RESEARCH ARTICLE

Optimization of environmental factors for simulating wild cultivation of *Ganoderma lucidum*: A case study of purple *Ganoderma* fungus

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The market demand for *Ganoderma lucidum* has increased, but wild resources are limited. Simulating wild cultivation techniques has become a solution. The aim of this study was to enhance the effectiveness of simulating wild planting of purple *Ganoderma* fungi by optimizing environmental factors to address the contradiction between increasing market demand and limited wild resources. This study used a method of establishing control and experimental groups combined with random grouping and repeated experiments to systematically analyze the effects of key environmental factors such as temperature, humidity, light intensity, and soil pH on the growth of purple *Ganoderma* fungi. This study also established a mathematical model between environmental factors and the growth of purple *Ganoderma* fungi. The results showed that, when the temperature was controlled at 25.5℃, humidity was 82.5%, light intensity was 1,250 lux, and soil pH was 6.6, the yield of purple *Ganoderma* fungi reached its maximum. The multiple regression model predicted a single-bag yield of 56.8 g with a dry weight of 56.8 g. Under the same environmental testing, three experimental groups were repeated with 30 cultivation bags in each group, and the average yield of the measured results was 57.3 g/bag. The results demonstrated that optimizing the combination of environmental factors can significantly improve the yield and quality of purple *Ganoderma* fungi with humidity having the most significant impact on growth. This study provided not only the scientific basis for the wild planting of purple *Ganoderma* fungi but also a theoretical support for the sustainable development of the *Ganoderma lucidum* industry.

Keywords: purple *Ganoderma fungus*; wild cultivation; optimization; temperature; humidity; light intensity; pH.

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Introduction

Ganoderma lucidum (GL), a precious traditional Chinese medicinal herb, has attracted much attention due to its unique medicinal value and health benefits [1]. In recent years, with people's increasing pursuit of a healthy lifestyle, the market demand for GL has also been constantly rising. The growth characteristics of GL prefer warmth and humidity, and it mostly grows on decaying trees in forests. Wild GL resources are limited and difficult to harvest, making it difficult to meet market demand [2]. Using simulating wild planting (SWP) technology to simulate its natural growth environment can effectively increase its yield and maintain its wild quality and medicinal efficacy. Its significant advantages not only alleviate the pressure on wild resources but also meet the market's demands for high-quality GL [3]. At present, research on GL-SWP has made

certain progress in various countries, mainly focusing on cultivation substrates, inoculation techniques, growth environment, and other aspects. Researchers attempt to improve the yield and quality of GL by adjusting these parameters [4]. However, there is still a lack of systematic and in-depth research on how environmental factors affect GL growth and how to optimize these environmental factors to achieve optimal planting results [5].

Numerous in-depth analyses and explorations have been conducted. Thakur *et al*. studied the morphology, cultivation techniques, and market potential of GL, providing comprehensive background information for GL-SWP and emphasizing the importance of optimizing cultivation techniques in improving GL's yield and quality [6]. Feng *et al*. conducted research on improving the efficient reproduction of GL and microbial droplet cultivation techniques, providing technical support for the refined management of GL-SWP, especially in the regulation of microorganisms [7]. Furthermore, Chen *et al*. conducted in-depth research on the biological characteristics and domesticated cultivation of wild GL, providing practical cases and references for the regulation of environmental factors in GL-SWP, especially in simulating wild environments [8]. Meanwhile, Poyeri and Ohimain compared the cultivation effects of commercial strains and wild strains and evaluated their productivity in rainforest areas of Nigeria, which provided new ideas for the regional adaptability research of GL-SWP [9]. Xu *et al*. investigated the effects of GL under story cultivation on soil organic carbon pools and physiological characteristics of microbial communities, focusing not only on the growth of GL itself but also on its impact on the ecological environment. The results provided ecological considerations for the sustainable development of GL-SWP [10]. These studies collectively constitute a rich background for GL-SWP research, but further in-depth research is needed on the optimal combination of environmental factors. Ahmad *et al*. studied the multiple health benefits of GL acid in GL and the factors affecting its production, emphasizing the importance of environmental factors such as temperature and humidity on GL acid production. The study provided a theoretical basis for the regulation of environmental factors in GL-SWP [11]. He *et al*. explored the relationship between GL germplasm resources and secondary metabolism regulation, pointing out the influence of environmental factors such as light and temperature on GL secondary metabolites, which provided a new idea for improving GL quality through environmental factor regulation [12]. In addition, İnce *et al*. investigated the effect of cultivation substrate composition on GL's biomass yield and quality parameters and found that different substrates had a significant impact on GL growth, providing experimental evidence for the selection and optimization of substrates in GL-SWP [13]. Schoder *et al*. investigated the effects of environmental and nutritional conditions on the growth of three types of basidiomycetes hyphae including GL. The study revealed the regulatory effects of environmental factors such as temperature, humidity, and light on the growth of GL hyphae, providing a reference for the optimization of environmental factors in GL-SWP [14]. Anothai *et al*. investigated the factors affecting GL growth and cellulase activity in wood and discovered that environmental factors such as soil pH and temperature had a significant impact on GL growth, which provided a scientific basis for the regulation of soil environment in GL-SWP [15].

Based on the results of previous studies, significant progress has been made in the field of SWP of GL, providing a reference for optimizing environmental factors. However, most of these studies focused on the effects of a single environmental factor or specific conditions, lacking exploration of the comprehensive effects and optimal combinations of environmental factors. Although there have been studies on the relationship between GL growth and soil environment, the specific mechanisms and optimization strategies for key factors such as soil pH are still unclear. The purpose of this study was to optimize environmental factors in SWP

through a combination of experimental design and data analysis. Based on comprehensive consideration of multiple factors such as temperature, humidity, light, and soil pH, this study explored the comprehensive impact of environmental factors on the growth of purple *Ganoderma* fungi (PGF) through systematic experiments and mathematical modeling. The study also predicted the optimal combination of environmental factors. This research provided a scientific basis for the precise regulation and sustainable development of GL-SWP technology, reduced dependence on wild GL resources, and alleviated ecological pressure.

Materials and methods

Experimental site and material resources

The experimental site was selected in Tianlin County, Baise City, Guangxi Zhuang Autonomous Region, China, which was a forest with convenient transportation, sufficient water sources, good ventilation, and sunny conditions [16]. The soil layer in this forest was deep and loose. The soil pH naturally fell within the range of 4 - 5, which was very suitable for the growth of PGF. Meanwhile, the shading degree of the forest land was over 75%, providing ideal shading conditions for PGF. The *Ganoderma lucidum* strain was provided by BioScience Inc. (Beijing, China). The cultivation substrates including 15 cm × 30 cm oak basswood and sawdust with particle size ≤ 5 mm were obtained from Green Growth Ltd. (Shanghai, China) and EcoTech Industries (Guangzhou, Guangdong, China), respectively. The auxiliary materials included bran (Agriculture Best, Chengdu, Sichuan, China) and gypsum powder with a purity ≥ 95% (Mineral Resources Co., Nanjing, Jiangsu, China), which were used to regulate the nutrition and breathability of the substrate.

Experimental groups and design

The samples were divided into a control group (CG) and an experimental group (EG) for comparison study. CG as the basic reference for the experiment had its environmental factor settings referred to existing research and planting experience to ensure it reflected the growth status of PGF under conventional conditions with the environmental parameters being set as temperature at 25 ± 2℃, humidity within the range of 70 - 80%, light intensity at 1,000 – 1,500 lux, and soil pH around 6.5 [17, 18]. EG was a variant of CG, which observed changes in PGF growth by adjusting environmental factors of temperature, humidity, light intensity, and soil pH. Eight EGs were designed in this study with each group being adjusted for one or more environmental factors (Table 1). To ensure the reliability and accuracy of the experimental results, this study adopted the methods of random grouping and repeated experiments. All bacterial rods were randomly assigned to CG and various EGs before the start of the experiment to eliminate the influence of initial condition differences on the experimental results. Each processing group was repeated three times. Briefly, the oak wood was sawed into small pieces and mixed with sawdust in a ratio of 3:1 before adding 5% bran and 2% gypsum powder and stirring thoroughly until uniform. The mixed substrate was put into a cultivation bag and sterilized in SterilPro YX-280A high-pressure sterilization pot (Medical Equipment Corp., Wuhan, Hubei, China) at 121℃ for 2 hours. The PGF strain was then evenly inoculated onto the sterilized substrate in a CleanAir Tech SW-CJ-1FD sterile inoculation box (BioSafety Solutions, Shenzhen, Guangdong, China) followed by sealing the cultivation bag. The PGF strain was cultivated under variable conditions with adjusted temperature, humidity, and lighting conditions in the cultivation room according to the experimental design for each group. The soil pH value was regularly checked and adjusted as needed to ensure the stability of experimental conditions.

Data collection and analysis

The data of this research included two parts as regular observation and sample collection. Regular observation included regularly observing the growth status of PGF under different environmental factors, recording in detail key

Table 1. Detailed parameter settings of experimental design.

Group	Temperature (°C)	Humidity (%)	Light intensity (Lux)	Soil pH
CG	25 ± 2	70 - 80	1,000 – 1,500	6.5
EG 1	28 ± 2	70 - 80	1,000 - 1,500	6.5
EG 2	22 ± 2	70 - 80	1,000 – 1,500	6.5
EG 3	25 ± 2	80 - 90	1,000 – 1,500	6.5
EG 4	25 ± 2	60 - 70	1,000 – 1,500	6.5
EG 5	25 ± 2	70 - 80	1,500 – 2,000	6.5
EG 6	25 ± 2	70 - 80	500 – 1,000	6.5
EG 7	25 ± 2	70 - 80	1,000 – 1,500	7.0
EG 8	25 ± 2	70 - 80	1,000 – 1,500	6.0

indicators such as hyphal growth rate, morphological changes of fruiting bodies, and final yield. These data will directly reflect the degree of impact of environmental factors on PGF growth. To gain a deeper understanding of the physiological characteristics of PGF under different environmental conditions, the samples at key growth stages were collected for laboratory analysis to obtain detailed information on the biochemical composition and enzyme activity of PGF under different environmental factors. The data analysis included statistical analysis, correlation analysis, and model establishment. Descriptive statistics were conducted to gain a preliminary understanding of the basic situation of PGF growth under different environmental factors [19] followed by a variance analysis to test whether the effects of different environmental factors on PGF growth indicators were statistically significant [20]. Correlation analysis was to explore the correlation between different environmental factors and between environmental factors and PGF growth indicators. This study measured the degree of linear correlation between variables by using Pearson correlation coefficient to identify the most critical environmental factors affecting PGF growth and their interactions, providing a basis for subsequent model construction. Further, this study used a multiple linear regression model to establish a quantitative relationship between environmental factors and PGF growth. The model took temperature (T), humidity (H), light intensity (L), and soil pH value (P) as independent variables and the final yield of PGF (Y) as the dependent variable. The regression equation below was obtained through data fitting and parameter optimization.

$$Y = aT + bH + cL + dP + e \quad (1)$$

where *a*, *b*, *c*, *d* were the regression coefficients of *T*, *H*, *L*, *P*. *e* was a constant term. The specific regression coefficient values were obtained through parameter estimation using the least squares method. By continuously adjusting model parameters and optimizing model structure, the model could more accurately reflect the complex relationship between environmental factors and PGF growth. By using the established model for prediction, the optimal combination of environmental factors was obtained, providing a scientific basis for the optimization of PGF simulating wild planting (PGF-SWP) technology.

Statistical analysis

ANOVA and Tukey HSD post hoc tests were employed for the comparisons of hyphal growth rate, fruiting body diameter, and final yield among the different groups with *P* value less than 0.05 as statistically significant differences and *P* value less than 0.01 as very significant differences.

Results

Descriptive statistics results

Table 2. Descriptive statistics of growth indicators of *Ganoderma lucidum*.

Group	Hyphal growth rate (mm/d)	Fruiting diameter (cm)	Final production (g)
CG	1.05 ± 0.12	11.1 ± 1.5	49.8 ± 5.2
EG 1	0.97 ± 0.08#	10.3 ± 1.1	42.3 ± 4.2#
EG 2	1.02 ± 0.11	10.8 ± 1.3	48.4 ± 4.9
EG 3	0.91 ± 0.09#	9.5 ± 1.1	38.1 ± 3.5#
EG 4	1.03 ± 0.10	10.3 ± 1.2	44.5 ± 4.4#
EG 5	0.93 ± 0.08#	9.8 ± 1.1	40.2 ± 3.9#
EG 6	0.99 ± 0.11	10.7 ± 1.4	46.8 ± 4.5#
EG 7	0.96 ± 0.12#	10.3 ± 1.3	43.3 ± 4.1#
EG 8	0.95 ± 0.08	10.1 ± 1.1	42.2 ± 4.3#

Note: # indicated $P < 0.05$ compared to the control group.

Table 3. Variance analysis of the effects of environmental factors on the growth of GL.

Variation	Hyphal growth rate (mm/d)	Fruiting diameter (cm)	Final production (g)
Temperature	F = 4.52 ($P < 0.05$)	F = 3.87 ($P < 0.05$)	F = 5.11 ($P < 0.05$)
Humidity	F = 6.23 ($P < 0.05$)	F = 5.78 ($P < 0.05$)	F = 7.12 ($P < 0.01$)
Light Intensity	F = 3.15 ($P > 0.05$)	F = 2.98 ($P > 0.05$)	F = 3.67 ($P < 0.05$)
Soil pH	F = 2.42 ($P > 0.05$)	F = 2.11 ($P > 0.05$)	F = 2.89 ($P > 0.05$)

The descriptive statistical results of PGF growth indicators under different environmental factors showed that the PGF of CG was slightly higher than that in most EG in terms of hyphal growth rate, fruiting body diameter, and yield. The hyphal growth rate demonstrated that CG was the fastest one at 1.05 mm/d, while EG3 was the slowest at 0.91 mm/d. The fruiting body diameter showed that CG was the largest, reaching 11.1 cm, while EG3 was the smallest at 9.5 cm. In terms of yield, CG had the highest yield at 49.8 g, while EG3 had the lowest yield at only 38.1 g (Table 2). There were also differences among various EGs, but overall, most EGs had similar growth indicators and were lower than CG. In addition, among the indicators of hyphal growth rate, there were significant differences between the CG and EG1, EG3, EG5, and EG7 ($P < 0.05$), indicating that environmental factor adjustments in these EGs had a significant impact on hyphal growth rate. In the fruiting body diameter, there was no significant difference between the CG and the EGs. In the final yield, except for the comparison between the CG and EG2 that showed no statistically significance difference, there were first out differences in all other comparisons ($P < 0.05$), indicating that the adjustment of environmental factors had a significant impact on the final yield. The results indicated that environmental factors had a significant impact on the growth of PGF, and optimizing environmental factors was expected to improve the yield and quality of PGF.

The influence of different environmental factors on the growth of PGF

(1) Variance analysis

The results of variance analysis including the effects of various environmental factors on hyphal growth rate, fruiting body diameter, F-value, and significance level of yield showed that temperature and humidity had significant effects on the hyphal growth rate, fruiting body diameter, and yield of PGF ($P < 0.05$) with humidity having the most significant effect ($P < 0.01$). The effect of light intensity on yield also demonstrated a significant difference ($P < 0.05$), while its effects on hyphal growth rate and fruiting body diameter were no significant difference. The effect of soil pH on PGF growth indicators was relatively weak and had not reached a significant level (Table 3). The results

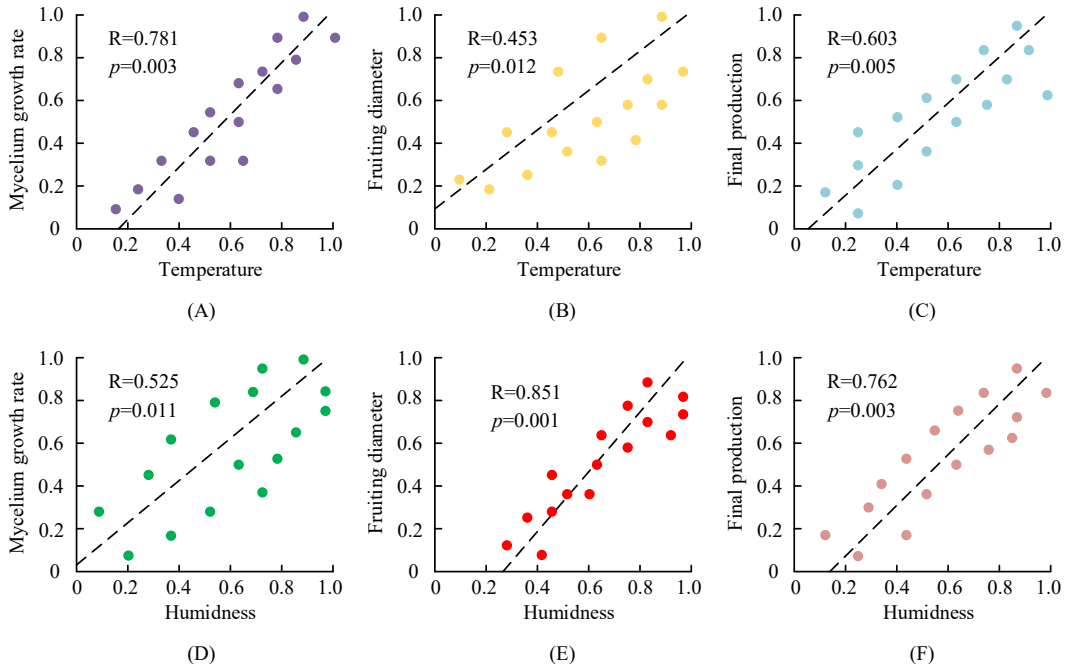

Figure 1. Correlation analysis results. **A.** between temperature and mycelial growth rate. **B.** between temperature and fruiting diameter. **C.** between temperature and final production. **D.** between humidity and mycelial growth rate. **E.** between humidity and fruiting diameter. **F.** between humidity and fruit production.

indicated that, in the PGF-SWP process, special attention should be paid to the regulation of temperature and humidity, while appropriately considering the influence of light intensity to achieve high yield and high quality of PGF.

(2) Correlation analysis

The correlation analysis results between temperature, humidity, and hyphal growth rate, solid diameter, and yield showed that the hyphal growth rate of PGF was positively correlated with temperature (R = 0.781) and positively correlated with fruiting body diameter and yield with weak correlations (Figure 1). In addition, the fruiting diameter was significantly positively correlated with humidity (R = 0.851). The correlation between light intensity, soil pH and hyphal growth rate, solid diameter, and yield demonstrated that the fruiting diameter was significantly negatively correlated with light intensity (R = -0.62, $P < 0.05$), while yield was positively correlated with temperature, humidity, and soil pH with the highest correlation observed with humidity (R = 0.76) (Figure 2). The results indicated that, in the PGF-SWP, the interaction between different environmental factors should be comprehensively considered to optimize planting conditions.

Determination of optimal environmental factor combination based on regression model

To visually demonstrate the impact of different environmental factors on PGFY and determine the optimal combination of environmental factors, this study compared the yield prediction values under each combination. The results showed that, when temperature was controlled at about 25.5°C, humidity was maintained at around 82.5%, light intensity was 1,250 lux, and soil pH was maintained at 6.6, the yield of PGF reached its maximum value with a predicted value of 56.8 g (Table 4). This combination had been determined as the optimal combination of environmental factors. Through model establishment and data analysis, this study successfully determined the optimal combination of environmental factors in the PGF-SWP. This combination provided a scientific basis

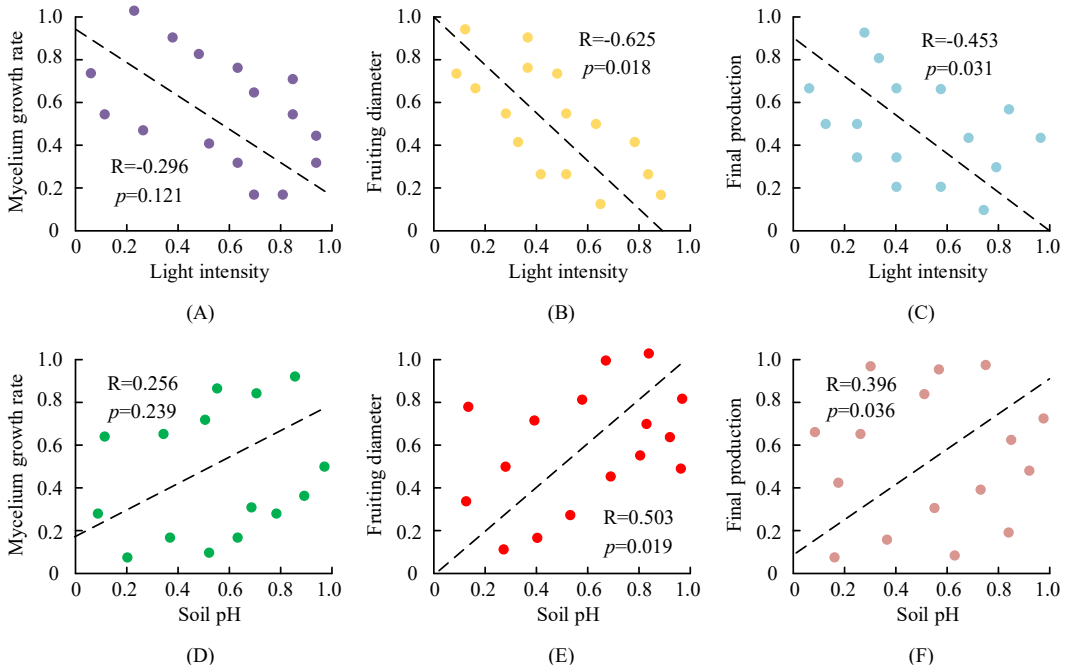

Figure 2. Correlation analysis results. **A.** between light intensity and mycelial growth rate. **B.** between light intensity and fruiting diameter. **C.** between temperature and final production. **D.** between soil pH and mycelial growth rate. **E.** between soil pH and fruiting diameter. **F.** between soil pH and final production.

Table 4. Comparison of the final production forecast values under each combination.

Combination of environmental factors	Temperature (°C)	Humidity (%)	Light intensity (Lux)	Soil pH value	Final production forecast (g)
Combo 1	25.0	80.0	1,200	6.5	55.0
Combo 2	26.0	75.0	1,300	6.7	53.5
Combo 3	24.0	85.0	1,100	6.3	52.0
Combo 4	24.5	80.0	1,300	6.4	54.2
Combo 5	26.5	85.0	1,200	6.8	53.8
Combo 6	25.0	77.5	1,150	6.6	54.6
Combo 7	25.5	82.5	1,250	6.5	56.2
Combo 8	25.5	82.5	1,200	6.6	56.5
Optimal combination	25.5	82.5	1,250	6.6	56.8

for the high yield and high quality of PGF and also laid a solid foundation for the sustainable development of the GL industry.

Result validation and optimization

(1) Verification experiment

To verify the accuracy of the research model, validation experiments under the optimal environmental factor combination were conducted with the same PGF strain and standardized cultivation substrates to ensure consistency in experimental conditions. The results showed that, under the optimal environmental factor combination, the average hyphal growth rate reached 1.12 ± 0.09 mm/day, indicating good growth of GL under optimized conditions. The color of the hyphal slightly changed in three repeated experiments with light yellow, pale yellow, and yellow, which indicated that there was some variability in pigmentation,

Table 5. Comparison between experimental results and model predictions.

Index	Verify experimental results (average)	Standard deviation of experimental results	Model predicted value	Prediction error (%)
Mycelium growth rate (mm/d)	1.12 ± 0.09	0.01	1.10	1.8
Fruiting body diameter (cm)	11.8 ± 0.8	0.1	11.7	0.8
Final production (g)	57.3 ± 2.6	0.3	56.8	0.9

Table 6. Comparison between the predicted value of the optimized and adjusted model and the verified experimental results.

Index	Verify experimental results (average)	Experimental results	The predicted value of the optimized model	Prediction accuracy (%)
Mycelium growth rate (mm/d)	1.12 ± 0.09	1.03 - 1.21	1.11	0.9
Fruiting body diameter (cm)	11.8 ± 0.8	11.0 - 12.6	11.8	0.0
Final production (g)	57.3 ± 2.6	54.7 - 60.0	57.2	0.2

but it did not significantly affect the growth rate. The mycelial branching was mainly multi-branched in the first and second repeated experiments, and a few branching patterns were observed in the third repeated experiment. The variability of this branching was not correlated with changes in growth rate or final yield. The average diameter of the fungal body in the experiment was 11.8 ± 0.8 cm, indicating uniform development between experiments. The color of the fungal body was consistently dark brown, which was a typical feature of mature PGF bodies. From a morphological perspective, the fungal entity presented an umbrella-shaped structure with slightly curled edges in the second repeated experiment, while it exhibited an umbrella-shaped structure with a central micro-protrusion in the third repeated experiment, demonstrating a typical developmental pattern. The average final yield of GL was 57.3 ± 2.6 g, indicating that optimizing cultivation conditions not only improved yield and quality but also provided a predictable harvest schedule. The results of the validation experiment with the predicted values of the model were compared in this study. The average yield value of 57.3 g was slightly higher than the model predicted value of 56.8 g with no significant difference, indicating that the model had high prediction accuracy. Meanwhile, the experimental values of hyphal growth rate and fruiting body diameter were consistent with the predicted values, further verifying the accuracy of the model (Table 5).

(2) Optimization and adjustment

Based on the verification results, the model was optimized and adjusted to improve prediction accuracy and reliability. In response to the slight difference between experimental values and predicted values, the coefficients in the regression equation were fine tuned to make them closer to the actual growth situation. Considering the possible fluctuations in environmental factors during the actual planting process, the model introduced more robust algorithms to reduce the impact of external interference on the prediction results. The comparison results of the optimized and adjusted model predictions with the validation results demonstrated that the optimized model showed higher accuracy in predicting hyphal growth rate, fruiting body diameter, and yield. Especially, the predicted value of 57.2 g in yield was very close to the experimental value of 57.3 g, indicating that the optimized and adjusted model had higher reliability and practicality (Table 6).

Discussion

This study explored the effects of environmental factors of temperature, humidity, light intensity,

and soil pH on PGF growth through systematic experimental design and data analysis and successfully established a mathematical model between environmental factors and PGF growth. The humidity had the most significant impact on the growth of PGF followed by temperature and light intensity, while soil pH had a relatively small effect. When the humidity was controlled at around 82.5%, temperature was 25.5°C, light intensity was 1,250 lux, and soil pH was 6.6, the yield of PGF reached its maximum value with a predicted value of 56.8 g, while the average yield of the validation experiments also reached 57.3 g, verifying the accuracy of the model. Despite significant achievements in the research, there were still some limitations. The experimental conditions were relatively idealized and did not fully consider the complexity and uncertainty of the natural environment such as the impact of climate fluctuations, pests and diseases on PGF growth. Further, this study only focused on the single variety PGF, and there might be differences in the response of different GL varieties to environmental factors. Therefore, future research should further expand to more GL varieties and conduct experiments under conditions closer to the natural environment to improve the universality and practicality of research results. Based on the results of this study, it was recommended that, in the actual SWP process, special attention should be paid to the regulation of humidity by maintaining an appropriate range of humidity, while adjusting temperature and light intensity according to specific circumstances. In addition, it was recommended to adopt an intelligent management system for real-time monitoring and precise regulation of the planting environment to improve planting efficiency and yield. Meanwhile, monitoring and prevention of pests and diseases during the growth process of GL should be strengthened to ensure its healthy growth and high yield and quality. This research confirmed the accuracy and reliability of the established model, which provided not only a scientific basis for optimizing the SWP technology of GL, but also theoretical support for the sustainable development of the GL industry.

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