intensity of light, and the acidity and pH of the soil [10]. It is very important to understand and control the specific role of these factors in the development of Morchella, so as to improve the yield and quality of Morchella.

In the analysis of the single factor of soil type, it was found that the growth performance of Morchella in soybean field was the most superior. In soybean fields, due to the nitrogen-fixing effect of rhizobia, the soil was rich in nitrogen, which was essential for the growth of Morchella. In addition, the carbon content in the soybean field was also quite rich and it was necessary for Morchella to thrive. In the cultivation process of Morchella, it was necessary to create an optimal growth environment by regulating the carbon and nitrogen ratio in the soil. The study of Wang et al. further pointed out that lactose and maltose served as optimal carbon sources for Morchella’s growth, and ammonium sulfate and potassium nitrate were the best nitrogen sources [11]. These outcomes agreed with the study's conclusions on Morchella's optimal growing conditions in soybean fields, and further demonstrated the significance of soil rich in carbon and nitrogen for Morchella growth. In summary, by carefully investigating how environmental conditions affected Morchella's growth, the growth law can be better mastered, so as to optimize the cultivation conditions and improve the yield and quality of Morchella.

The results of this study suggested that the growth rate of Morchella mycelia was strongest under the condition of soil depth of 2 cm. The effects of soil humidity and depth on Morchella growth were examined individually. This finding suggested that the right soil thickness is crucial for the growth of Morchella [12]. Too thick soil may cause mycelia to be compressed during growth, limiting its expansion, while too thin soil may not provide enough support and nutrition for mycelia, affecting its normal growth. Therefore, in the cultivation process of Morchella, it was necessary to precisely control the soil depth to create an optimal growth environment for it. In terms of soil humidity, this study found that the environment with soil humidity of 80% was more conducive to the growth of Morchella. This finding aligns with the research conducted by Liu et al., which demonstrated that high humidity levels were optimal for the Morchella germination [6]. This, however, was at odds with Zhang et al.'s results, which suggested that the culture material's humidity be kept between 60% and 65% [13]. Since different species of morels may require varying amounts of humidity, this discrepancy could be the result of the varied types of morels being cultivated. In addition, the habitat conditions of the cultivation site may also have an impact on the growth of Morchella, such as temperature, light and other factors may work together with humidity to affect the growth of Morchella. In summary, soil depth and soil humidity were the key factors affecting the growth of Morchella. By precisely controlling these environmental factors, an optimal environment can be created for the growth of Morchella, thereby improving its yield and quality [14]. However, due to the diversity of Morchella species and the complexity of cultivated habitat, it was necessary to further study the interaction between these factors in order to better grasp the growth law of Morchella.

In the optimization process of orthogonal experiment, this study obtained the best combination of conditions for cultivating Morchella by comprehensively considering multiple environmental factors: soybean soil with a 70% humidity level and a soil depth of 2 cm. This conclusion provided more accurate and comprehensive guidance for the culture of Morchella. It was worth noting that the optimal soil humidity (70%) obtained in the orthogonal experiment was inconsistent with the results obtained in the single-factor experiment (80%). This difference may be due to the complexity of multiple factors in the actual cultivation of Morchella. In the actual cultivation environment, soil moisture is not the only factor affecting the growth of Morchella mycelia, but interacted with many factors such as oxygen content and soil depth [8]. Furthermore, the outcomes of the experiment may also be impacted by various operational procedures and experimental setups. The orthogonal experiment thoroughly examined the effects of many parameters, whereas the single factor experiment focused only on the impact of soil moisture on Morchella growth. Therefore, although the results of the two experiments are different in terms of soil humidity, it was not contradictory, but reflected the different characteristics of Morchella growth under different experimental conditions. In summary, the optimal combination of cultivation conditions optimized by orthogonal experiment can more comprehensively and accurately reflect the actual needs of Morchella growth. In the actual cultivation process, the role of multiple factors should be considered according to the specific situation to create an optimal environment for the growth of Morchella.

In this study, an artificial neural network (ANN) model was developed to predict the mycelial weight of Morchella across various soil types, soil depth and soil humidity. The ANN model could accurately predict the mycelial weight of Morchella under various soil conditions. It can be seen from the results that no matter the training set, verification set or test set, the MSE value of the model was low, and the R^2 value was close to 1. It indicated that the ANN model had high accuracy and reliability in predicting the mycelium weight of Morchella. In addition, through the model training and optimization process, the influence weight and contribution degree of soil type, soil depth and soil humidity on the mycelial weight of Morchella were obtained. These results provided an important reference for further understanding of the growth mechanism and cultivation management of Morchella. Although soil humidity was one of the key factors affecting the mycelium weight of Morchella, soil humidity was not the only determinant in the actual cultivation process [2]. Soil type and soil depth also have important effects on the growth of Morchella. This suggested that in the actual cultivation process, it was necessary to consider many factors comprehensively and optimized the growing environment of Morchella through scientific cultivation and management measures.

5. Conclusion

In this study, the effects of different growth conditions on the growth of Morchella were analyzed. The growth of Morchella was obviously affected by culture conditions, especially soil type, soil depth and soil humidity. Through correlation analysis, soil type had the most significant effect on Morchella mycelia growth, while soil humidity had relatively little effect. A neural network model was used to further study the relationship between different culture conditions and the mycelial growth of Morchella, and the model fit was as high as 0.995, which showed the accuracy and reliability of the model in predicting the growth of Morchella. These conclusions provided more comprehensive and scientific guidance for the artificial cultivation of Morchella, and had important theoretical significance and practical value for promoting the further development of culture technology of Morchella.

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