



EFFECT OF DIFFERENT CULTURE MEDIA AND TEMPERATURE ON RADIAL GROWTH OF MILKY MUSHROOM (*CALOCYBE INDICA*) P & C. STRAINS

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Abstract

Milky mushroom also known as “white summer mushroom” because it is suitable to grow in tropical regions. It is 4th largest cultivated mushrooms in India after button and Oyster and paddy straw mushrooms. Milky mushrooms are edible and are rich in essential nutrients such as proteins, carbohydrates, vitamins, mineral, fat, fibres and various amino acids. Variations in mycelial growth of milky mushroom strains were observed in diversified range of media and temperature. In present study we have evaluated the three strains of *Calocybe indica* (CIP-18, CIP-19 and CIP-20) for seven different culture media and temperature ranges. The results shown that strain CIP-18 shown maximum growth on PDA and WEA media was 8.83 cm followed by MEA (8.70 cm), BAF (8.13 cm), CMA (7.77 cm) and YEPDA (7.73 cm) while minimum growth observed from CEA media (7.20 cm). The strain CIP-19 shown maximum growth on MEA media (8.93 cm) followed by PDA (8.73 cm), WEA (8.33 cm), CMA (8.27 cm), YEPDA (7.43cm) and BAF (7.17 cm) while minimum CEA media (7.03 cm). The best performance of strain CIP-20 observed on PDA medium (9.00 cm) than on MEA (8.83 cm), WEA (8.77 cm), CEA (8.50 cm), BAF (8.47 cm) and YEPDA (8.03 cm) while minimum on CMA medium (7.90 cm). The maximum mycelial growth in the effect of different temperature ranges for strain CIP-18 observed at 30°C (8.10 cm) than at 34°C (7.63 cm), 28°C (7.60 cm), 26°C (7.36 cm), 32°C (7.30 cm) and at 36°C (2.30 cm) while temperature 38°C shown minimum growth (2.3 cm). The strain CIP-19 shown maximum mycelial growth at 30°C (9.00 cm), than at 32°C (8.90 cm), 34°C (8.63 cm), 26°C (8.00 cm), 28°C (7.93 cm) and at 36°C (5.83 cm) but minimum at 38°C (2.3 cm). The strain CIP-20 strain shown best performance on different temperature effect with full petri-plate mycelial growth 9.00 cm at temperature 30°C and 32°C followed by 26°C and 28°C shown 8.93 cm than at 34°C (8.13 cm) and 36°C (3.66 cm) while minimum mycelial growth was observed at 38°C (2.23 cm).

Introduction

Agriculture is backbone of our country. Green revolution provided the required food security as produced sufficient amount of food grain by every year but struggling to achieve nutritional sufficiency. The Mushrooms cultivation technology has always catch the attention due to its multidimensional usage such as eliminating malnutrition, recycling of residue and providing chances of employment for youth. The mushroom cultivation can also be done by land less labour and it is an eco-friendly alternative for agro-waste recycling and provide better nutrition for the vegetarian population. In some societies, the consumption of mushroom was considered a royal food due to its pleasant flavor and texture (Chang and Miles, 1990). The large size fruiting body which can be considered as mushroom was found in approximately 14000 described species from the millions of fungi existing in the world (Kirk *et al.*, 2008). Out of them about 7000 macro-fungi having varying degree of edibility and 200 species were successfully cultivated in laboratory but now a day's hardly 10 species were cultivated at industrial

label by farming communities of different countries (Ferreira *et al.*, 2016). In 2019 total mushroom production of India accounted 20 million tonnes, in which Button mushroom and oyster mushroom production was contributed about 73% and 16% of total mushroom production respectively while milky mushroom contributed only 3% of total mushroom production in India. (ICAR, DMR, 2019).

Milky mushroom (*Calocybe indica*) strains are robust, fleshy and milky white in colour even after flattening. The milky mushroom strains were suitable for cultivation even in hot humid climate, tropical regions with temperature ranges 25°C to 35°C and 70 to 90 percent humidity hence this mushroom is highly suitable for production in most of the plains of India almost throughout the year. Milky mushroom strains were grown on several agricultural wastes and on wide range of temperatures and pH. The *Calocybe indica* strains can grow on wide range of culture media and temperature. Thus, it is essential to evaluate most favorable culture media and temperature range for efficient mycelial growth of milky

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mushroom strains. So in this present study, seven different culture media and temperature ranges were evaluated to determine their effects on mycelial growth of three cultivated strains of milky mushroom, viz. CIP-18, CIP-19 and CIP-20.

Material and Method

Collection of Mushroom Culture

The Pure culture of three cultivated strains CIP-18, CIP-19 and CIP-20 of *Calocybe indica* were introduced from Dr. Rajendra Prasad Central Agricultural University, Samastipur, Bihar. These cultures were sub-cultured and maintained on PDA medium in a B.O.D. incubator at $30 \pm 2^\circ\text{C}$ temperature for further investigation in "Mushroom Spawn Laboratory" Department of Mycology and Plant Pathology, I.A.S., B.H.U., Varanasi.

Preparation of culture media

The pure culture of cultivated strain of milky mushrooms was obtained on the following media to evaluate best suitable media for milky mushroom cultivation. These media generally used as substrate for isolation, sub-culture, maintenance and preservation of mushroom cultures.

Potato Dextrose Agar (PDA) Media: Take 250g Peeled and sliced potatoes were boiled in distilled water for 20-25 minutes till those become soft. Then filter the extract with a muslin cloth. After that 20 gm dextrose and 20gm agar powder were added to the filtrate over a hot plate by continues stirring. The final volume of the medium was adjusted to one liter by adding required amount of distilled water. The medium was taken in flask were plugged with non-absorbent cotton and sterilized in autoclave at 121°C (151 b/sq. inch pressure) for 30 minutes. After autoclaving melt medium was poured in sterilized Petri-plates. These poured Petri-plates were used for sub-cultured of milky mushroom. Slant culture tubes were also prepared for above purpose

Malt Extract Agar Medium: The procedure for preparation of Malt Extract Agar (MEA) medium was similar to PDA medium, only added 25 g malt extracts instead of peeled potato and dextrose but 20 g agar- agar was also added for preparation of one liter of media.

Wheat Extracts Agar Medium: 100 g Wheat grains were boiled in 1 liter distilled water for one hour. Filter the extract after 24 hour. 20 g Agar was added to the supernatant by stirring with a glass rod over a hot plate. The final volume of medium was adjusted to 1 liter by adding required amount of distilled water. The medium was then autoclaved as above.

Compost Extract Agar Medium: Boiled the 100 g ready synthetic compost in 1 liter of water for 1 hour and then filter it after 24 hour. 20 g Agar was added to the supernatant by stirring with a glass rod over a hot plate. The final volume of medium was adjusted to 1 liter by adding required amount of distilled water. The medium was then autoclaved as above.

Yeast Extract Potato Dextrose Agar (YPDA): Take 250 g peeled and sliced potatoes than boiled in distilled water for 20-25 minutes till these become soft. Filter the extract with a muslin cloth. Add 1.0 g of yeast extract, 20 g dextrose and 20 g agar powder to the filtrate over a hot plate by stirring. The final volume of the medium should be adjusted to one litre by adding required amount of distilled water.

Biotin-Aneurin-Folic Acid Agar (BAF): For the preparation of BAF media we required these components Glucose- 30 g, Pepton- 2 g, Yeast extract (Difco)- 0.2 g, KH_2PO_4 - 0.5 g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ - 0.5 g, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ - 10.0 mg, $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ - 1.0 mg, $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ - 5.0 mg, CaCl_2 - 100.0 mg, Thiamin HCl- 0.05 mg, Biotin- 0.001 mg, Folic acid- 0.1 mg, Inositol- 50 mg, Water- 1 litre and Agar- 15 g. Take 600 ml water in flask and dissolve all the ingredients at 60°C . Agar also dissolved in 300 ml boiling water in separate flask and cool down than mix both and adjust the 1 L final volume of medium with sterilized distilled water. Sterilize this medium in autoclave for 20-25 minutes at 121°C .

Cornmeal Agar extract: Take 60 g maize grain in pan having 1 L water and boiled it for 1 hour than filter with muslin cloth. Add 20 g agar in filtrate and make final volume 1 L with distilled water than sterilized in autoclave.

Maintaining the Temperature

In case of evaluate the effect temperature on different cultivated strains (CI-15-02, CIP-18, CIP-19 and CIP-20) of *Calocybe indica*. The inoculums were transferred in Petri-plates which poured by autoclaved PDA in laminar flow. Then the inoculated Petri-plates took in incubator where different temperature range 26°C , 28°C , 30°C , 32°C , 34°C , 36°C and 38°C were adjusted and then observing the radial growth of milky mushroom.

Result and Discussion

Effect of different culture media on mycelial growth of milky mushroom (*Calocybe indica*) strains

The effect of seven (MEA, PDA, BAF, YEPDA, CMA, CEA and WEA) different media on radial growth for three strains (CIP-18, CIP-19 and CIP-20) of milky mushroom (*Calocybe indica*) shown in Table 1. The result presented in Table 1 shown that on 3rd day observations the strain CIP-18 obtained maximum growth 3.67 cm on WEA media followed by PDA (3.6 cm), BAF (3.27 cm), CMA (3.1) while minimum growth was observed on CEA media (2.57 cm). The strain CIP-19 shown maximum radial growth on PDA media that was 3.43 cm followed by CMA, MEA, and CEA 3.3 cm, 3.0 cm and 2.90 cm, respectively. The minimum mycelial growth for strain CIP-19 was observed on BAF (2.67 cm). On 3rd day maximum mycelial growth was reported for strain CIP-20 on PDA media (4.0 cm) than on BAF (3.53 cm), WEA (3.47) while minimum on MEA (2.9 cm).

The data presented in Table 1 for 7th day observation found that for strain CIP-18 maximum mycelial growth obtained from PDA media (7.23 cm) followed by WEA (7.10 cm), BAF (6.33 cm) and MEA (5.87 cm) while minimum growth observed on CEA media (5.37 cm). The strain CIP-19 shown maximum mycelial growth on PDA media 6.77 cm than MEA (6.67 cm), CMA (6.60 cm) and WEA (6.0 cm) while minimum on CEA media (5.13 cm). Result shown that for strain CIP-20 of *Calocybe indica* maximum radial growth on 7th day observed from PDA media (8.20 cm) followed by WEA (7.17 cm), BAF (7.13 cm), CEA (6.57 cm), MEA (6.33 cm) and YEPDA (6.23 cm) while minimum growth was observed from CMA media (6.00 cm). The observations for 10th day were presented in Table 1. The results shown that the maximum mycelial growth observed for milky mushroom strain CIP-18 on PDA and WEA media was 8.83 cm followed by MEA (8.70 cm), BAF (8.13 cm), CMA (7.77 cm) and YEPDA (7.73 cm), while minimum growth observed from CEA media (7.20 cm). The strain CIP-19 shown maximum mycelial growth on MEA media (8.93 cm) followed by PDA (8.73 cm), WEA (8.33 cm), CMA (8.27 cm), YEPDA (7.43 cm) and BAF (7.17 cm) while minimum mycelial growth observed from CEA media (7.03 cm). The maximum mycelial growth in the influence of seven different media was observed on PDA media for milky mushroom strain CIP-20 on 10th day. Than on MEA (8.83 cm), WEA (8.77 cm), CEA (8.50 cm), BAF (8.47 cm) and YEPDA (8.03 cm), while minimum mycelial growth observed on CMA media (7.90 cm).

Effect of different Temperature on mycelial growth of milky mushroom (*Calocybe indica*) strains

Seven different temperature range (26°C, 28°C, 30°C, 32°C, 34°C, 36°C and 38°C) were evaluated for mycelial growth of three strains (CIP-18, CIP-19 and CIP-20) of milky mushroom (*Calocybe indica*). The observation for mycelial growth taken at 3rd day, 7th day and 10th day were presented in Table 2. The results shown that on day 3rd strain CIP-18 shown maximum mycelial growth 34°C (3.43 cm) followed by 30°C (2.70 cm), 36°C (2.66 cm), 32°C (2.63 cm), 28°C (2.50 cm) and 26°C (2.26 cm) while the minimum mycelial growth observed from 38°C (1.66 cm). The strain CIP-19 shown maximum radial growth on 3rd day observation from 30°C (3.73 cm) than at 32°C (3.46 cm), 36°C (3.33 cm) while minimum growth was observed at 38°C (1.60 cm). The strain CIP-20 shown maximum mycelial growth for 3rd day observation at 30°C (4.3 cm) than at 32°C (3.90 cm), 26°C (3.16 cm), 28°C (3.06 cm) and 34°C (3.00 cm) but minimum growth was observed at 38°C (1.5 cm).

Observations data for 7th day mycelial growth of *Calocybe indica* strains presented in Table 2. The results from Table 2 shown that maximum mycelial growth for strain CIP-18 recorded at 34°C (6.16 cm) than at 30°C (6.06 cm), 28°C (5.46 cm), 32°C (5.43 cm), 26°C (5.13 cm) and 36°C (4.66 cm) while minimum growth at 38°C (2.13 cm). The strain CIP-19 shown maximum mycelial growth for 7th day observed at 30°C (8.13 cm) followed by 32 (7.73) 34°C

(7.36 cm), 26°C (6.33 cm), 25°C (5.80 cm) and 36°C (5.53 cm) while minimum growth at 38°C (2.13 cm). The milky mushroom strain CIP-20 shown maximum radial growth for 7th day at 30°C and 32°C (8.50 cm) than at 34°C (7.10 cm), 26°C (6.86 cm), 28°C (6.66 cm) and 36°C (3.43 cm) but minimum radial growth observed at 38°C (2.00 cm).

The 10th day observation data for mycelial growth of three milky mushroom strains presented in Table 2. The result shown that maximum mycelial growth in the effect of different temperature ranges for strain CIP-18 observed at 30°C (8.10 cm) than at 34°C (7.63 cm), 28°C (7.60 cm), 26°C (7.36 cm), 32°C (7.30 cm) and at 36°C (2.30 cm) while temperature 38°C shown minimum mycelial growth (2.3 cm). The strain CIP-19 shown maximum mycelial growth at 30°C (9.00 cm), than at 32°C (8.90 cm), 34°C (8.63 cm), 26°C (8.00 cm), 28°C (7.93 cm) and at 36°C (5.83 cm) but minimum growth observed at 38°C (2.3 cm). The milky mushroom strain CIP-20 strain shown best performance on different temperature effect which shown maximum full petri-plate mycelial growth 9.00 cm at temperature 30°C and 32°C followed by 26°C and 28°C shown 8.93 cm than at 34°C (8.13 cm) and 36°C (3.66 cm) while minimum mycelial growth was observed at 38°C (2.23 cm). Kerketta *et al.* (2017) were also found similar results for mycelial growth of milky mushroom strains (CI-1, CI-4, CI-522, CI-524 and CI-530). They reported that PSA (Potato sucrose agar) medium followed by PDA (Potato dextrose agar) medium shown best performance and Similarly 30°C temperature found to be suitable for maximum mycelial growth of different milky mushroom strains.

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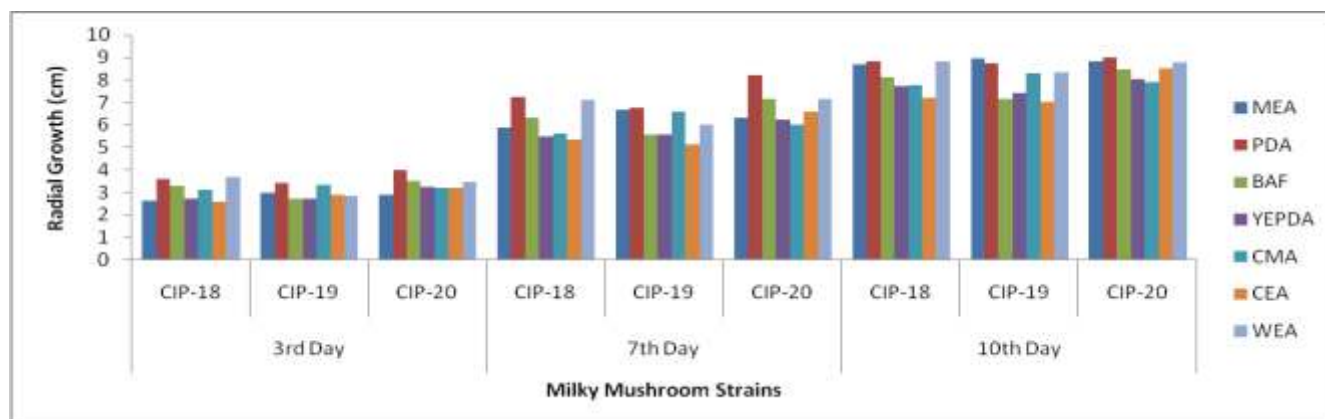


Fig. 1: Effect of different culture media on mycelial growth of milky mushroom (*Calocybe indica*) strains

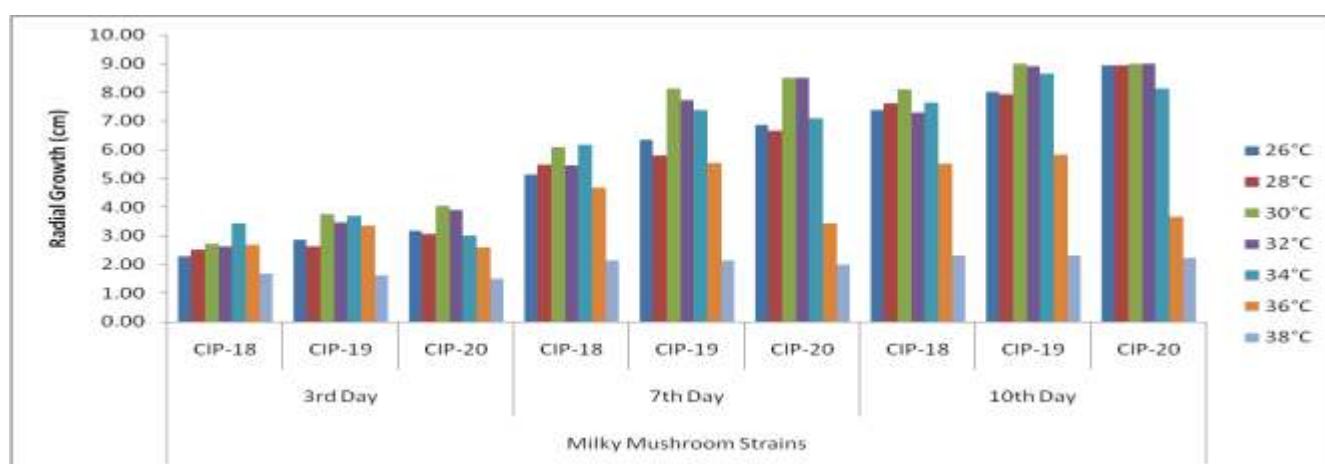


Fig. 2: Effect of different Temperature on mycelial growth of milky mushroom (*Calocybe indica*) strains

Table 1: Effect of different culture media on mycelial growth of milky mushroom (*Calocybe indica*) strains

Strain/ Days	Radial Growth (cm)								
	3 rd Day			7 th Day			10 th Day		
Media	CIP-18	CIP-19	CIP-20	CIP-18	CIP-19	CIP-20	CIP-18	CIP-19	CIP-20
MEA	2.63±0.20 ^d	3±0.43 ^{abc}	2.9±0.20 ^c	5.87±0.20 ^{bc}	6.67±0.64 ^{ab}	6.33±0.25 ^c	8.7±0.10 ^{ab}	8.93±0.12 ^a	8.83±0.15 ^a
PDA	3.6±0.2 ^{ab}	3.43±0.20 ^a	4±0.20 ^a	7.23±0.42 ^a	6.77±0.45 ^a	8.2±0.46 ^a	8.83±0.15 ^a	8.73±0.31 ^{ab}	9±0.00 ^a
BAF	3.27±0.40 ^{abc}	2.67±0.15 ^c	3.53±0.30 ^{ab}	6.33±0.40 ^b	5.57±0.25 ^{cd}	7.13±0.25 ^b	8.13±0.50 ^{bc}	7.17±0.32 ^c	8.47±0.25 ^{ab}
YEPDA	2.73±0.11 ^{cd}	2.73±0.15 ^c	3.23±0.45 ^{bc}	5.47±0.20 ^c	5.57±0.25 ^{cd}	6.23±0.55 ^c	7.73±0.45 ^{cd}	7.43±0.42 ^c	8.03±0.71 ^b
CMA	3.1±0.6 ^{bcd}	3.3±0.26 ^{ab}	3.3±0.26 ^{bc}	5.63±0.45 ^c	6.6±0.56 ^{ab}	6±0.30 ^c	7.77±0.45 ^{cd}	8.27±0.40 ^b	7.9±0.40 ^b
CEA	2.57±0.15 ^d	2.9±0.20 ^{bc}	3.2±0.40 ^{bc}	5.37±0.30 ^c	5.13±0.15 ^d	6.57±0.15 ^{bc}	7.2±0.36 ^d	7.03±0.25 ^c	8.5±0.20 ^{ab}
WEA	3.67±0.20 ^a	2.83±0.25 ^c	3.47±0.49 ^{abc}	7.1±0.40 ^a	6±0.26 ^{bc}	7.17±0.45 ^b	8.83±0.29 ^a	8.33±0.25 ^b	8.77±0.32 ^a
SE(m)	0.181	0.174	0.208	0.205	0.234	0.213	0.208	0.179	0.206
CD (5%)	0.549	0.446	0.610	0.621	0.709	0.647	0.630	0.543	0.626

Table 2: Effect of different Temperature on mycelial growth of milky mushroom (*Calocybe indica*) strains

Strain/ Days	Radial Growth (cm)								
	3 rd Day			7 th Day			10 th Day		
Temp.	CIP-18	CIP-19	CIP-20	CIP-18	CIP-19	CIP-20	CIP-18	CIP-19	CIP-20
26°C	2.26±0.51 ^{bc}	2.86±0.41 ^{bc}	3.16±0.32 ^{bc}	5.13±1.34 ^a	6.33±0.89 ^{bc}	6.86±0.15 ^b	7.36±1.72 ^a	8±0.87 ^a	8.93±0.11 ^a
28°C	2.5±0.26 ^{bc}	2.63±0.55 ^c	3.06±0.20 ^c	5.46±0.15 ^a	5.8±1.99 ^c	6.66±0.15 ^b	7.6±0.3 ^a	7.93±1.67 ^a	8.93±0.11 ^a
30°C	2.7±0.52 ^{ab}	3.73±0.15 ^a	4.03±0.11 ^a	6.06±0.85 ^a	8.13±0.25 ^a	8.5±0.1 ^a	8.1±0.88 ^a	9±0.1 ^a	9±0 ^a
32°C	2.63±0.75 ^{ab}	3.46±0.05 ^a	3.9±0.26 ^{ab}	5.43±1.80 ^a	7.73±0.25 ^{ab}	8.5±0.1 ^a	7.3±1.49 ^a	8.9±0.17 ^a	9±0 ^a
34°C	3.43±0.11 ^a	3.7±0.1 ^a	3±0.06 ^c	6.16±0.23 ^a	7.36±0.37 ^{ab}	7.1±2.25 ^{ab}	7.63±0.25 ^a	8.63±0.32 ^a	8.13±1.50 ^a
36°C	2.66±0.80 ^{ab}	3.33±0.28 ^{ab}	2.6±0.8 ^c	4.66±0.80 ^a	5.53±0.15 ^c	3.43±0.83 ^c	5.5±0.6 ^b	5.83±0.32 ^b	3.66±1.10 ^b
38°C	1.66±0.15 ^c	1.6±0.1 ^d	1.5±0.1 ^d	2.13±0.15 ^b	2.13±0.15 ^d	2±0.1 ^c	2.3±0.26 ^c	2.3±0.17 ^c	2.23±0.11 ^c
SE(m)	0.144	0.108	0.130	0.183	0.110	0.182	0.176	0.117	0.136
CD (5%)	0.904	0.516	0.737	1.689	1.496	1.600	1.696	1.297	1.241