



"Mathematical Modeling and Analysis of Temperature and pH Effects on Mycelial Growth in *Calocybe indica*"

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ABSTRACT

Calocybe indica is characterized by its white sporophore, large-sized fruiting bodies, and delicious flavor. It exhibits high temperature tolerance, moderate protein content, good biological efficiency, and a long shelf life. We have a mathematical review to analyze the growth rate of the effect of temperature and different pH on mycelial growth. The maximum growth rate approx 1 cm/day was observed at 30°C in strain Cl-5, at a pH level of 8.

Kew Words: Mushroom, Temperature, pH, Mathematical Model, linear and Non-linear equations, curve fitting, regression analysis etc.

1. Introduction:

Calocybe indica, commonly known as the milky mushroom, is a notable species in a small genus of about 40 species, including both the St. George mushroom and the milky mushroom, which are widely cultivated in India. While some species in this genus are native to Britain, about nine species thrive in subtropical regions. The name of the genus *Calocybe* is derived from the ancient Greek words kalos, meaning "beautiful" and kybos, meaning "head", which refers to the attractive appearance of these mushrooms. Mushrooms, in general, are broadly classified as "macro-fungi with distinctive fruiting bodies that are visible to the naked eye and large enough to be picked by hand" (Chang and Miles, 1992). This group is highly diverse in size, shape and colour, with significant variations in physiological characteristics, edibility and appearance. The white milky mushroom, *C. indica*, is a prized edible species recognized for its white sporophore, robust and large fruiting bodies, and delightful flavor. This mushroom is particularly resilient, able to tolerate high temperatures between 25°C and 35°C, achieving a biological efficiency of 60–70% under ideal conditions. Its sporocarps have an extended shelf life, making it suitable for commercial cultivation. Nutritionally, *C. indica* is rich in essential minerals including potassium, sodium, phosphorus, iron, and calcium (Chang and Miles, 2004). With its alkaline ash and high fiber content, it provides health benefits, especially for individuals with hyperacidity and constipation (Doshi et al., 1995). In the case of *Lentinula edodes* (Berk.) Pegler, or shiitake mushroom, optimum radial growth was observed at 25°C and pH 5.0. Among the evaluated carbon and nitrogen sources, starch and urea facilitated the most effective mycelial growth. Spawn cultivation for *L. edodes* was carried out on sawdust of *Dalbergia sissoo*, *Acacia* and *Populus* species, with *Dalbergia sissoo* sawdust providing the highest efficacy. These sawdust types were further mixed with chopped wheat straw, rice straw and cotton waste as substrates. Sawdust mixed with cotton waste provided better mycelial growth than mixtures of wheat or rice straw. The initial protein concentration in the substrate ranged from 0.94% to 3.75% and increased as the mycelium colonized the substrate (Khan et al., 1991). The primary objective was to evaluate the physiological factors affecting radial growth in *L. edodes* and to identify locally obtained forest and agricultural waste substrates that could make shiitake mushroom cultivation viable as a cottage industry.

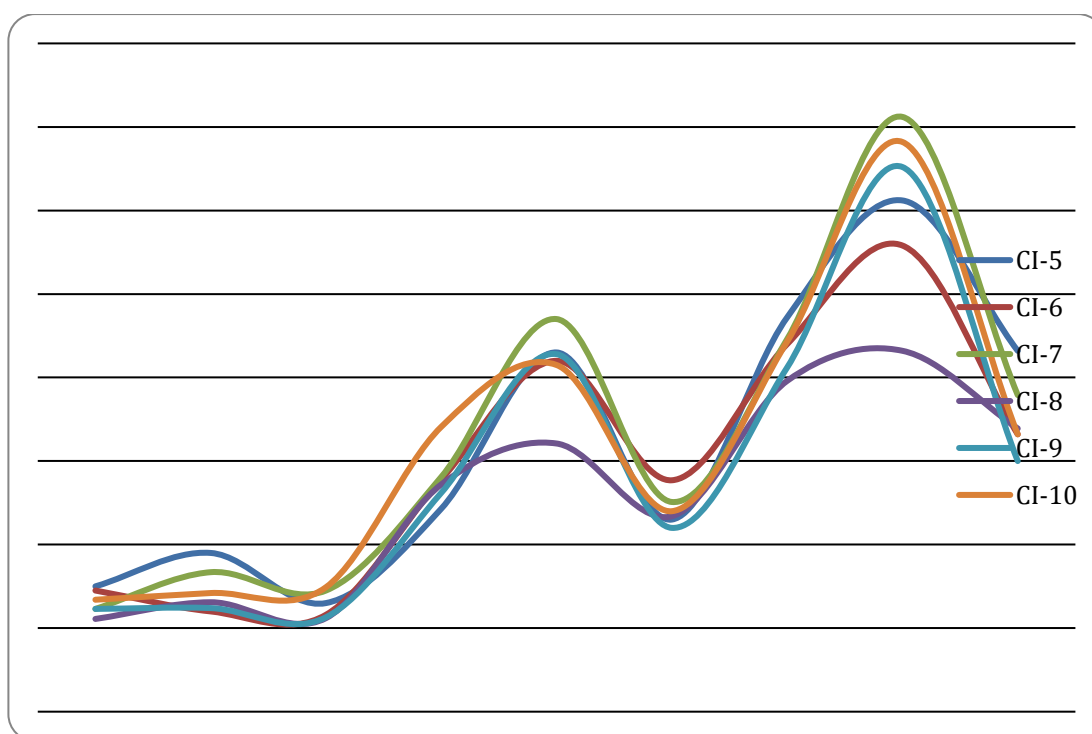
It is essential to investigate the physiological requirements and colony characteristics of *C. indica* before large-scale cultivation. Therefore, the present study aimed to explore the effects of temperature and pH on mycelial growth and colony morphology in different strains of *C. indica* (Bahukhandi et al., 1991; Shukla, Dayal, & Jetley, 2014).

Material and Methods:**Table-1 Colony characters of different strains of *Calocybe indica*:**

Serial no.	Strain	Appearance	Colour	Shape	Margin
1	CI-5	Fluffy	white	Circular	Even
2	CI-6	Cottony	white	Circular	Even
3	CI-7	Fluffy	white	Circular	Even
4	CI-8	Cottony	white	Circular	Even
5	CI-9	Fluffy	white	Irregular	Uneven
6	CI-10	Fluffy	White	Circular	Even

Table-2. Mycelial growth of *Calocybe indica* on different Temperature:

S.No.	Strains	Days:								
		3rd			6th			9th		
		25 °C	30 °C	35 °C	25 °C	30 °C	35 °C	25 °C	30 °C	35 °C
1	CI-5	1.5	1.9	1.3	2.43	4.3	2.3	4.72	6.12	4.32
2	CI-6	1.45	1.2	1.16	2.78	4.2	2.77	4.4	5.58	3.32
3	CI-7	1.23	1.67	1.45	2.81	4.7	2.51	4.46	7.12	3.79
4	CI-8	1.11	1.31	1.12	2.72	3.21	2.34	3.96	4.32	3.39
5	CI-9	1.23	1.24	1.13	2.62	4.28	2.2	4.12	6.52	3
6	CI-10	1.34	1.42	1.49	3.41	4.15	2.4	4.43	6.82	3.32

**Fig.1 (For Table 2)****Table- 3 Mycelial growth of *Calocybe indica* on different pH:**

S.No.	Strains	3 rd			6 th			9 th		
		pH6	pH7	pH8	pH6	pH7	pH8	pH6	pH7	pH8
1	CI-5	1.6	1.58	1.61	1.73	3.2	3.4	4.72	5.45	5.64
2	CI-6	1.5	1.78	1.74	2.14	3.3	3.48	5	5.15	5.63
3	CI-7	1.62	1.73	1.77	2.74	2.98	3.2	3.8	4.64	5.74
4	CI-8	1.7	1.71	1.82	2.55	3.4	4.16	4.7	6.57	6
5	CI-9	1.74	1.64	1.77	2.63	3.3	3.74	4.3	5.19	6.15
6	CI-10	1.62	1.79	1.72	3.22	3.45	3.44	4.1	4.16	5.65

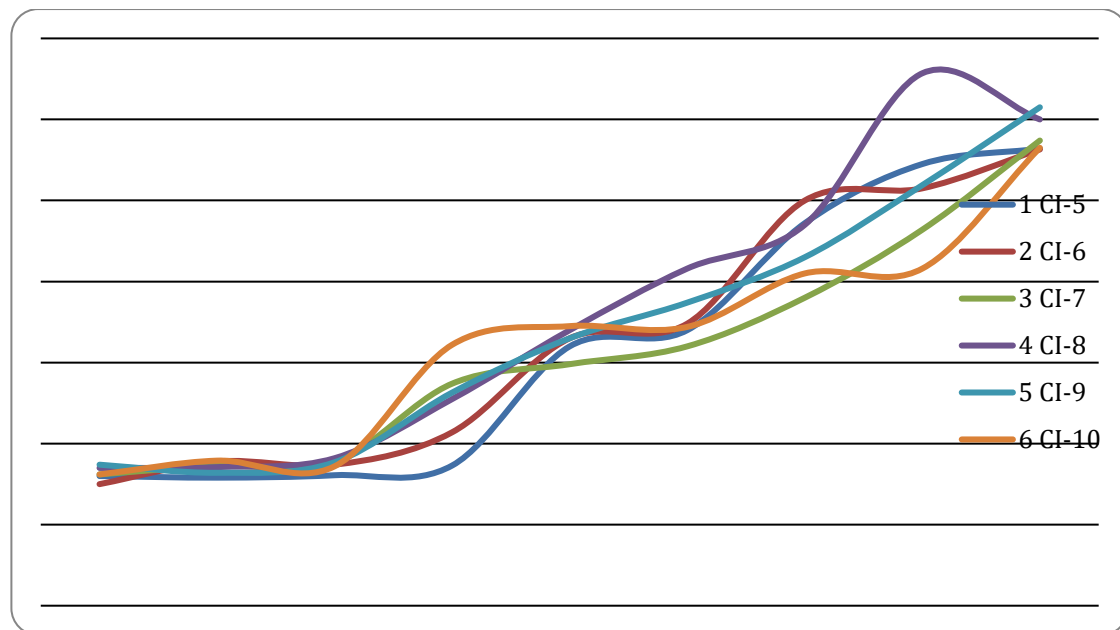


Fig.2 (for Table 3)

2. Mathematical Representation:

3.1 Mathematical Model-1:

To create a mathematical model that represents the growth data of *Calocybe indica* (CI strains) at various temperature points and over different days, we can set up a function based on the given table-2. This function will ideally relate the variables: strain, temperature, and time (days) to the growth values. (Fig 1 of table 2)

$G(T,D,S)$ represent the growth (response variable) as a function of temperature T , days d , and strain s . Where “ T ” is the temperature (25°C, 30°C, 35°C), “ D ” is the day (3rd day, 6th day, 9th day), “ S ” is the strain (CI-5, CI-6, CI-7, CI-8, CI-9, and CI-10).

The growth might be linearly or quadratic dependent on temperature T and time D . We can define a general linear or quadratic form as-

$$G(T,D,S) = a_s + b_s T + c_s D + d_s T^2 + e_s D^2 + f_s TD \quad \dots(1)$$

Where: $a_s, b_s, c_s, d_s, e_s, f_s$ are strain-specific coefficients, T is temperature (25°C, 30°C, 35°C), D is the day (3, 6, 9). The quadratic terms T^2 and D^2 capture non-linear growth patterns, and the interaction term $T \cdot D$ accounts for how the effects of temperature and time may influence each other.

For example, for CI-5, we have:

- Day 3, growth data: $G(25,3,CI-5)=1.5$, $G(30,3,CI-5)=1.9$, $G(35,3,CI-5)=1.3$
- Day 6, growth data: $G(25,6,CI-5)=2.43$, $G(30,6,CI-5)=4.3$, $G(35,6,CI-5)=2$
- Day 9, growth data: $G(25,9,CI-5)=4.7$, $G(30,9,CI-5)=6.12$, $G(35,9,CI-5)=4.32$
- These values would be plugged into the function to solve for the coefficients $a_s, b_s, c_s, d_s, e_s, f_s$ for strain CI-5. (Table 2)

For each strain, substitute the values of T (temperature) and D (days) into the general function. For example, for CI-5(s)

Day 3, Temperature 25°C:

$$1.5 = a_s + 25b_s + 3c_s + 25^2 d_s + 3^2 e_s + 25 \times 3 f_s$$

Day 3, Temperature 30°C:

$$1.9 = a_s + 30b_s + 3c_s + 30^2 d_s + 3^2 e_s + 30 \times 3 f_s$$

Day 3, Temperature 35°C:

$$1.3 = a_s + 35b_s + 3c_s + 35^2 d_s + 3^2 e_s + 35 \times 3 f_s$$

Day 6, Temperature 25°C:

$$2.43 = a_s + 25b_s + 6c_s + 25^2 d_s + 6^2 e_s + 25 \times 6 f_s$$

Day 6, Temperature 30°C:

$$4.3 = a_s + 30b_s + 6c_s + 30^2 d_s + 6^2 e_s + 30 \times 6 f_s$$

Day 6, Temperature 35°C:

$$2.3 = a_s + 35b_s + 6c_s + 35^2 d_s + 6^2 e_s + 35 \times 6 f_s$$

Day 9, Temperature 25°C:

$$4.72 = a_s + 25b_s + 9c_s + 25^2 d_s + 9^2 e_s + 25 \times 9 f_s$$

Day 9, Temperature 30°C:

$$6.12 = a_s + 30b_s + 9c_s + 30^2 d_s + 9^2 e_s + 30 \times 9 f_s$$

Day 9, Temperature 35°C:

$$4.32 = a_s + 35b_s + 9c_s + 35^2 d_s + 9^2 e_s + 35 \times 9 f_s$$

Now we are finding these 6 constants by using these 9 equations written as

$$1.5 = a_s + 25b_s + 3c_s + 625 d_s + 9e_s + 75 f_s$$

$$1.9 = a_s + 30b_s + 3c_s + 900 d_s + 9e_s + 90 f_s$$

$$1.3 = a_s + 35b_s + 3c_s + 1225 d_s + 9e_s + 105 f_s$$

$$2.43 = a_s + 25b_s + 6c_s + 625 d_s + 36e_s + 150 f_s$$

$$4.3 = a_s + 30b_s + 6c_s + 900 d_s + 36e_s + 180 f_s$$

$$2.3 = a_s + 35b_s + 6c_s + 1225 d_s + 36e_s + 210 f_s$$

$$4.72 = a_s + 25b_s + 9c_s + 625 d_s + 81e_s + 225 f_s$$

$$6.12 = a_s + 30b_s + 9c_s + 900 d_s + 81e_s + 270 f_s$$

$$4.32 = a_s + 35b_s + 9c_s + 1225 d_s + 81e_s + 315 f_s$$

We use least square approximation for the solution of the equations:

Now with these equations we can construct a matrix form $A.X = b$

Where A is the coefficient matrix, written by above 9 equations:

$$A = \begin{bmatrix} 1 & 25 & 3 & 625 & 9 & 75 \\ 1 & 30 & 3 & 900 & 9 & 90 \\ 1 & 35 & 3 & 1225 & 9 & 105 \\ 1 & 25 & 6 & 625 & 36 & 150 \\ 1 & 30 & 6 & 900 & 36 & 180 \\ 1 & 35 & 6 & 1225 & 36 & 210 \\ 1 & 25 & 9 & 625 & 81 & 225 \\ 1 & 30 & 9 & 900 & 81 & 270 \\ 1 & 35 & 9 & 1225 & 81 & 315 \end{bmatrix}$$

b is the vector of constants:

$$b = \begin{pmatrix} 1.5 \\ 1.9 \\ 1.3 \\ 2.43 \\ 4.3 \\ 2.3 \\ 4.72 \\ 6.12 \\ 4.32 \end{pmatrix}$$

And X is a vector of unknowns:

$$X = \begin{pmatrix} a_s \\ b_s \\ c_s \\ d_s \\ e_s \\ f_s \end{pmatrix}$$

"Since matrix A is not a square matrix, we cannot directly compute its inverse. Therefore, we apply the Least Squares method, which minimizes the sum of squared errors between the predicted values from the equations and the actual constants (the right-hand side values)

The solution is given by:

$$X = (A^T A)^{-1} A^T b$$

From the above least square method we calculate all unknowns:

$$a_s = -46.67, \quad b_s = 3.224, \quad c_s = 0.2811, \quad d_s = -0.0538,$$

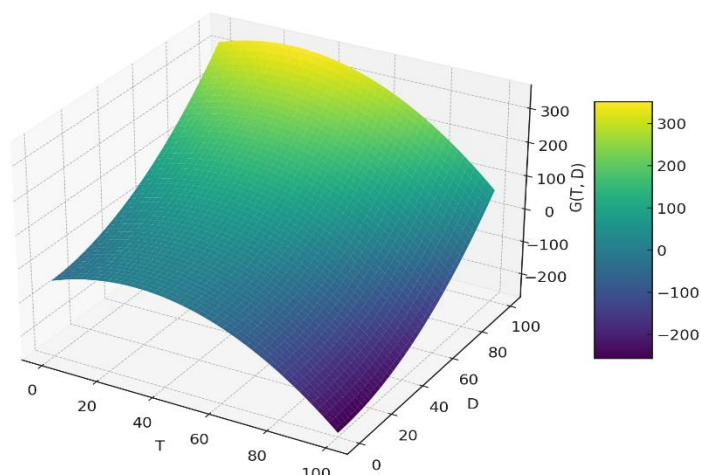
$$a_s = -46.67, \quad b_s = 3.224, \quad c_s = 0.2811, \quad d_s = -0.0538, \quad e_s = 0.0333, \quad e_s = 0.0333, \quad f_s = -0.0033$$

$$f_s = -0.0033$$

Putting these values in equation (1)

$$G(T, D, S) = -44.67 + 3.224 T + 0.2811 D - 0.0538 T^2 + 0.0333 D^2 - 0.0033 TD \dots(2)$$

Graphical Representation of G(T, D)



(Fig.3-for G(T,D,S))

The surface plot shows how the values of G change with respect to T and D. Where S is represent only the starian CI-5, so the function G(T,D,S) is represent in graph G(T,D)

2.2 Method Model-2:

To describe a mathematical relation for the mycelial growth of *Calocybe indica* across different pH levels, the data in the table-3 represents the mycelial growth of different strains of *Calocybe indica* under varying pH levels across three time points (3rd, 6th, and 9th days). The graph depicts this growth visually. Here is a breakdown of the mathematical function and analysis, the table shows six different strains (CI-5 to CI-10) and The mycelial growth is measured at three pH levels (6, 7, 8) over three time points (3rd, 6th, and 9th days).

Now we describe a mathematical function as:

The Micelial growth for each strain modeled the function of time “t” and pH denoted by “P”

That is

$$G(P, t) = at^\alpha + c \cdot P + d \quad \dots(3)$$

Where “t” represents the time points (3rd, 6th, 9th days), P represents the pH value (6, 7, 8) and a, b, c and α are constants that can be derived by fitting the data for each strain. The form at^α indicates a possible non-linear growth over time (since growth appears to accelerate between 6th, 7th and 9th days, and c·P represents the linear effect of pH on the growth. Use curve fitting method in table 3 we get the value of a = 0.100 , $\alpha = 1.689$, b = 4.0424, c = -1.914, use these values in above equation 2, we get:

$$G(P, t) = 0.10 t^{1.689} + 4.0424 P - 1.914 \quad \dots(4)$$

Graphical Representation of this growth:

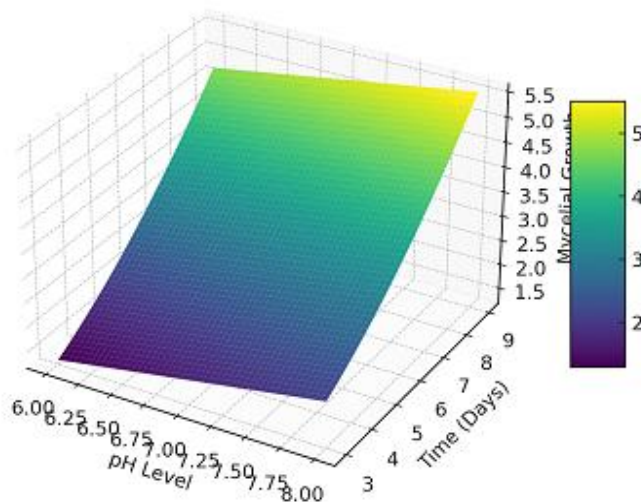


Fig.4 for (G(pH,t))

Where pH: Varies from 6 to 8, Time Varies from 3 to 9 days. Growth: The color bar indicates the mycelial growth values, where yellow represents higher growth and purple represents lower growth. This visualization shows how growth accelerates with time and increases linearly with pH level.

3. Result and Discussion:

The equation $G(T,D,S) = -44.67 + 3.224 T + 0.2811 D - 0.0538 T^2 + 0.0333 D^2 - 0.0033 TD$, describes the growth (g) of *Calocybe indica* mycelium, specifically strain CI-5, in response to different temperatures (T) and days (d) of incubation. This formula gives insight into how both temperature and the passage of time affect mycelial growth, capturing the balance between favorable and limiting conditions. The equation starts with a base value of -44.67, which indicates that in the absence of optimal temperature and time, growth would theoretically remain low. However, as temperature (T) increases, growth is significantly boosted due to the positive linear coefficient of 3.224. This term highlights the strong initial effect of temperature, suggesting that warm conditions within the appropriate range are favorable to mycelial growth. Similarly, the term $-0.0538 \cdot T^2$ shows that excessive temperatures eventually reduce growth, reflecting the biological principle that too high a temperature can stress or damage the mycelium. In contrast, the positive quadratic term $0.0333 \cdot D^2$ suggests a moderate acceleration in growth over time, reflecting an initial favorable response to incubation period. However, the growth rate slows as days increase due to factors such as nutrient depletion or environmental stress on the mycelium. Additionally, the interaction term $-0.0033 \cdot TD$ suggests that prolonged exposure to high temperatures is particularly stressful to the mycelium, as it reduces growth more than the sum of temperature and time. That is, this equation provides a predictive model for finding the optimal balance between temperature and incubation time for growing *Calocybe indica*. At moderate temperatures and appropriate duration, growth rates are maximized. However, too high temperatures or excessive incubation times begin to suppress growth, and if both are increased simultaneously, the reduction in growth becomes even more pronounced. This equation is therefore a valuable tool for growers, helping them adjust environmental conditions to reach ideal growth levels and avoid negative effects from excessively high temperatures or unnecessarily long incubation times. And here we see in Model 2 above that equation 4 describes that in all strains, growth increases steadily from the 3rd to the 9th day. There is particularly high growth between the 6th and 9th days at pH 7 and pH 8. At each time point, growth is generally higher at either pH 7 or pH 8 than at pH 6. Strain CI-8 shows particularly high growth at pH 8 on the 9th day (6.57). Strain CI-5 has a relatively constant growth across pH levels. Strain CI-10 shows high growth at pH 6 between the 3rd and 6th days, but shows moderate growth after the 6th day compared to the other strains.

A controls the rate of growth with respect to time t with the effect of pH. The positive value indicates increasing growth with time, and the exponent (approximately 1.689) suggests that the growth is non-linear and accelerates as time increases. b Shows the effect of pH on growth. The positive value indicates that higher pH values contribute positively to mycelial growth, c is the intercept or baseline growth when both time and pH are zero. This model captures the interaction between pH and time in predicting mycelial growth, where growth accelerates with time and is also affected by pH levels

4. Conclusion:

The equation (2) describing the growth of *Calocybe indica* mycelium for strain CI-5 illustrates how temperature and incubation time affect growth. It provides a predictive model that shows that growth is highly sensitive to both environmental temperature and duration, with an initial increase in growth as temperature increases, although extremely high temperatures reduce growth. This shows that while warm conditions are generally favorable for mycelium growth, there is an optimum temperature threshold, beyond which growth is reduced due to biological stress on the mycelium. This equation also shows how growth changes over time, with a gradual increase occurring during the incubation period, which is initially rapid but slows as time progresses. This slowdown is likely due to factors such as nutrient depletion and environmental stress. Furthermore, the interaction between temperature and time suggests that prolonged exposure to high temperatures has a particularly negative effect, reducing growth more than temperature or time alone. This model is important for cultivators, as it highlights the importance of maintaining balanced conditions. At moderate temperatures and within a reasonable time frame, growth reaches its peak. However, when temperature or incubation time is increased beyond this optimal range, growth decreases, emphasizing the need to carefully control both factors. When observing different strains at different pH levels (e.g. pH 6, 7 and 8), growth patterns show that pH levels also significantly affect mycelial growth in equation 4. Growth peaks between the 6th and 9th day at pH 7 and 8, with strain CI-8 performing particularly well at pH 8 on the 9th day. Strain CI-5 exhibits relatively stable growth at all pH levels, while strain CI-10 initially performs better at pH 6, but later grows more moderately. This variation between strains highlights that pH, along with temperature and time, plays a key role in determining growth outcomes. The second model further elucidates the relationship between time and pH on mycelial growth. Here, growth rate is positively affected by pH over time, with a non-linear, accelerated growth trend, indicating that higher pH levels may be beneficial. This growth model shows the combined effect of time and pH, and provides a nuanced understanding of how optimal growth conditions can be achieved by balancing multiple environmental factors, temperature, incubation time and pH – while minimizing potential stresses.

5. Future Applications:

On the basis of above models 1 and 2, future plans can focus on optimizing and applying these findings in real-world mushroom cultivation and related research. Here are some possible applications: Refinement of environmental parameters, Development of adaptive cultivation systems, Discovery of new strains and varieties, Extending the model to predict yield and quality, Data-driven decision making in commercial cultivation etc.

6.1 Refinement of environmental parameters: Based on the model, further experiments can be designed to pinpoint the exact temperature and incubation time that maximizes growth while minimizing energy costs. Controlled trials across a range of temperatures, time periods, and pH levels would allow a clear definition of ideal growth conditions, particularly for strain Cl-5 and other *Calocybe indica* strains. This would enable farmers to fine-tune their conditions for optimal yields.

6.2 Development of adaptive cultivation systems: With these growth predictions, it is possible to create automated or semi-automated cultivation systems. Such systems would adjust temperature, humidity, and other environmental parameters based on real-time feedback to maintain optimal growth conditions. This could improve efficiency and reduce manual intervention, making large-scale production more feasible and consistent.

6.3 Discovery of new strains and varieties: The model has shown variation in growth among different strains at different pH levels. Future research could explore additional *Calocybe indica* strains or related mushroom species to investigate how these environmental factors affect each. Selective breeding programs could aim to develop strains that are more resilient or have better growth characteristics under specific conditions.

6.4 Extending the model to predict yield and quality: In addition to growth rate, parameters such as mushroom size, biomass yield, and nutrient composition are important for commercial cultivation. Extending the model to correlate these factors to environmental conditions would provide a comprehensive framework for maximizing both quantity and quality in mushroom production.

6.5 Data-driven decision making in commercial cultivation: The model could serve as the basis for data-driven decision tools for farmers. An app or software solution could suggest to growers when to adjust temperature, time, or pH based on the strain, expected yield, or specific growth goals. This digital tool can predict optimal harvest times or suggest minor adjustments to maximize productivity.

7. References:

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