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Standardizing the Media and *In Vitro* Growth Conditions for *Macrocybe gigantea*

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Abstract

Macrocybe gigantea (Callistosporiaceae) is a large, edible mushroom of nutritional and economic significance, valued for its protein, fibre, vitamins, polysaccharides, and essential minerals. Despite its potential for commercialization, optimized cultural conditions for its large-scale production remain under explored. In this study, a pure culture of *Macrocybe gigantea* isolated from Thrissur, Kerala, was molecularly characterized (GenBank accession number: PX068171) and evaluated *in vitro* for vegetative growth under different conditions. Among the different media tested, sorghum potato dextrose supported maximum mycelial growth in both solid and liquid forms. Sucrose proved to be the most efficient carbon source. The fungus exhibited optimum growth at 30 ± 2 °C and pH 5.0, under both dark and room light regimes. These results provide baseline information on the nutritional and environmental requirements of *Macrocybe gigantea*, offering a framework for its commercial cultivation and contributing to its sustainable utilization as a nutritionally rich food source.

***Key words*:** *Macrocybe gigantea*, Culture media, pH, Temperature, Light

The genus *Macrocybe* [1], belonging to the family Callistosporiaceae (Basidiomycota), comprises large, edible, fleshy mushrooms predominantly found in tropical and subtropical regions. *Macrocybe gigantea* was first reported from West Bengal [1], where it is locally known as "Boro dhoodh chattu" due to its distinctive dehydrated milk-like aroma [2]. Initially placed under the genus *Tricholoma*, it was later separated into *Macrocybe* based on distinct morphological and molecular characteristics, with phylogenetic studies revealing its close affinity to *Calocybe* and clear separation from *Tricholoma* through features such as the absence of siderophilous granulations, saprophytic growth, and clamped hyphae [3-4]. In natural habitats, *Macrocybe gigantea* occurs in large caespitose clusters and is consumed as a seasonal delicacy in several Asian countries. Its large size, palatability, extended shelf life, and nutritional richness particularly in protein, dietary fibre, vitamins, polysaccharides, and essential minerals like calcium, magnesium, and zinc make it a promising candidate for commercial cultivation [5-6].

Macrocybe gigantea, a tropical edible mushroom, has attracted considerable attention owing to its unique combination of nutritional richness, palatability, and market potential. It is a rich source of proteins, essential amino acids, vitamins, minerals, and bioactive compounds, making it highly valuable as a functional food with both dietary and therapeutic benefits. Additionally, it offers a viable livelihood option for farmers and entrepreneurs in regions where mushroom cultivation is expanding as an agribusiness. However, unlike well-studied mushrooms such as *Pleurotus* or *Lentinula*, standardized protocols for the in vitro cultivation of *M. gigantea* remain underdeveloped. The species exhibits specific growth requirements in terms of media composition, pH balance, temperature range, light exposure, and substrate compatibility, which directly influence its mycelial vigor, fruiting efficiency, yield, and overall quality. Inconsistent or suboptimal conditions not only limit productivity but also raise costs and reduce the commercial viability of large-scale operations. Therefore, scientific optimization of cultural parameters—ranging from laboratory-level mycelial growth to spawn development and substrate colonization—is essential. Establishing reproducible and cost-effective protocols will pave the way for reliable mass cultivation, ensure year-round availability, and enhance its market competitiveness. Ultimately, optimizing growth conditions for *Macrocybe gigantea* is not just a biological necessity but also an economic imperative for promoting food security, rural income generation, and sustainable mushroom-based industries.

Given its economic and nutritional significance, optimizing cultural conditions for *Macrocybe gigantea* is critical to support large-scale production and commercialization. Routine media like potato dextrose agar often fails to support fast growth of *Macrocybe gigantea* unless supported with suitable supplementation and maintenance of optimal growing conditions. In this context, the present study was undertaken to evaluate the influence of different solid and liquid media, carbon sources, pH levels, temperature, and light regimes in order to determine the optimum conditions for promoting vegetative growth of *Macrocybe gigantea* in vitro.

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MATERIALS AND METHODS

The present study was carried out using a pure culture of *Macrocybe gigantea* isolated from the Thrissur district of Kerala, India. The fungal isolate was molecularly characterized through ITS sequencing and confirmed as *Macrocybe gigantea* (GenBank accession number: PX068171). All experiments were conducted in the Department of Plant Pathology, College of Agriculture, Vellanikkara, Kerala Agricultural University, Kerala, India.

Effect of different solid and liquid media for culturing Macrocybe gigantea in vitro

To evaluate the effectiveness of different media in supporting the mycelial growth of *Macrocybe gigantea*, five different media viz., potato dextrose, malt extract, oatmeal, sorghum potato dextrose, and czapek-dox were tested in both solid and liquid forms. For optimization of solid media, a 5 mm disc from a 15 days old fungal culture was aseptically placed at the Centre of each Petri plates with solidified media, which was then sealed and incubated at room temperature. Radial mycelial growth (in cm) was measured daily and observations were taken until complete growth was achieved in all the plates. In case of liquid media, 150 ml of sterilized broth in 250 ml flasks was inoculated with four 5 mm culture discs and incubated at room temperature for 21 days. After incubation, the mycelial mats were filtered using Whatman No. 1 filter paper and dried in a hot air oven at 60 °C for 10–13 hours until a constant weight was achieved, and the final dry weight was recorded to determine the most suitable liquid medium for *Macrocybe gigantea*. Best medium was selected based on radial growth of mycelia per day in cm and dry weight of mycelial mat in 150 ml broth.

Effect of carbon sources on mycelial growth of M. gigantea

Sorghum potato dextrose medium served as the basal medium to evaluate the most suitable carbon source for the growth of *Macrocybe gigantea*. Various carbon sources like lactose, mannitol, maltose and sucrose were individually substituted in place of dextrose in the medium. These modified media were tested under both solid and liquid culture conditions.

Effect of pH on mycelial growth of M. gigantea

Sorghum potato sucrose medium was used to assess the impact of pH on the growth of *M. gigantea*, with the pH adjusted to five levels viz., 4, 5, 6, 7, and 8 using hydrochloric acid (HCl) and sodium hydroxide (NaOH) prior to sterilization. Mycelial growth was evaluated in both solid and liquid cultures.

Effect of temperature on mycelial growth of M. gigantea

Sorghum potato sucrose medium adjusted to pH 5 was used to study the effect of temperature on the mycelial growth of *M. gigantea.* Inoculated Petri plates and conical flasks were incubated at different temperatures (4°C, 24°C, 26°C, 28°C, 30°C, 32°C, and 34°C) using incubators with thermostat control to maintain the desired temperature.

Effect of light on mycelial growth of M. gigantea

Sorghum potato sucrose medium adjusted to pH 5 was used to standardize the light conditions for *M. gigantea*. Five different treatments were imposed by incubating the inoculated culture plates and conical flasks under the following conditions: room light, alternating light and dark cycles, white light, blue light, and complete darkness.

Statistical analysis

Statistical analysis was carried out using a Completely Randomized Design (CRD). All analyses were performed with KAU GRAPES software version 1.1.0 [7], and the critical difference (C.D.) was calculated at the 5% significance level.

RESULTS AND DISCUSSION

Understanding the nutritional and environmental requirements of *Macrocybe gigantea* is crucial for optimizing its cultivation, as these factors directly influence mycelial growth, biomass production, and overall yield potential. In this investigation, the effects of different culture media, carbon sources, temperature, pH, and light regimes on the vegetative growth of *M. gigantea* were assessed. The findings are presented and discussed below in relation to earlier reports.

Effect of different solid and liquid media for culturing M. gigantea in vitro

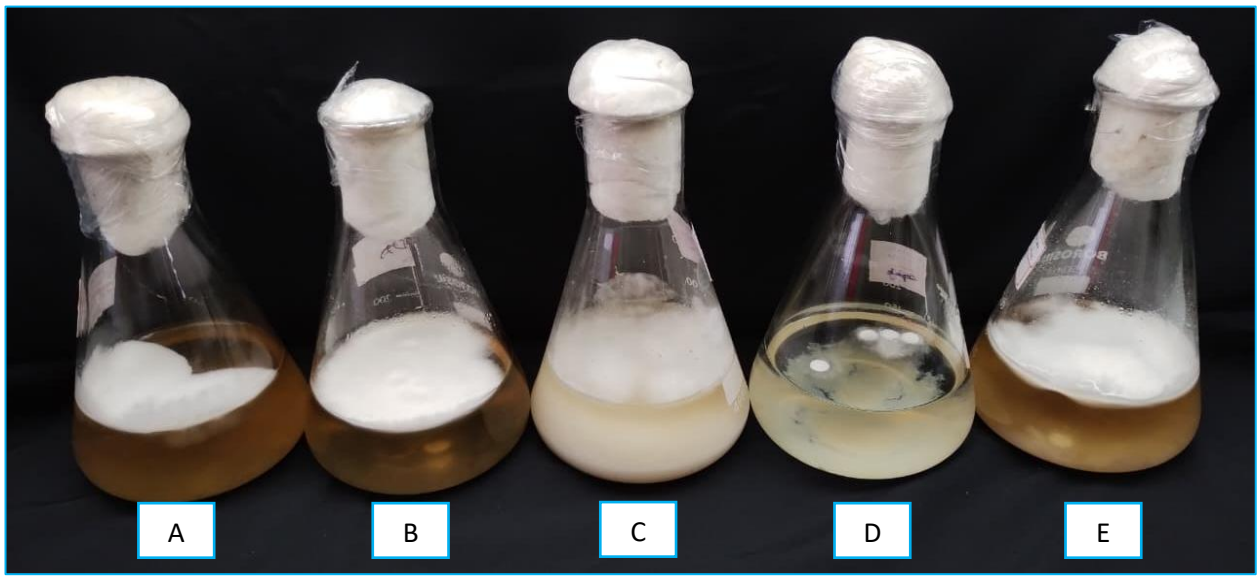
Sorghum potato dextrose medium supported the highest mycelial growth, recording 0.455 cm per day on solid medium with the development of a thick, white mycelial mat, and producing 1.5 g/150 ml biomass in liquid culture. This was closely followed by oat meal agar (0.452 cm/day), which also favoured good radial growth (Table 1, Fig 2). In liquid culture, malt extract broth performed next best after sorghum potato dextrose, yielding 1.25 g/150 ml (Fig 1). Potato dextrose medium supported relatively lower growth with 0.405 cm per day on solid medium and 1 g/150 ml in liquid culture. These results highlight the ability of sorghum-based medium to support both extensive colony growth and higher biomass yield compared to other media tested. Based on these findings, sorghum potato dextrose was selected for further studies.

Table 1 Effect of culture media on mycelial growth of *Macrocybe gigantea*

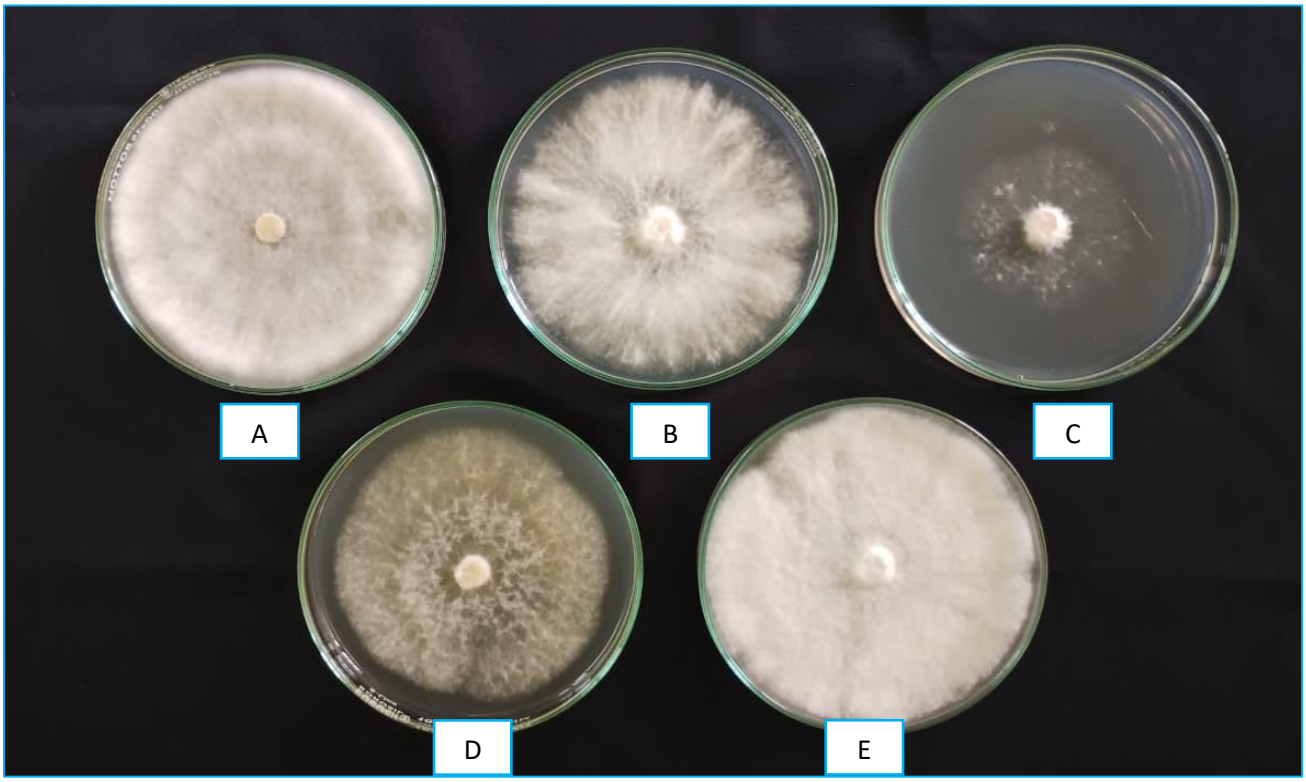
Media	Solid media	Liquid media
	Radial growth of mycelia per day (cm)	Dry weight of mycelial mat in 150 ml of broth (g)
Sorghum potato dextrose	0.455[a]	1.500 [a]
Oats meal	0.452[a]	1.000 [a]
Potato dextrose	0.405[b]	1.000 [a]
Malt extract	0.391[b]	1.250 [a]
Czapeck dox	0.235[c]	0.147 [b]
CD (0.05)	0.04	0.539

The superior performance of sorghum potato dextrose, along with the enhanced growth observed on PDA supplemented with sorghum powder, suggests that sorghum provides additional nutrients that promote vigorous growth of *Macrocybe gigantea*. This agrees with earlier reports highlighting the influence of nutrient-rich substrates on the mycelial development of *Macrocybe* spp. For instance, Inyod *et al.* [8] reported rapid growth of *M. crassa* on MEA at alkaline pH, while Roy and Krishnappa [9] and Suman *et al*. [10] noted PDA and MEA as highly supportive for *M. gigantea*

colonization. Similarly, Ghafoor *et al*. [11] and Kaur *et al*. [12] emphasized the effectiveness of PDA and MEA in promoting dense and rapid growth. The present findings not only corroborate these observations but also indicate that supplementing conventional media with sorghum can further enhance growth, making sorghum potato dextrose a promising medium for both radial extension and biomass production in *Macrocybe gigantea.*



Note: A (Malt Extract), B (Potato Dextrose), C (Oats Meal), D (Czapek Dox) and E (Sorghum Potato Dextrose)
Fig 1 Mycelial growth of *M. gigantea* in different liquid media



Note: A (Oats Meal), B (Potato Dextrose), C (Czapek Dox), D (Malt Extract) and E (Sorghum Potato Dextrose)
Fig 2 Mycelial growth of *M. gigantea* in different solid media

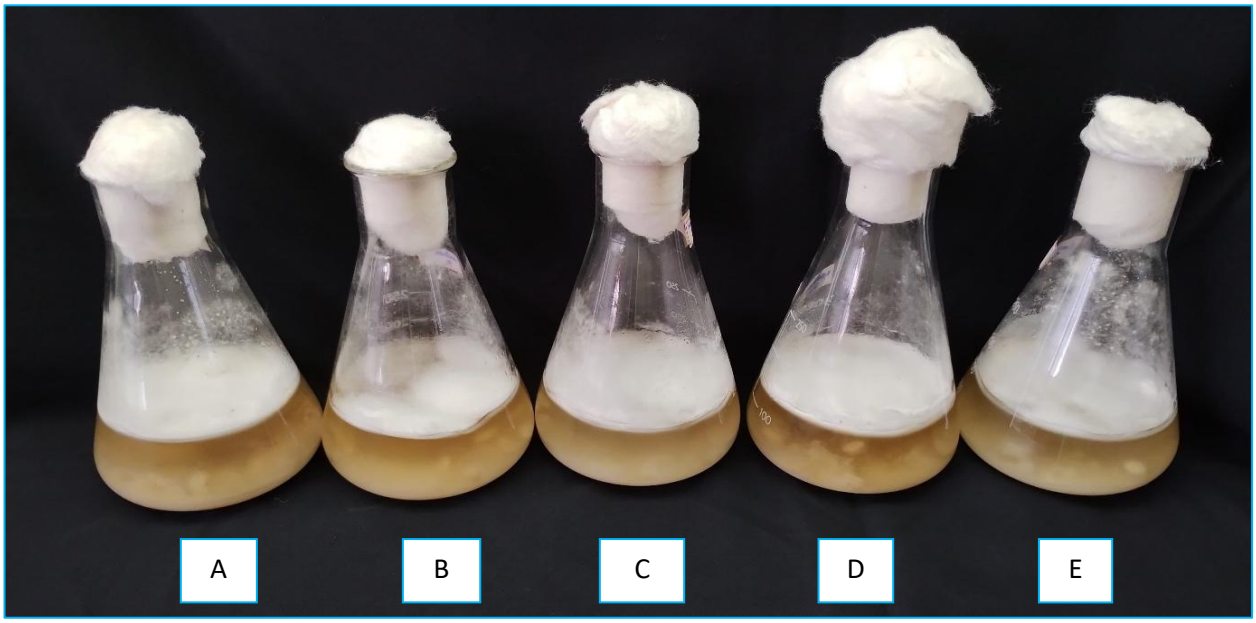
Effect of carbon sources on mycelial growth of M. gigantea

Sucrose proved to be the most effective carbon source, supporting both rapid radial growth (0.439 cm per day) and the highest biomass production (1.31 g/150 ml), followed by dextrose (0.389 cm/day). Interestingly, maltose, though less effective for radial extension, supported comparatively higher biomass yield in liquid culture (1.25 g/150 ml) after sucrose (Table 2, Fig 3-5). These results emphasize that the type of carbon source plays a crucial role in regulating both colony expansion and biomass accumulation in *M. gigantea*. Similar findings have been reported in related species, where carbon availability significantly influenced growth performance. Kinjo and Miyagi [13] observed that soluble starch supported the maximum mycelial growth of *Tricholoma giganteum*, followed by mannose, while Prathibha [14] noted that mannitol, dextrose, and sucrose were equally effective in supporting complete radial growth of *T. giganteum* within 14 days. Collectively, the results highlight sucrose as an efficient energy source, making it particularly suitable for promoting vigorous mycelial growth and biomass production in *M. gigantea*.

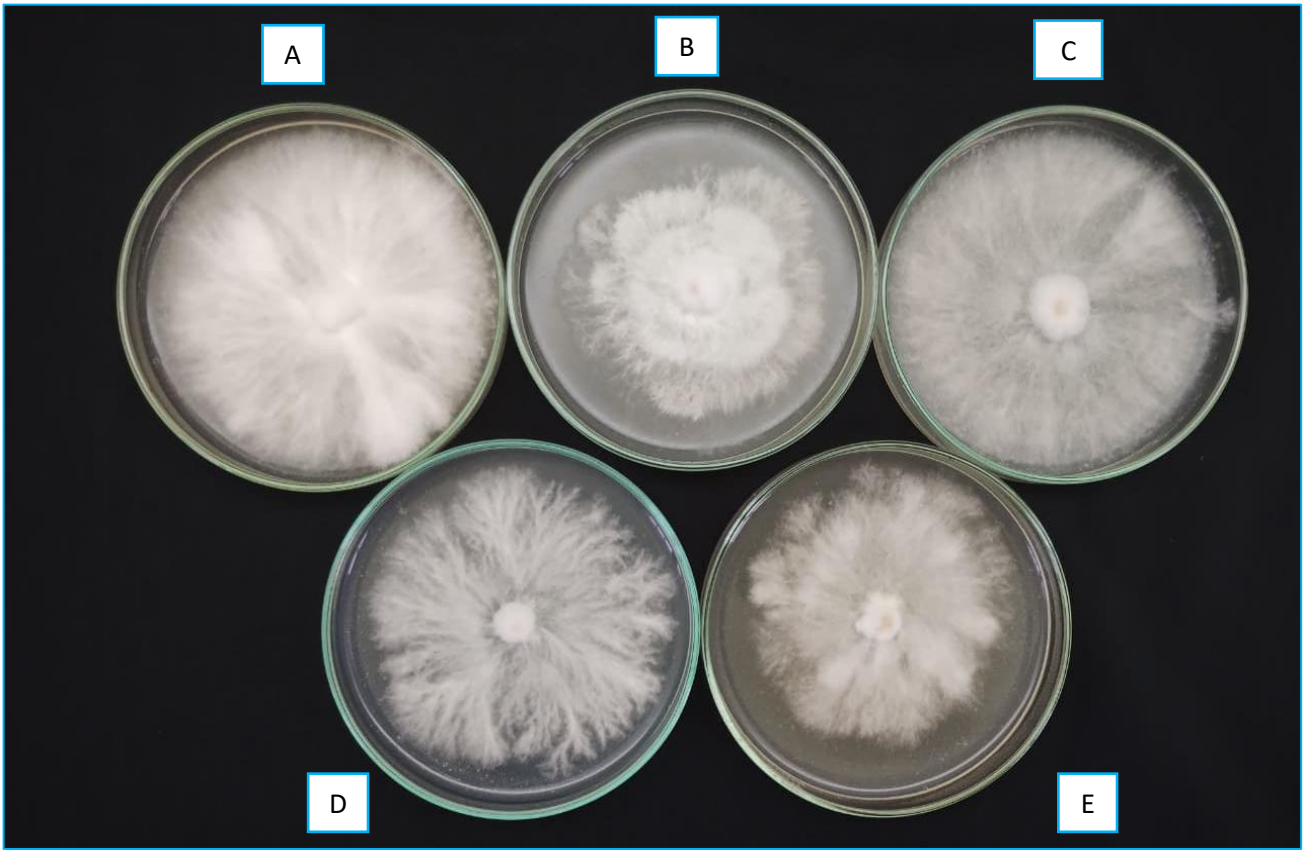
Table 2 Effect of carbon source on mycelial growth of *Macrocybe gigantea*

Carbon source	Solid media Radial growth of mycelia per day (cm)	Liquid media Dry weight of mycelial mat in 150 ml of broth (g)
Sucrose	0.439[a]	1.312
Dextrose	0.389[ab]	0.875
Maltose	0.349[bc]	1.250
Mannitol	0.321[c]	1.000
Lactose	0.300[c]	1.000
CD (0.05)	0.065	NS

NS - Not Significance



Note: A (Dextrose), B (Lactose), C (Mannitol), D (Maltose) and E (Sucrose)
Fig 3 Mycelial growth of *M. gigantea* in liquid media with different carbon sources



Note: A (Dextrose), B (Lactose), C (Sucrose), D (Mannitol) and E (Maltose)
Fig 4 Mycelial growth of *M. gigantea* in solid media with different carbon sources

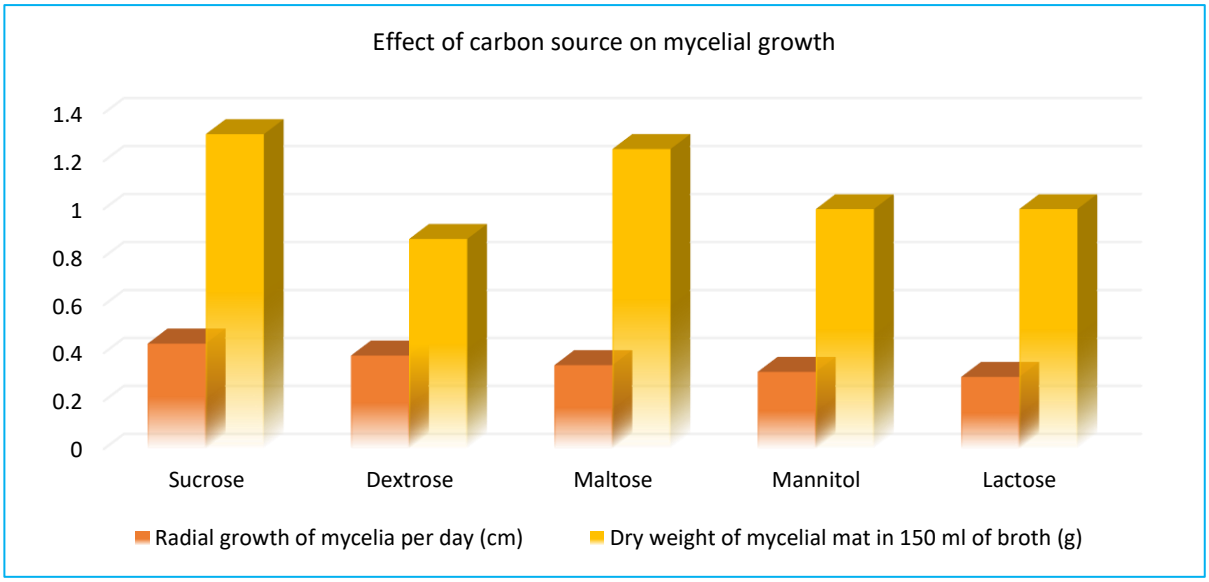


Fig 5 Effect of carbon sources on mycelial growth of *M. gigantea*

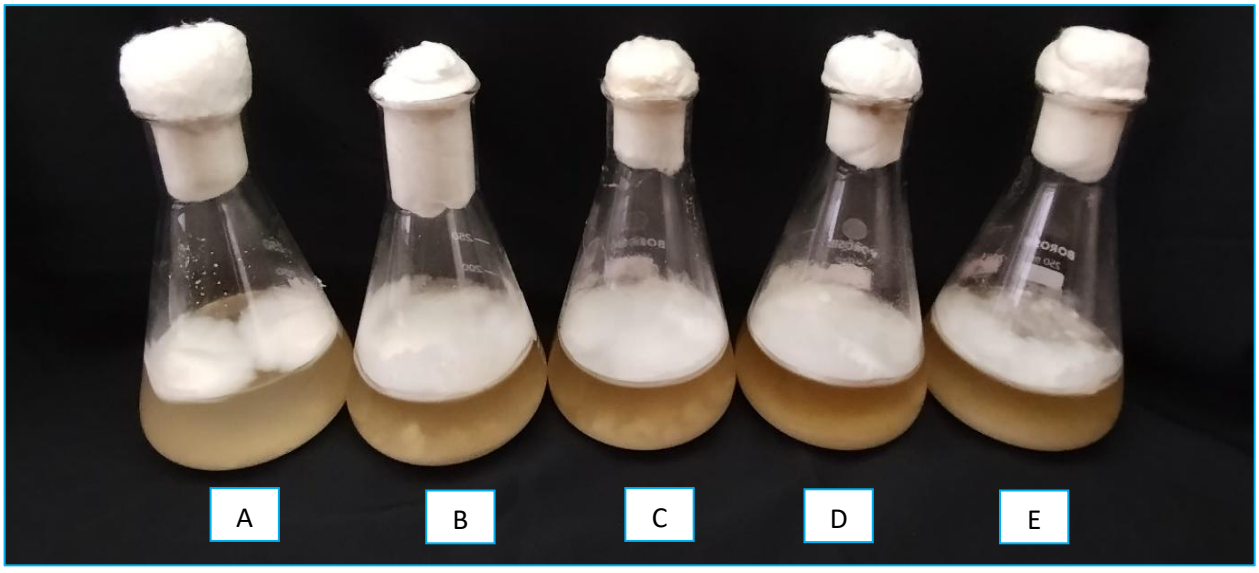
Effect of pH on mycelial growth of Macrocybe gigantea

Optimum mycelial growth was achieved at pH 5.0, with a growth rate of 0.44 cm per day on solid medium and a biomass yield of 1.25 g/150 ml in liquid culture (Fig 6-8, Table 3). Although pH 4 supported nearly similar radial extension, the biomass accumulation in liquid broth was considerably lower (0.75 g/150 ml). Interestingly, pH levels 5, 6, and 8 all supported comparable biomass yields of 1.25 g/150 ml (Table 3), suggesting that *Macrocybe gigantea* is capable of tolerating a relatively wide pH range, but its optimum growth is achieved in a mildly acidic environment. These observations are consistent with Roy and Krishnappa [9], who reported maximum growth of *Macrocybe gigantea* at pH 5.6 on PDA, MEA, and SDA, and with Kaur *et al*. [12], who observed the highest colony diameter and biomass production at pH 5.0 on MEA. However, some variation has been noted across studies, with Pamitha [15] reporting optimum growth at pH 7 and Inyod *et al*. [8] showing better performance of *Macrocybe crassa* at alkaline pH levels (8–10). Such differences may be attributed to species-specific responses or substrate composition, but collectively the findings emphasize that an acidic environment around pH 5 provides the most favourable conditions for vigorous mycelial growth of *Macrocybe gigantea*.

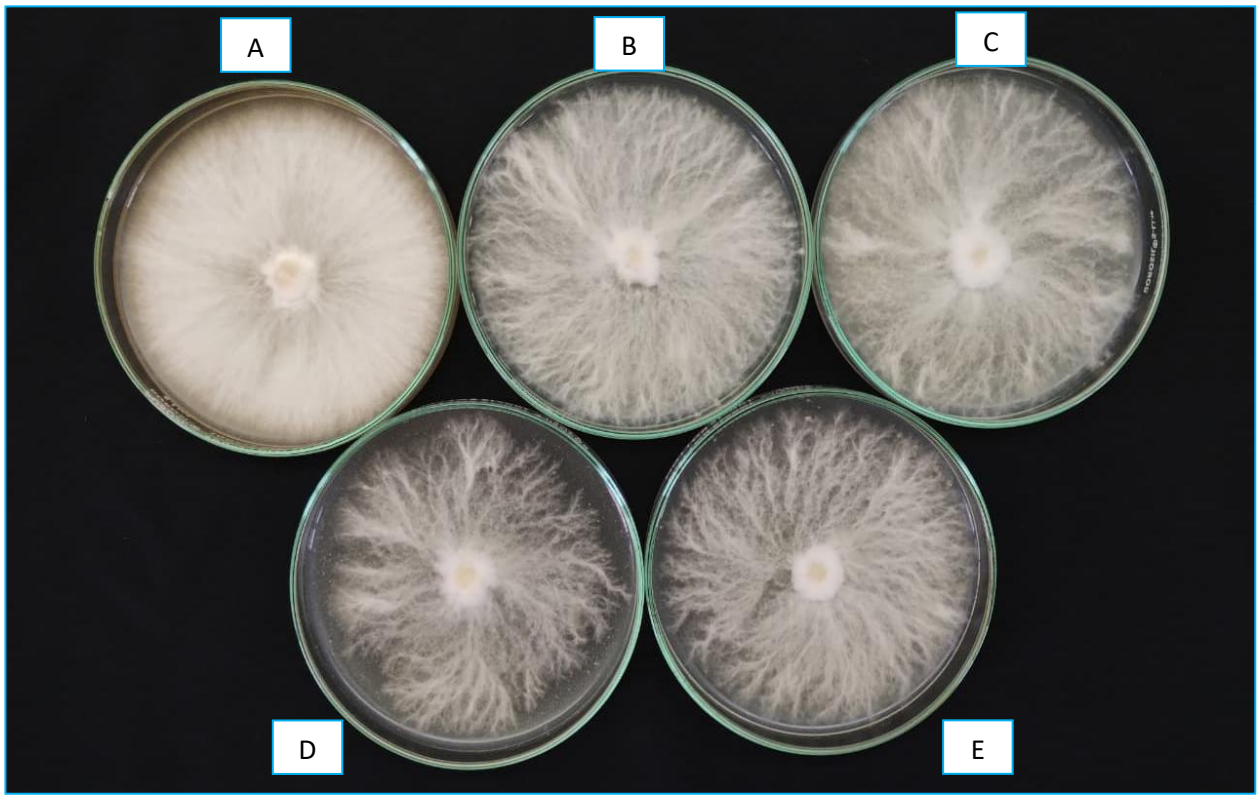
Table 3 Effect of pH on mycelial growth of *Macrocybe gigantea*

pH	Solid media Radial growth of mycelia per day (cm)	Liquid media Dry weight of mycelial mat in 150 ml of broth (g)
pH 4	0.440[a]	0.75
pH 5	0.440[a]	1.25
pH 6	0.424[ab]	1.25
pH 7	0.390[bc]	1.00
pH 8	0.376[c]	1.25
CD 0.05	0.041	NS

NS - Not Significance



Note: A (pH 4), B (pH 5), C (pH 6), D (pH 7) and E (pH 8)
Fig 6 Mycelial growth of *M. gigantea* in liquid media under different pH levels



Note: A (pH 4), B (pH 5), C (pH 6), D (pH 7) and E (pH 8)
Fig 7 Mycelial growth of *M. gigantea* in solid media under different pH levels

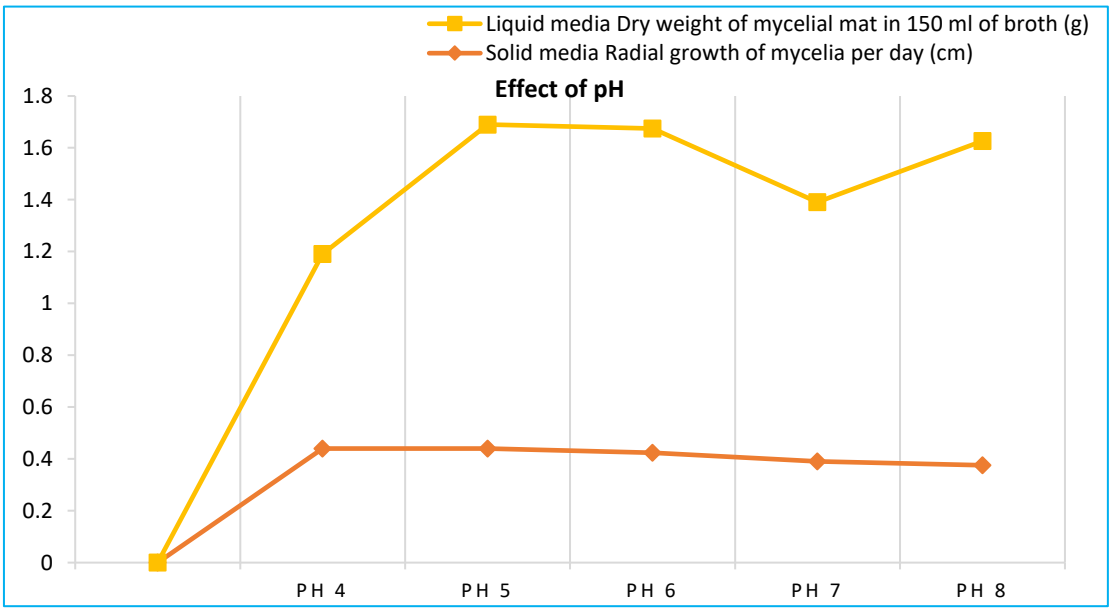


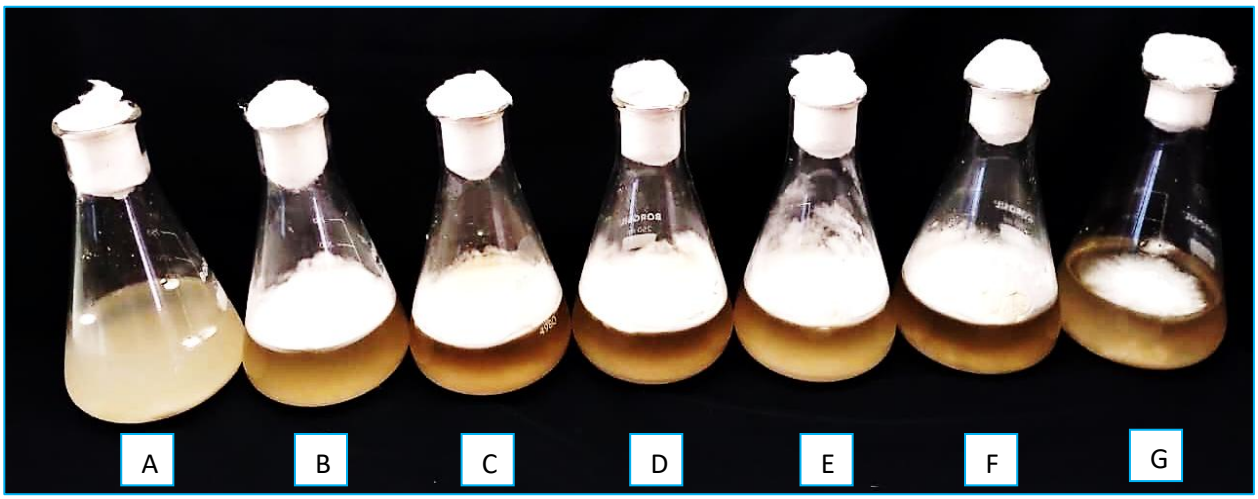
Fig 8 Effect of pH on mycelial growth of *M. gigantea*

Effect of temperature on mycelial growth of Macrocybe gigantea

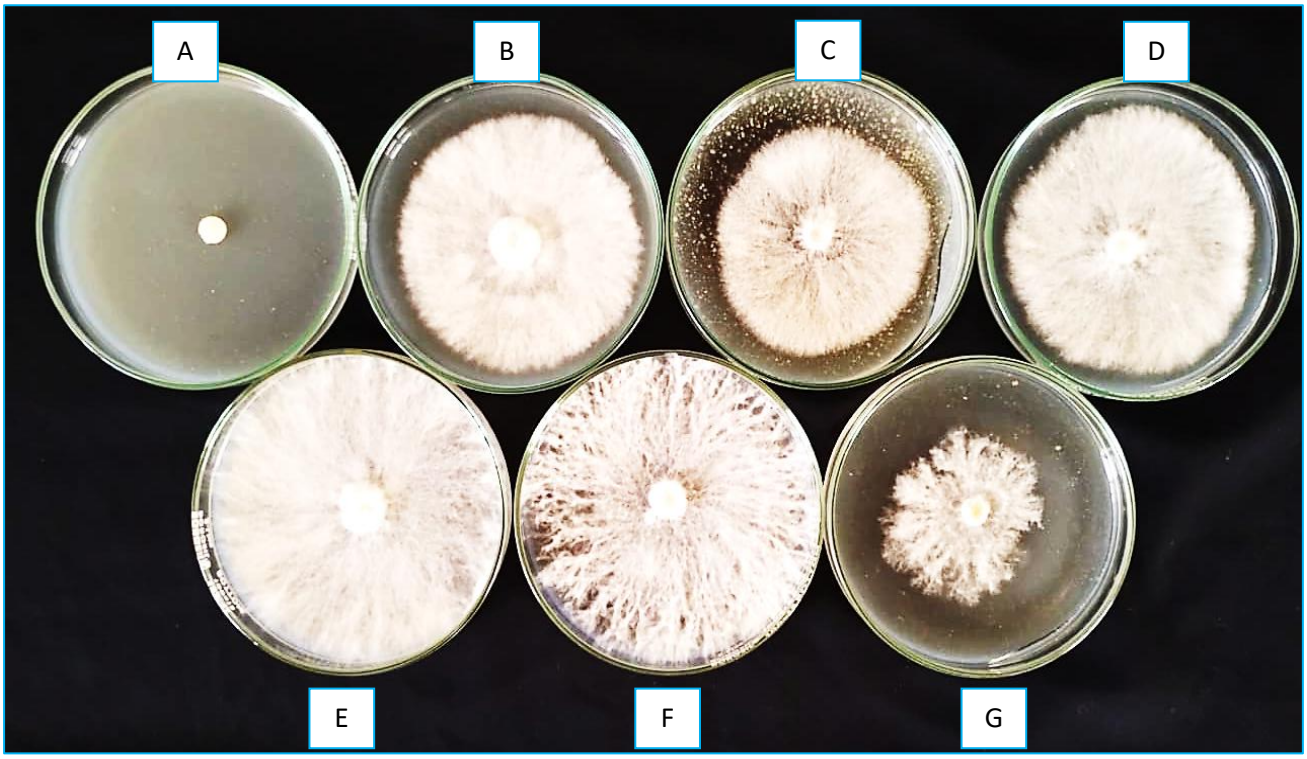
Temperature had a marked influence on mycelial growth, with 32 °C supporting the highest radial extension (0.430 cm per day) on solid medium and a biomass yield of 1 g/150 ml in liquid culture. A slightly lower growth rate (0.388 cm/day) was observed at 30 °C, while liquid cultures maintained at 24, 26, 30, 32, and 34 °C all produced similar biomass yields of 1 g/150 ml. Interestingly, at 28 °C, radial growth was restricted to 0.22 cm per day, yet biomass accumulation was comparatively higher (1.25 g/150 ml), indicating that temperature can differentially regulate colony expansion and biomass production in *M. gigantea* (Table 4, Fig 9-11). Comparable trends have been reported in earlier studies. Pamitha [15] observed maximum growth of *M. gigantea* at 35 °C on SDA medium, whereas Inyod *et al*. [8] found 30 °C most favourable for *M. crassa*, achieving 8.43 cm colony diameter within 15 days, with no growth recorded at 33 °C. Similarly, Ghafoor *et al*. [11] demonstrated that PDA at 30 °C supported the highest growth rate of *M. gigantea* (13.96 ± 0.033 mm/day), while Kaur *et al*. [12] reported optimum vegetative growth at 30 ± 2 °C, with 6.3 cm radial growth on solid medium and 6.43 mg/ml biomass in liquid culture. Taken together, these results suggest that while *M. gigantea* can tolerate a broad temperature range, with 30 ± 2 °C providing the most favourable balance for sustaining vigorous mycelial growth and biomass production.

Table 4 Effect of temperature on mycelial growth of *Macrocybe gigantea*

Temperatures	Solid media Radial growth of mycelia per day (cm)	Liquid media Dry weight of mycelial mat in 150 ml of broth (g)
4ºC	0.000[c]	0.00[b]
24ºC	0.360[a]	1.00[a]
26ºC	0.201[b]	1.00[a]
28ºC	0.222[b]	1.25[a]
30ºC	0.388[a]	1.00[a]
32ºC	0.430[a]	1.00[a]
34ºC	0.182[b]	1.00[a]
CD (0.05)	0.078	0.278



Note: A (4 °C), B (24 °C), C (26 °C), D (28 °C), E (30 °C), F (32 °C) and G (34 °C)
Fig 9 Mycelial growth of *M. gigantea* in liquid media under different temperatures



Note: A (4 °C), B (24 °C), C (26 °C), D (28 °C), E (30 °C), F (32 °C) and G (34 °C)
Fig 10 Mycelial growth of *M. gigantea* in solid media under different temperatures

Effect of light on mycelial growth of Macrocybe gigantea

Light conditions influenced the mycelial growth and morphology of *Macrocybe gigantea*. Cultures exposed to room light recorded the highest radial growth on solid medium (0.555 cm/day) with a biomass yield of 1 g/150 ml in liquid culture, closely followed by blue light (0.554 cm/day). Cultures incubated in darkness showed slightly lower radial extension (0.545 cm/day) but developed denser mycelial mats, while biomass production remained uniform (1 g/150 ml) across room light, white light, blue light, and dark conditions (Table 5, Fig 12-14). These results suggest that illumination influenced

colony morphology more than biomass accumulation. Similar variations in light response have been reported earlier: Suman *et al*. [10] observed that blue and yellow light favoured faster mycelial growth in *Macrocybe gigantea* strains on PDA, while Kaur *et al*. [12] reported superior growth under dark conditions compared to light on both solid and liquid media. Taken together, these findings indicate that although light quality can modulate growth dynamics, both room light and darkness provide favourable conditions for sustaining vigorous vegetative growth in *Macrocybe gigantea*.

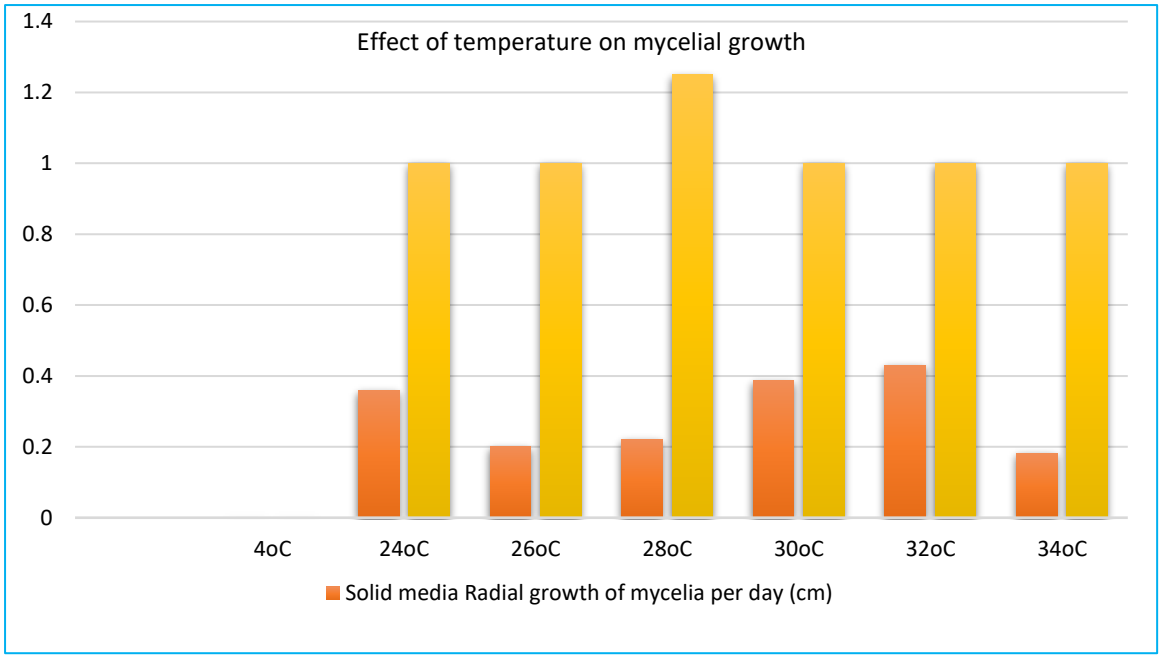
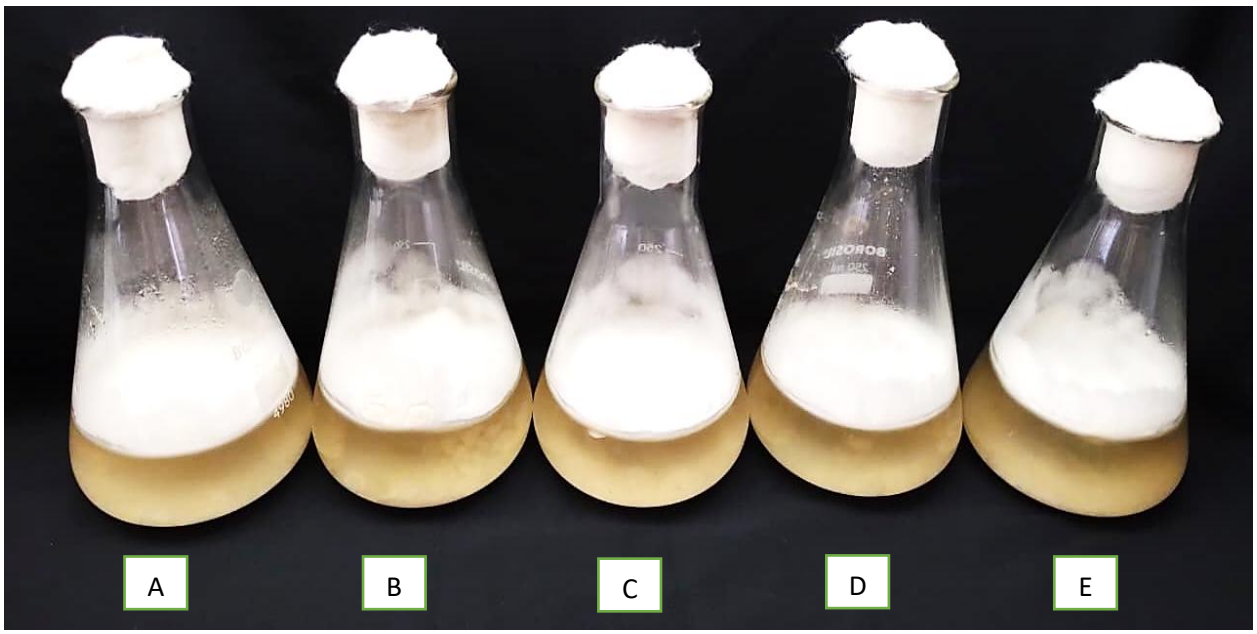


Fig 11 Effect of temperatures on mycelial growth of *M. gigantea*

Table 5 Effect of light on mycelial growth of *Macrocybe gigantea*

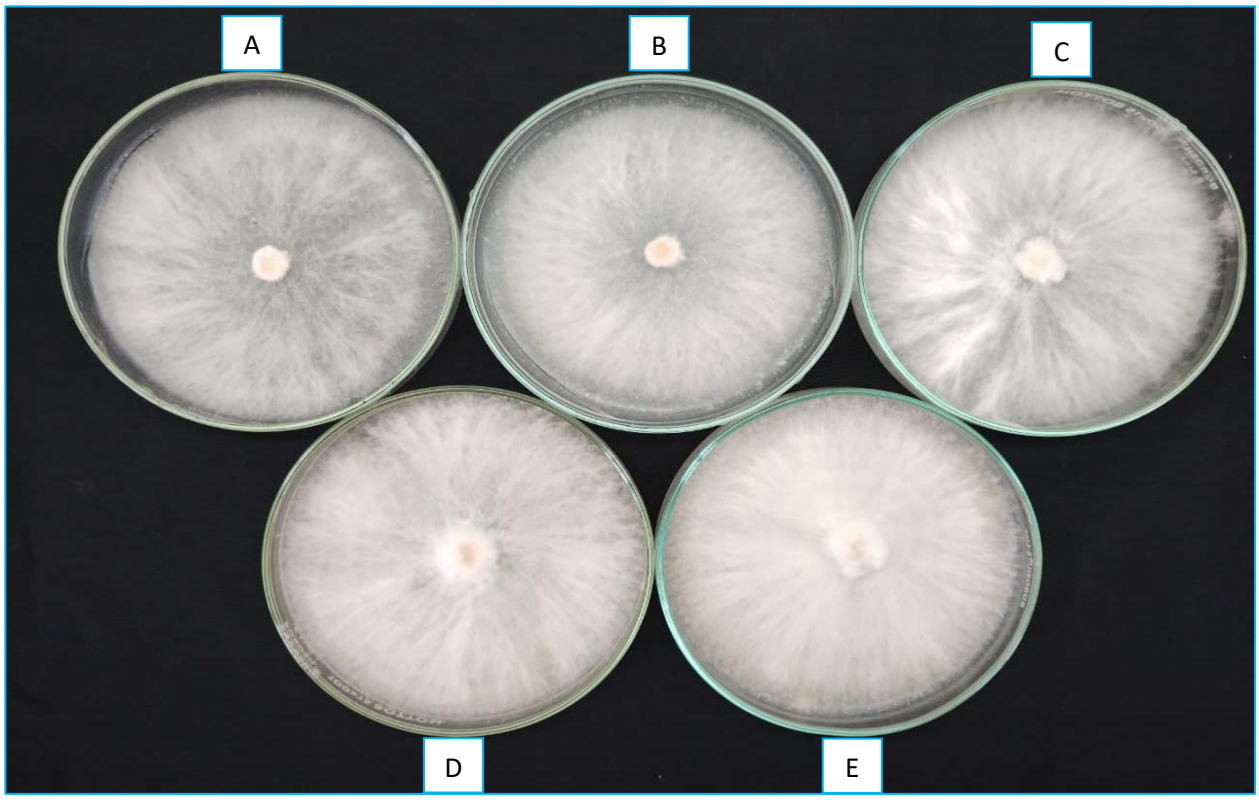
Light conditions	Solid media Radial growth of mycelia per day (cm)	Liquid media Dry weight of mycelial mat in 150 ml of broth (g)
Room light	0.555[a]	1.00
Alternate dark and light	0.434[c]	0.65
White light	0.488[b]	1.00
Blue light	0.554[a]	1.00
Dark	0.545[a]	1.00
CD (0.05)	0.039	NS

NS - Not Significance



Note: A (Room light), B (Blue light), C (White light), D (Alternate dark and light condition) and E (Dark)

Fig 12 Mycelial growth of *Macrocybe gigantea* in liquid media under different light conditions



Note: A (White light), B (Alternate dark and light condition), C (Blue light), D (Room light) and E (Dark)
Fig 13 Mycelial growth of *Macrocybe gigantea* in solid media under different light conditions

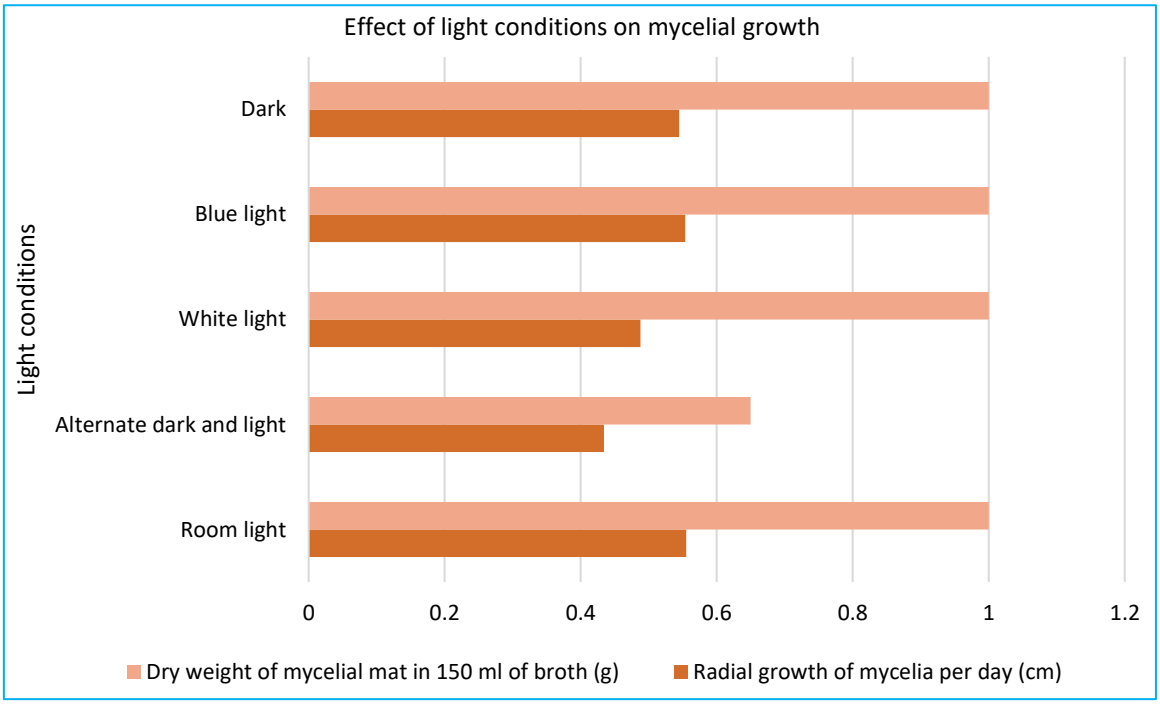


Fig 14 Effect of light conditions on mycelial growth of *M. gigantea*

CONCLUSION

The study revealed that sorghum potato dextrose medium, in both solid and liquid forms, was most effective in supporting the vegetative growth of *Macrocybe gigantea*. Among the carbon sources tested, sucrose proved to be the most efficient in promoting mycelial development. Optimum growth was achieved at 30 ± 2 °C and pH 5.0, with both dark and room light conditions equally supporting biomass production. These findings not only provide insights into the cultural requirements of *M. gigantea* but also establish baseline information that could facilitate its large-scale cultivation and commercial exploitation.

Acknowledgement

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