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Full Length Research Paper

Mathematical representation for effect of temperature on mycelial growth of *Calocybe indicia*

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Mushroom is characterized by having white sporophore, large sized fruiting bodies and delicious flavor. The temperature tolerance has been found to be high, with moderate protein content and good biological efficiency with long shelf life. There is mathematical model to describe mycelia growth on effect of temperature. The maximum mycelial growth rate (1.12 cm/day) at 30°C was observed in the strain *CI*-7 at 8.0 pH level.

Key words: Laplace transformation, integration, white milky mushroom, temperature tolerance, protein content, growth.

INTRODUCTION

Calocybe Indica is a small genus of about 40 species of mushroom, including St. George's mushroom and milky mushroom, which are edible and cultivated in India. Very less species of this genus are growing in Britain. The name is derived from the Ancient Greek term kalos "pretty" and cubos "head". Around nine species are found in subtropical regions. Mushroom is broadly defined as "a macro fungus with distinctive fruiting body which can be either epigeal or hypogeal; large enough to be seen with the naked eye and to be picked up by hand (Chang and miles, 1992). The mushroom comprises a large heterogeneous group having various shapes, size and colors. All are quite different in characters, appearance and edibility. White milky mushroom (*C. indica*) is an edible mushroom having white sporophore, large sized fruiting bodies and delicious flavor. Mushroom could tolerate high temperature ranges of 25-35°C with a biological efficiency of 60-70% under optimum conditions. Their sporocarps have long shelf-life. This mushroom contains most of the mineral salts required by the human body, such as potassium, sodium, phosphorus, iron and calcium (Chang and Millis, 2004), due to its alkaline ash and high fibre content, it is highly suitable for people with hyperacidity and constipation (Doshi et al., 1988).

It was essential to investigate the physiological requirements and colony characters of this fungus in culture before going its large scale cultivation. Hence the present investigation was carried out to observe the effect of temperature and pH on mycelial growth and colony characteristics of different strains of *C. indica.*

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Table 1. Colony characters of different strains of *C. indica.*

Strain	Appearance	Colour	Shape	Margin
CI-5	Fluffy	White	Circular	Even
CI-6	Cottony	White	Circular	Even
CI-7	Fluffy	White	Circular	Even
CI-8	Cottony	White	Circular	Even
CI-9	Fluffy	White	Irregular	Uneven
CI-10	Fluffy	White	Circular	Even

MATERIALS AND METHODS

Cultures and their maintenance

The culture of different strains of *C. indica* namely *CI*-5, *CI*-6, *CI*-7, *CI*-8, *CI*-9, and *CI*-10 were taken from the Mushroom Research and Training Center, Pantnagar (Uttarakhand, India) and the cultures were revived on fresh Malt Extract Agar medium (MEA) using hyphal tip method by incubation at 25°C temperature.

Effect of different temperatures on mycelial growth:

The effect of temperature on mycelial growth was studied on MEA media. The Petri-plates containing 25 ml medium were inoculated with 4 mm disc of actively growing mycelium with the help of sterilized cork borer. The inoculated Petri-plates were incubated at different temperature viz., 25, 30 and 35°C. Three replicates were taken for each strain. Cardinal growth was recorded up to the growth of strains to fill one of the Petri plates. The radial growth was recorded in cm in two directions at right angles to each other and the average was calculated.

Effect of different pH on mycelial growth:

The effect of different pH viz., 6, 7, 8 and 9 for mycelial growth was observed on MEA medium. The pH was adjusted using concentrated HCl and NaOH solutions for lower and higher pH values respectively. Each treatment (pH level) was replicated three times for each strain. These Petri plates containing 25 ml medium of desired pH level were inoculated as like in temperature trials and incubated at their optimum temperature. The observations were recorded in the same manner as for temperature.

Colony characters on MEA

The strains were grown on malt extract agar medium at their optimum temperature and pH to study the colony characteristics. Petri plates containing 25 ml of the medium were inoculated at the centre with 4 mm disc of actively growing mycelium under aseptic conditions, maintaining 3 replicates, for each strain. The observations on colony type (appearance), color, shape and margin were recorded.

Mycelial growth vs temperature

There are various factors which affect the growth in different manner. For example the pH value was different for strains *CI*-5, *CI*-6, *CI*-7, *CI*-8, *CI*-9 and *CI*-10 (Table 3), similarly the growth varied with the temperature (Table 2) and so the shape color appearance and margin were different for each strain of *C. India* (Table 1). The temperature vs. growth graph of each strain on different days are shown (Figure 1) which support the idea of temperature as a decision making factor for growth. However the effect of temperature is not only the cause.

Mathematical formulation

Let us consider a function,

$$f(t)=\begin{pmatrix} 0 & for\, 0\le t \prec 25 \\ m_1 t + c_1 & for\, 25\le t\le 30 \\ m_2 t + c_2 & for\, 30\le t\le 35 \\ 0 & t \succ 35 \end{pmatrix}$$

Where m_1 and m_2 are the slope of growth rate at given temperature, t denote the temperature and c_1, c_2 are arbitrary constants.

Taking Laplace of both sides

$$L\{f(t)\}=\int_0^{\infty} e^{-st} f(t)dt$$

$$L\{f(t)\}=\int_0^{25} e^{-st}0dt+\int_{25}^{30} e^{-st}(m_1t+c_1)dt+\int_{30}^{35} e^{-st}(m_2t+c_2)dt+\int_{35}^{\infty} e^{-st}0dt$$

$$L\{f(t)\}=\left[(m_1t+c_1)(\frac{e^{-st}}{-s})-(m_1)(\frac{e^{-st}}{s^2})\right]_{25}^{30}+\left[(m_2t+c_2)(\frac{e^{-st}}{-s})-(m_2)(\frac{e^{-st}}{s^2})\right]_{30}^{35}$$

$$L\{f(t)\}=\left[\left\{(30m_1+c_1)(\frac{e^{-30s}}{-s})-(m_1)(\frac{e^{-30s}}{s^2})\right\}-\left\{(25m_1+c_1)(\frac{e^{-25s}}{-s})-(m_1)(\frac{e^{-25s}}{s^2})\right\}\right]$$

$$+\left[\left\{(35m_2+c_2)(\frac{e^{-35s}}{-s})-(m_2)(\frac{e^{-35s}}{s^2})\right\}-\left\{(30m_2+c_2)(\frac{e^{-30s}}{-s})-(m_2)(\frac{e^{-30s}}{s^2})\right\}\right]$$

Therefore,

$$L\{f(t)\} = -\frac{1}{s}\left[e^{-30s}(30m_1 + c_1 - 30m_2 - c_2) - (25m_1 + c_1)e^{-25s} + (35m_2 + c_2)e^{-35s}\right]$$

$$-\frac{1}{s^2}\left[(m_1 - m_2)e^{-30s} - m_1e^{-25s} + m_2e^{-35s}\right]$$

Remarks: Since $m_1 = m_2 = m$ (say) at 30°c temperature, because of for better cultivation, the growth would be maximum at the given temperature. Then, this equation shows the relation between mycelial growth rate on given temperatures.

$$L\{f(t)\} = -\frac{1}{s}\left[e^{-30s}(30m + c_1 - 30m - c_2) - (25m + c_1)e^{-25s} + (35m + c_2)e^{-35s}\right]$$

$$-\frac{1}{s^2}\left[(m - m)e^{-30s} - me^{-25s} + me^{-35s}\right]$$

$$L\{f(t)\} = -\frac{1}{s}\left[e^{-30s}(c_1 - c_2) - (25m + c_1)e^{-25s} + (35m + c_2)e^{-35s}\right]$$

$$-\frac{1}{s^2}\left[-me^{-25s} + me^{-35s}\right]$$

RESULTS AND DISCUSSION

Effect of different temperatures on mycelial growth of strains of *C. indica*

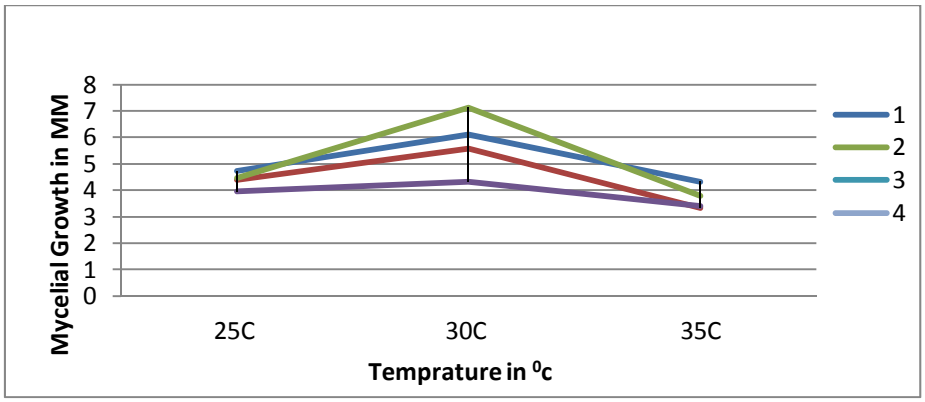
From the data (Table 2) all the strains showed maximum mycelial growth at 30°C followed by 25°C and minimum at 35°C on 4th, 6th and 8th day. However, on 2nd day some of the strains like *CI*-5 and *CI*-6 showed maximum mycelial growth at 25°C while that of other strains were at 30°C. The mycelial growth of the each strain was varied significantly at all the tested temperatures (Bhugarski et al, 2002). At 30°C, on 8th day strains *CI*-7 showed maximum full diametric growth of mycelium (9.0 cm). The other strains showed significant variation to each other giving diametric growth ranges from 6.4-8.7 cm (with average growth rate of 0.80-1.09 cm per day). Then the strain in order to superiority was *CI*-7 giving 6.56 cm mycelial growth. At par growth was recorded from the strain *CI*-5 and *CI*-6 however *CI*-10 produces minimum growth. The least growth of the strains was recorded at temperature 35°C on which highest mycelial diametric growth was (5.40) obtained for *CI*-5. The minimum growth of 3.8 and 3.9 cm was recorded from *CI*-10 and *CI*-9 respectively. These results are in accordance with the findings of Doshi and Sharma (1995), Furlan et al. (1997). They reported optimum temperature range between 20 - 35°C for the mycelial growth of *C. indica*.

Effect of different pH on mycelial growth of strains of *C-indicia*

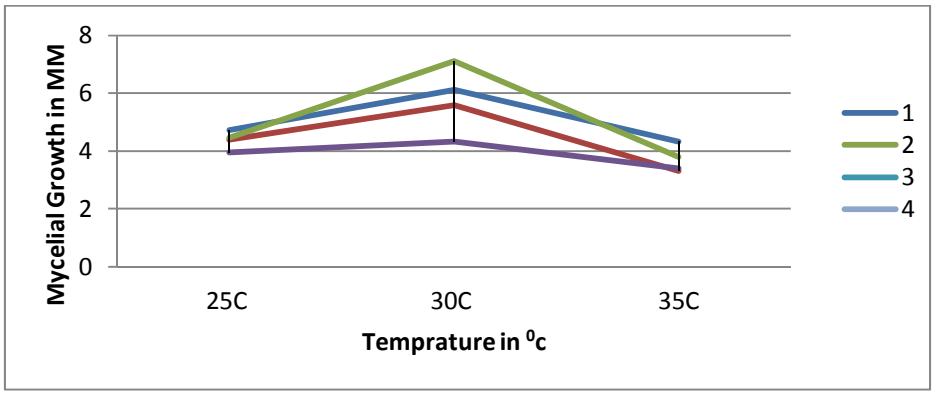
The data (Table 3) shows that the maximum diametric mycelial growth was at pH 8.0 followed by at 7.0. The minimum mycelial growth for all the strains was observed at pH level 6.0 and 9.0. At pH 8.0, the strain *CI-7* has completed its growth on the 8th day that is 9.0 cm. The next strains in order to superiority was *CI-10.* The poor mycelial growth was recorded in the case of strain *CI-5*, that is 6.8 cm which was near with the growth obtained for strains *CI-9* and *CI-8*. It was observed that the strains *CI-9* grew faster at all the tested pH. At pH 7 the strains showed diametric growth ranges from 6.40 - 8.30 cm. The growth pattern of the strains at pH 7.0 was similar to pH 8.0 but at slow growth rate. The results are in accordance with the finding of Khan et al. (1991) which reported pH 7.0 as the most suitable pH level for radial mycelial growth of the *Lentinula edodes*.

Colony characters of different strains of *C. indica*

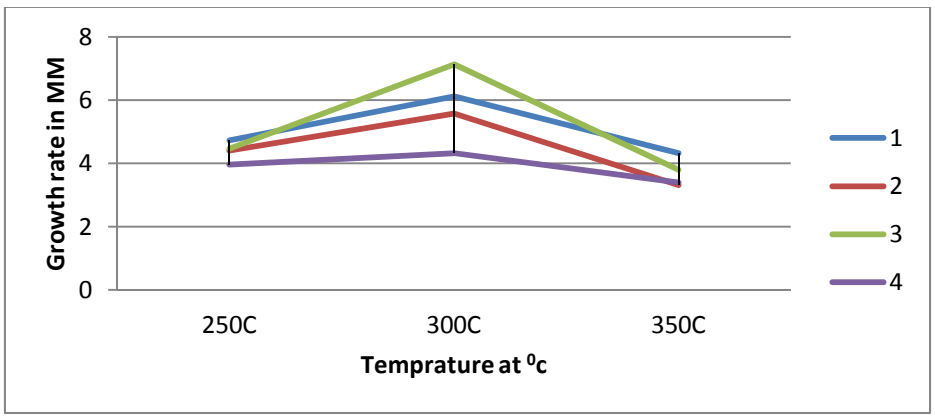
All the six strains were grown on malt extract Agar (MEA) medium to study their colony character viz., colony appearance, color, shape and margin. From the data (Table 1), two types of colony viz., fluffy and cottony



a



b



c

Figure 1. a. 2nd day of observation. **b.** 4rd day of observation. **c.** 6th day of observation.

were observed among the strains. Fluffy growth appeared for strain *CI*-5*, CI-7, CI-9* and *CI-10* while strains *CI-6* and *CI-8* showed cottony appearance. All the strains showed white colour of colonies. Shape of the colonies varied from circular to irregular. All the Strain showed circular colony except *CI-9* which showed irregular colony. Margin of colonies were observed: even and uneven types. The strains *CI*-5, *CI*-6, *CI*-7, *CI-8* and *CI-10* showed even margin whereas and *CI-9* showed uneven margin of the colonies.

The results obtained are in accordance with the finding of Jin and Xu (1990), Goltapeh and Kapoor (1989). They reported fluffy and apprised types of the colonies of *Agaricus bisporus*. Bahukhandi et al. (1991)

Table 2. Effect of temperature on mycelial growth of different strains of *C. indica.*

Strain	Days											
	2nd			4th			6th			8th		
	25°C	30°C	35°C	25°C	30°C	35°C	25°C	30°C	35°C	25°C	30°C	35°C
CI-5	1.70	1.80	1.29	2.41	4.10	2.40	4.72	6.12	4.32	6.39(0.79)	8.47(1.06)	5.40(0.68)
CI-6	1.30	1.19	1.10	2.82	4.00	2.72	4.40	5.58	3.32	6.40(0.80)	7.40(0.93)	4.10(0.56)
CI-7	1.23	1.66	1.39	2.89	4.80	2.49	4.46	7.12	3.79	6.56(0.82)	9.00(1.12)	4.69(0.58)
CI-8	1.20	1.25	1.10	2.72	3.16	2.30	3.96	4.32	3.39	5.20(0.65)	6.40(0.80)	4.30(0.54)
CI-9	1.15	1.40	1.10	2.50	4.26	2.00	4.12	6.52	3.00	5.00(0.63)	5.00(0.94)	3.90(0.49)
CI-10	1.35	1.60	1.50	3.36	4.12	2.60	4.43	6.82	3.32	6.20(0.78)	6.20(1.06)	3.80(0.60)

Table 3. Effect of different pH on diametric mycelial growth (in cm.) of *Calocybe indica.*

Strain	2nd				4th				6th				8th			
	pH6	pH7	pH8	pH9	pH6	pH7	pH8	pH9	pH6	pH7	pH8	pH9	pH6	pH7	pH8	pH9
CI-5	1.50	1.57	1.60	1.50	1.70	3.00	3.36	2.90	4.67	5.40	5.60	4.00	6.20	7.50	6.80	6.00
CI-6	1.70	1.73	1.73	1.70	2.10	3.35	3.40	3.17	4.90	5.10	5.60	4.20	6.00	6.40	8.00	5.90
CI-7	1.72	1.70	1.75	1.70	2.70	2.93	3.00	1.90	3.60	4.60	5.70	4.40	5.40	7.00	9.00	5.70
CI-8	1.75	1.70	1.80	1.60	2.50	3.00	4.10	2.93	4.67	6.50	5.90	4.10	6.20	8.30	7.90	5.10
CI-9	1.70	1.60	1.78	1.74	2.60	3.10	3.83	3.20	4.10	5.10	6.10	4.60	5.60	6.90	7.20	5.60
CI-10	1.60	1.70	1.73	1.65	3.20	3.33	3.40	2.90	4.00	4.10	5.60	3.20	5.50	6.80	8.50	5.30

observed the differentiation in colony characters of strains of *A. bisporus* in respect of colony diameter, appearance, color, shape, zonation, margin, sectoring, pigment and exudates formation.

Present investigations have been helpful in understanding the physiological requirements and colony character of different strains of this fungus. These preliminary studies are being extended for the cultivation trials of *C. indica.*

Conclusion

The growth rate of mycelium was evident at high values of temperature (even tends to infinity) using the above mathematical expressions. It helps us to define further growth rate by statistical assignments. These expressions can be solved in algebraic and differential equation because the Laplace transform provides the facility to define any function from zero to infinity in continuous and piece wise continuous manners.

Conflict of Interests

The author(s) have not declared any conflict of interests

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