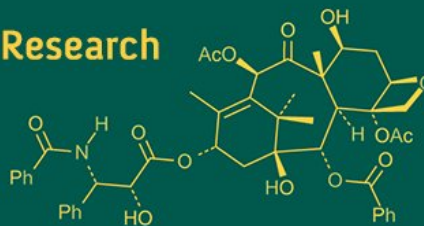


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Growth dynamics and morphological response of *Macrocybe gigantea* to different casing soil treatments.

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Abstract

Macrocybe gigantea is a large, edible tropical mushroom of considerable nutritional and commercial value, and the casing layer applied after spawn run is one of the key factors governing its growth and fruit body development. The present investigation was carried out at All India Coordinated Research Project on Mushroom, Govt. College of Agriculture, Pune (MPKV), to evaluate the effect of five casing soil formulations, viz., T₁ (Coir pith + Lime, 90:10), T₂ (Coir pith + Soil, 80:20), T₃ (Coir pith + SMS, 70:30), T₄ (Coir pith + Soil, 70:30) and T₅ (Coir pith + FYM, 80:20) as control, on the growth behaviour and fruit body characters of *Macrocybe gigantea* grown on wheat straw substrate under polythene bag cultivation. Among growth parameters, treatment T₄ (Coir pith + Soil, 70:30) recorded the fastest spawn run (15.94 days), earliest pinhead initiation (14.94 days) and quickest first harvest after pinhead formation (5.83 days), whereas T₁ (Coir pith + Lime) was the slowest for all three parameters (16.63, 17.06 and 8.58 days, respectively). With respect to fruit body characters, basidiocarps remained white to creamy white with a mild, milk-like fungoid odour across all treatments, while pileus diameter (5.01-7.37 cm), stipe length (8.82-12.48 cm) and stipe girth (6.96-9.82 cm) varied significantly, being maximum in T₄ and minimum in T₁. The findings indicate that coir pith enriched with soil in a 70:30 ratio (T₄) is the most favourable casing formulation for promoting rapid growth and superior fruit body development in *Macrocybe gigantea*, providing useful guidance for standardising casing practices for its commercial cultivation.

Keywords: *Macrocybe gigantea*, casing soil, growth parameters, mushroom, cultivation, Stipe.

Introduction

According to Chang and Miles (1992) ^[5], mushrooms are macro fungi characterized by distinctive fruiting bodies visible to the naked eye and are manually harvested. Historically, mushrooms have been gathered from natural habitats for both food and medicine, and are regarded as a culinary marvel and a forgotten source of nutrients likened to “white vegetables” (Feeney *et al.*, 2014; Raut and Adhikari, 2021) ^[1, 19]. Owing to their delicacy and flavour, mushrooms are also known as vegetable meat, and their thread-like mycelial structures allow efficient nutrient absorption, making mushroom cultivation an important income-generating venture in many countries. Mushrooms possess a wide range of medicinal benefits, including anti-inflammatory, antidiabetic, antiallergic, anticancer, hepatoprotective, antioxidant and antimicrobial effects (Jeitler *et al.*, 2020) ^[10], and have shown effectiveness against diabetes, high cholesterol, stress and cancer. Ancient civilizations valued mushrooms highly - the Greeks regarded them as a royal dish, the Egyptians as food of the Gods, and the Chinese as an elixir of life (Kotowaski, 2019). References to mushrooms are found in Ayurveda, the Vedas, and Islamic and Biblical texts alike (Adhikari, 1981; Wasson, 1971; Marwat *et al.*, 2009) ^[1, 21]. Purkayastha and Chandra (1974) ^[18] were the first to report *Calocybe indica* (morphologically resembling *Macrocybe gigantea*), a popular edible mushroom locally known as ‘Dudh chatta’ (milk umbrella) in West Bengal, while the giant mushroom itself is commonly referred to as ‘Boro Dudh Chattu’ or ‘Bada Dudh Chattu’. *Macrocybe gigantea* belongs to Kingdom Fungi, Phylum Basidiomycota, Class Agaricomycetes, Order Agaricales and Family Callistosporiaceae (Kui *et al.*, 2021; Kirk *et al.*, 2008), and was first documented in West Bengal (Pegler *et al.*, 1998) ^[17]. It is found throughout the tropical regions of Asia and Africa, is characterised by its pure white colour,

and is larger and more robust than both *Calocybe indica* and *Agaricus bisporus* (Akhtar *et al.*, 2018) [2]. The genus *Macrocybe*, segregated from *Tricholoma* on morphological and molecular grounds (Pegler *et al.*, 1998; Kui *et al.*, 2021) [17], comprises eight species, most of which are edible, with *M. gigantea* also valued for tolerating a high temperature range of 30-38°C (Amin *et al.*, 2010) [3]. *Macrocybe gigantea* is rich in carbohydrates, fats, proteins, amino acids and mineral elements, particularly Ca, Mg and Zn (Wang *et al.*, 2004; Liu *et al.*, 2012) [15], comprising 24.1% protein, 10.2% carbohydrate and 5.6% glycogen on a dry weight basis (Pamitha and Latha, 2014). It also possesses notable antioxidant, antibacterial and antitumour properties (Gaur and Rao, 2016; Mau *et al.*, 2002; Dai *et al.*, 2009; Giri *et al.*, 2012; Chatterjee *et al.*, 2011). By converting otherwise valueless agro-waste into an effective growing substrate, mushroom cultivation provides additional income and labour-intensive employment to farmers while making productive use of agricultural residues. *Macrocybe gigantea*, like other agaric mushrooms, requires a 'casing layer' once the mycelium has fully colonised the substrate. The casing layer, applied 14-16 days after spawning, harbours microorganisms that trigger the shift of the fungus from vegetative growth to the reproductive (fruiting) stage (Gülser and Pekşen, 2003; Pardo-Giménez *et al.*, 2020a), and is regarded as one of the key growing parameters influencing the productivity, quality and uniformity of commercial mushroom crops (Kaur and Rampal, 2017). Cocopeat is generally preferred as a casing base owing to its water-holding capacity, neutral pH and granular structure, which favour gaseous exchange and the release of hormone-like microbial substances that stimulate fruit body formation (Eger, 1972). Spent mushroom substrate can also be used as casing to lower production costs and reduce pollution (Pardo *et al.*, 2011). Effective casing materials should possess high water-holding capacity, porosity, bulk density and a good air-space ratio, and the quality of the casing material determines fruiting body initiation and regulates moisture, temperature and other physical, chemical, microbiological and nutritional factors that govern the suitability of a material for casing *Macrocybe gigantea*. Different casing soil formulations for *Macrocybe gigantea* cultivation were tested, using varying proportions of coir pith combined with lime, soil, SMS, and FYM. The treatments ranged from 70:30 to 90:10 ratios, with the control using coir pith and FYM in an 80:20 proportion. These formulations aimed to identify the optimal casing material for the mushroom growth.

Materials and Methods

The present investigation was carried out at the All India Coordinated Research Project (AICRP) on Mushroom, Govt. College of Agriculture, Pune (MPKV), Maharashtra, India during 2026. The materials and methodology used for evaluating the growth and fruit body characters of *Macrocybe gigantea* under different casing soil formulations are described below.

Procurement of culture of *Macrocybe gigantea*

The pure culture of *Macrocybe gigantea* was obtained from AICRP on Mushroom, Govt. College of Agriculture, Pune, and maintained on Potato Dextrose Agar (PDA) medium, with Malt Extract Agar (MEA) used for mass multiplication.

Preparation of spawn and spawning process: The bold seeded whole wheat grains were weighed and rinsed 2-3 times in tap water. The vessel was filled with water until the grains were just immersed. The grains were boiled in hot water for 25-30 minutes to prevent starch from escaping. After cooking, the grains were spread over a large wire gauze mesh to drain extra water. Overnight, the grains were maintained in a ventilated atmosphere. Based on the weight of these boiled grains, 4% calcium carbonate and calcium sulphate were mixed in the next morning to maintain pH to 6.5 to 7 and to prevent the gains from sticking together.

For mother spawn preparation, sterilized conical flask having wheat grain mixed with CaCO₃ were inoculated with small mycelial fragments of seven days old *Macrocybe gigantea* culture grown on test tube and quarter size bottle slants. The flasks were gently swirled to evenly distribute the inoculum, ensuring extensive contact between grains and mycelium. The flasks were then incubated at 25±1°C for approximately 10-12 days and the resulting spawn used as master spawn to prepare commercial spawn.

The mother spawn was inoculated into sterilized polypropylene bags containing autoclaved wheat grains under laminar airflow conditions. Aseptic procedures were meticulously followed throughout the process. Mother spawn was added to each polypropylene bag, which contained 500 grams of wet sterilized wheat grains using 4% spawn rate. After the inoculation, the bags were re-plugged and the contents were gently shaken to ensure even distribution of the inoculum throughout the substrate. These bags were then incubated for 12-15 days at 25 ± 1°C in an incubator. Once the commercial spawn substrate was fully colonized by the mycelium, the spawn was considered ready for use.

Preparation of substrate

Wheat straw was chopped into 2-3 cm pieces and chemically pasteurised by soaking in a solution of formalin (125 ml) and Bavistin (7.5 g) in 100 litres of water for 18 hours, after which excess water was drained.

Bed Preparation

Macrocybe gigantea was cultivated using the polythene bag method. Polythene bags of size 35x55 cm were disinfected with 5% formalin solution. Beds were prepared by the layer method of spawning at 4% on wet weight basis of straw. The necks of the bags were tightly tied with thread. About 35-40 pinholes were made all over the surface of the bags. Four replications for each casing treatment were kept for incubation (spawn run) in the incubation room.

Casing of substrate bags

Casing is a top dressing applied to the mycelium colonised substrate with soil or other casing material having a high water-holding capacity and a neutral pH (Amin *et al.*, 2010) [3]. Once the substrate was completely run, a layer of 2-4 cm casing material consisted of coir pith combined with lime, soil, SMS, and FYM. The treatments ranged from 70:30 to 90:10 ratios, with the control using coir pith and FYM in an 80:20 proportion. Before use, casing materials were sterilized in an autoclave at 15 lbs psi for 60 minutes to ensure pathogen free environment (Panda and Biswas, 2021). Spraying of water was done 1-2 times a day. The temperature was maintained in the range of 23-25°C and

relative humidity of more than 80 percent with the help of humidifier throughout the cropping period.

Harvesting of mushroom

Sporophores were harvested on maturation when caps became flat with incurved margins (Fig. 1 and Fig. 2). Harvesting of fruit bodies was done by gently twisting the stipes of the fruiting bodies both clockwise and anti-clockwise, which ensured that young sporophores growing nearby remain unaffected.

Fresh weight of mushroom

Biological efficiency (B. E.) = ----- X 100

Dry weight of substrate used

Treatment Details

T ₁	Coir pith + Lime	(90:10)
T ₂	Coir pith + Soil	(80:20)
T ₃	Coir pith + SMS	(70:30)
T ₄	Coir pith + Soil	(70:30)
T ₅	Control (Coir pith + FYM)	(80:20)

Results and Discussion

Fruit body observations of *Macrocybe gigantea* mushroom

4.1.1 Pileus diameter

The data on the effect of different casing materials on pileus diameter (Table 1, Fig.1, Plate 1) revealed values ranging from 5.01 to 7.37 cm. The longest pileus diameter was recorded in T₄ (Coir pith + Soil, 70:30) at 7.37 cm, followed closely by T₅ (Coir pith + FYM, 80:20) at 7.35 cm. Treatments T₃ (Coir pith+ SMS, 70:30) at 6.66 cm and T₂ (Coir pith + Soil, 80:20) at 6.07 cm produced intermediate diameters, while the smallest pileus diameter was recorded in T₁ (Coir pith + Lime, 90:10) at 5.01 cm. Therefore, Coir pith in 70:30 combination with Soil was highly suitable as a casing material. The present observations are concordant with earlier studies by Galappaththi *et al.*, (2022) ^[9], who reported that *Macrocybe gigantea* mushroom's pileus diameter ranged from 4 to 7 cm. However, Akhtar *et al.*, (2019) ^[2] observed morphological characteristics of four isolates of *Macrocybe gigantea* and recorded that the average pileus diameters range between 8.46 and 11.63 cm

Colour

No significant effect of different casing materials on the colour of *Macrocybe gigantea* basidiocarps was observed. Regardless of the casing type, the basidiocarps remained mostly white to creamy white, with the cap turning pale cream or buff upon maturity, while the gills and stipe stayed white to cream. The findings align with those of G Suresh

and R Sutha Raja Kumar (2022) ^[20] who reported that the pileus of *Tricholoma gigantea* was creamy white, with milky white gills.

Odour

The odour of *Macrocybe gigantea* was not influenced by the casing materials. Fresh basidiocarps across all treatments emitted a mild, pleasant, dehydrated milk-like, and characteristic fungoid (mushroom-like) scent, with no noticeable differences between casing types. As reported by Dutta and Acharya (2014) ^[8], *M. gigantea*, *M. lobayensis* and *M. crassa*, are locally known in India as 'boro dhoodh chhatu,' or 'dhoodh chhatu' meaning they emit a milk-like smell when dried.

Stipe length

The data (table 1, Fig 1 and Plate 2) highlighted that the longest stipe length of 12.48 cm was noted in the T₄ coir pith with soil (70:30) treatment, with the control treatment T₅ (Coir pith + FYM in 80: 20 ratio) ranking next measuring 12.46 cm and at par with T₄. The other treatments, T₃ coir pith with SMS (70:30) and T₂ coir pith with soil (80:20), produced intermediate stipe lengths of 10.9 cm and 10.31 cm, respectively. Conversely, the shortest average stipe length was recorded in the T₁ coir pith with lime (90:10) treatment, measuring 8.82 cm.

The findings of the present study are consistent with Akhtar *et al.*, (2019) ^[2] who reported that the average stipe length of four different isolates of *Macrocybe gigantea* ranged from 9.73 to 12.53 cm. while, Anurag Kerketta *et al.*, (2019) ^[12] reported that stalk length varied between 2.63 and 3.45 cm and remained statistically comparable across the casing materials i.e. cocopeat, vermicompost + cocopeat (1:1), vermicompost + soil (1:1), cocopeat + soil (1:1) vermicompost + cocopeat + soil (1:1:1) and soil + FYM + sand (1:1:1).

Stipe girth

The highest average stipe girth (Table 1, Fig. 1 and Plate 3.) of 9.82 cm was observed in T₄, closely followed by T₅ (9.40 cm), which were statistically at par. Treatments T₂ and T₃ produced stipe girths of 8.88 and 7.82 cm, respectively, while the lowest girth (6.96 cm) was recorded in T₁. The conclusion drawn by Galappaththi *et al.*, (2022) ^[9] states that the girth of *Macrocybe gigantea* mushroom ranges between 2.5 to 4. Mownika *et al.*, (2024) ^[16] described the cylindrical stipe of *Macrocybe gigantea* as about 6 cm in diameter, while Pegler & Lodge (1998) ^[17] stated the stipe is centrally positioned, solid, and measures 4 - 8 cm in thickness.

Table 1: Effect of casing soil on morphological characters of *Macrocybe gigantea* mushroom

Treatment No.	Treatment Details	Average Pileus diameter (cm)	Average stipe length (cm)	Average stipe girth (cm)
T ₁	Coir pith + Lime	5.01	8.82	6.96
T ₂	Coir pith + Soil	6.07	10.31	8.88
T ₃	Coir pith + SMS	6.66	10.9	7.82
T ₄	Coir pith + Soil	7.37	12.48	9.82
T ₅ (Control)	Coir pith + FYM	7.35	12.46	9.40
SE (m) ±	0.13	0.13	0.32	0.24
C.D. (0.01)	0.40	0.40	0.98	0.72

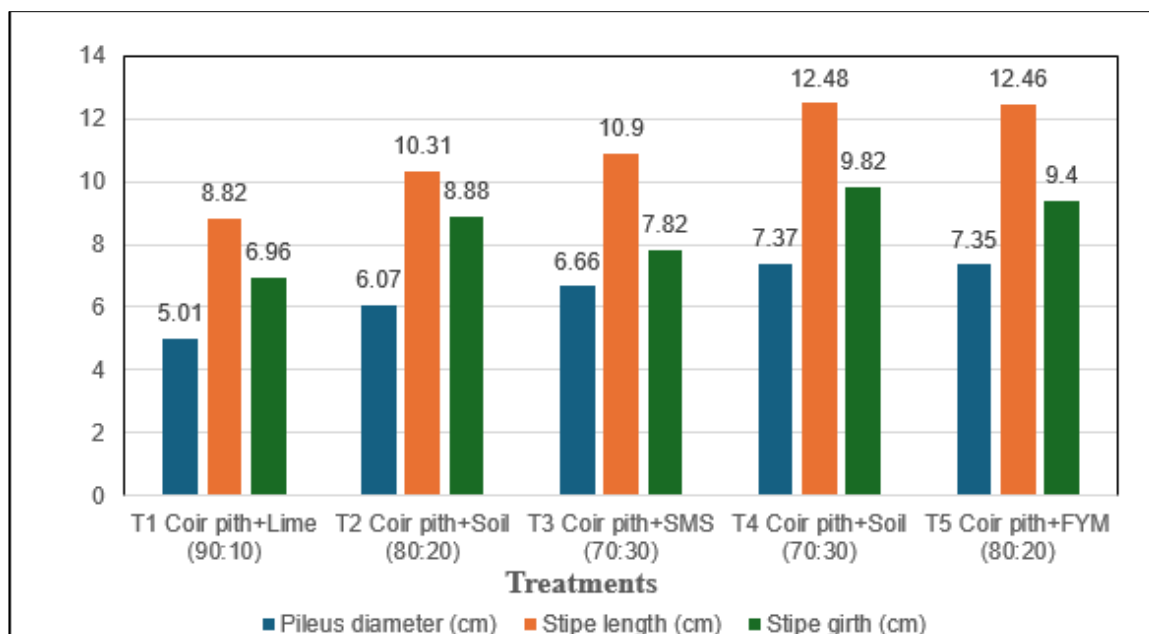


Fig 1: Morphological characters of *Macrocybe gigantea* on different casing materials

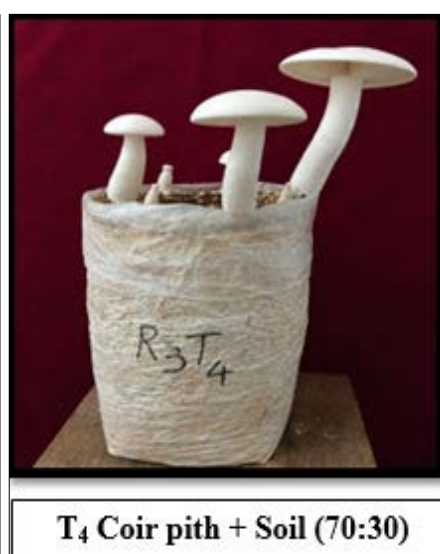


Plate 1: Pileus diameter of *Macrocybe gigantea* on different casing materials

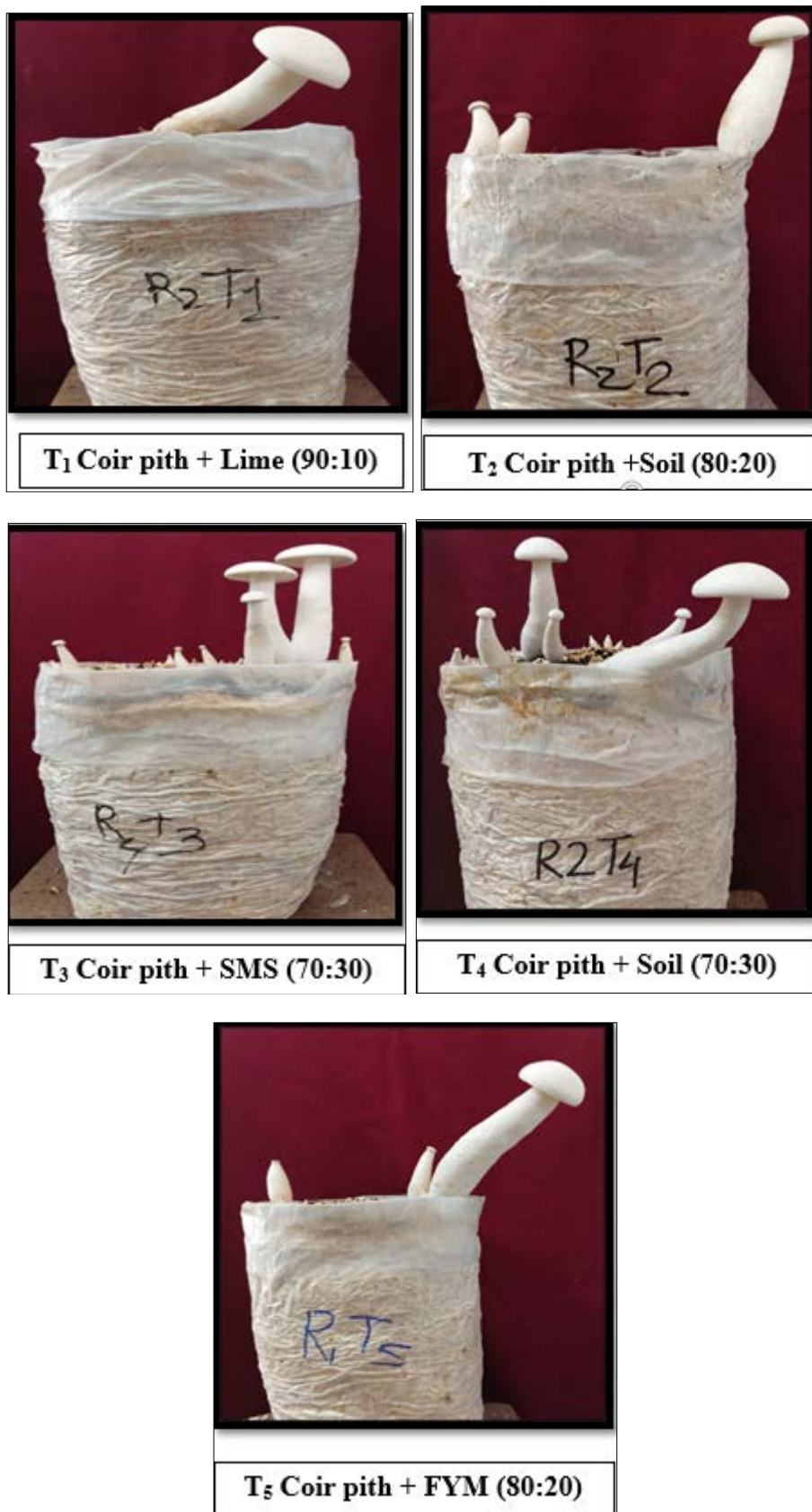


Plate 2: Stipe length of *Macrocybe gigantea* mushroom on different casing materials.

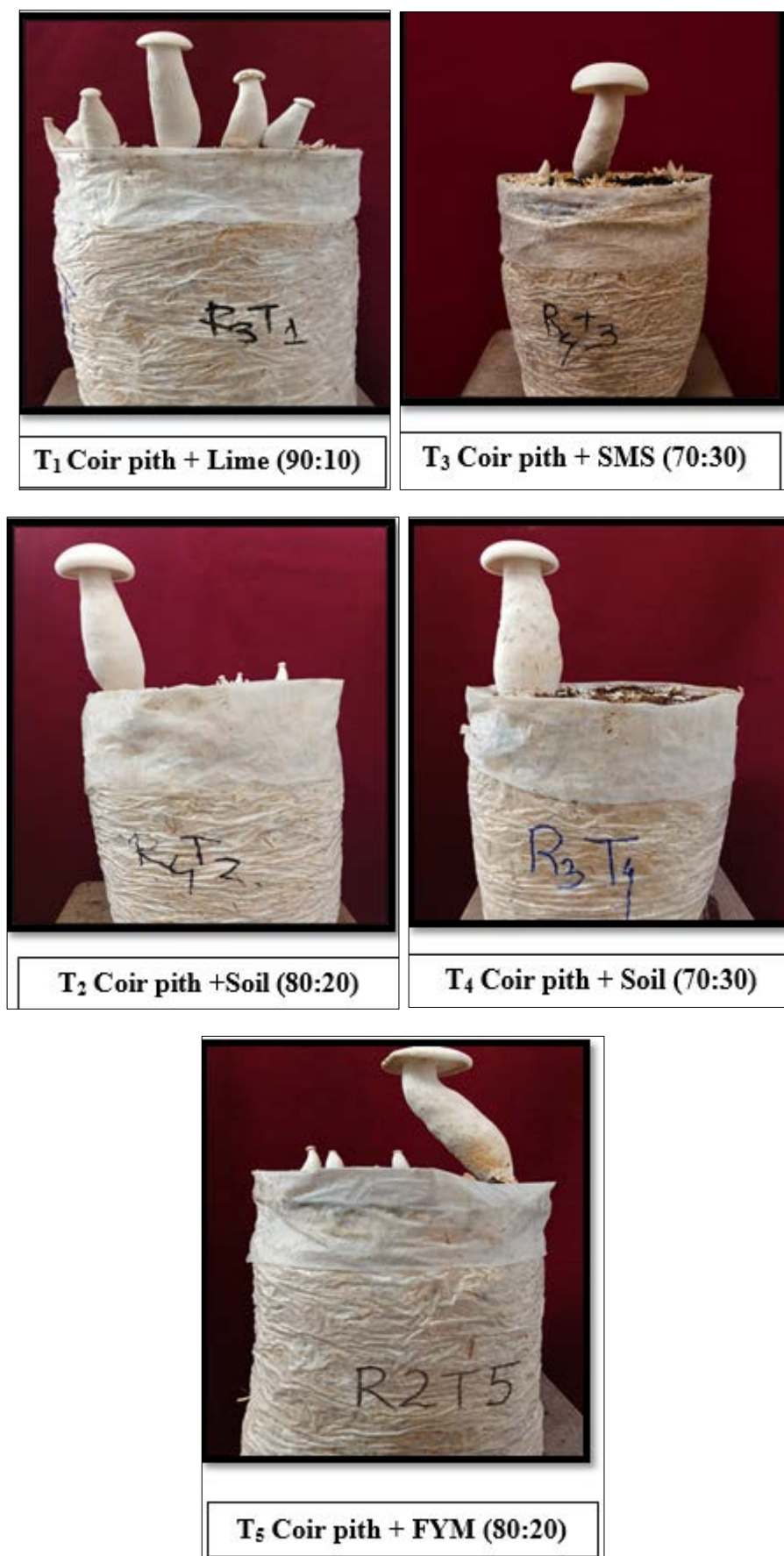


Plate 3: Stipe girth of *Macrocybe gigantea* mushroom on different casing materials

Growth observations

Average number of days required for spawn run of *Macrocybe gigantea* mushroom. The lowest number of days for spawn run (Table 2, Fig. 2) was recorded in T4

(Coir pith + Soil, 70:30) at 15.94 days, indicating the fastest spawn run, while the highest was recorded in T2 (Coir pith + Soil, 80:20) at 18.04 days. Treatments T1 (16.63 days), T3 (16.38 days) and T5 (16.25 days) were statistically at par

with T₄. Devi and Sumbali (2025) ^[6] stated that spawn run for *Macrocybe gigantea* mushroom with wheat straw as substrate took 17 days and with Paddy straw 20 days in monsoon whereas it took 22 days in summer. On contrary to that Galappaththi *et al.*, (2022) ^[9] tested rubber saw dust-based substrate for *Macrocybe gigantea*, recording spawn run completion in 55 days. Akhtar *et al.* (2019) ^[2] and Bharti (2019) ^[4] reported similar spawn run times of 18–21 days and 21–25 days on various agricultural waste substrates.

Average number of days required for Pinhead formation of *Macrocybe gigantea* mushroom.

The shortest duration was evident in T₄ (Coir pith + Soil in 70:30) at 14.94 days, indicating the fastest pinhead initiation. The longest duration was recorded in T₁ (Coir pith + Lime in 90:10 ratio) at 17.06 days, suggesting slower pinhead formation. Treatments T₂ (16.73 days), T₃ (16.79 days), and T₅ (15.61 days) showed intermediate results, which are statistically at par with one another. This means the treatments showed no statistically significant differences in the time required for pinhead formation.

The findings of Anurag Kerketta *et al.*, (2019) ^[12] showed that among six casing materials use for button mushroom growth and yield evaluation, the pinhead initiation was fastest in cocopeat + soil (12.25 days) and delayed in soil +

FYM + sand (15.62 days). Kushwaha *et al.*, (2016) ^[14] reported that *Macrocybe gigantea* pinheads appeared 13–16 days after applying the casing mixture.

Average number of days required for first harvest after pinhead formation of *Macrocybe gigantea* mushroom

Table 2, Fig. 2 indicates that shortest period was recorded in T₄ (Coir pith + Soil) at 5.83 days, indicating the earliest harvest following pinhead formation. The longest time period was noted in T₁ (Coir pith + Lime) at 8.58 days, showing a slower harvest process. Treatments T₂ (6.75 days), and T₅ (6.33 days) showed intermediate durations which are statistically at par to T₄. Overall, the Coir pith + Soil in 70:30 ratio (T₄) as a casing material was most effective in reducing the time to first harvest after pinhead formation. According to Kushwaha *et al.*, (2016) ^[14], harvest occurred 4 to 6 days after the pinhead stage. Devi and Sumbali (2021) ^[6] recorded 9 to 10 days required for harvest after the pinhead stage of *Macrocybe gigantea*. Duong *et al.*, (2017) ^[7] observed that *Macrocybe titans* sporophores matured 7–10 days after pinhead formation. Karnawadi (2006) ^[11] tested different casing materials for milky mushroom cultivation using paddy straw showed significantly fewer days for the first harvest, ranging from 7.4 to 14.2 days.

Table 2: Effect of different casing materials on growth parameters of *Macrocybe gigantea* mushroom

Treatment No.	Treatment Details	Average days for spawn run	Average days for pinhead formation	Average days for first harvest after pinhead formation
T ₁	Coir pith + Lime	16.63	17.06	8.58
T ₂	Coir pith + Soil	18.04	16.73	6.75
T ₃	Coir pith + SMS	16.38	16.79	7.33
T ₄	Coir pith + Soil	15.94	14.94	5.83
T ₅ (Control)	Coir pith + FYM	16.25	15.61	6.33
SE (m) ±		0.39	0.19	0.48
C.D. (0.01)		1.20	0.57	1.45

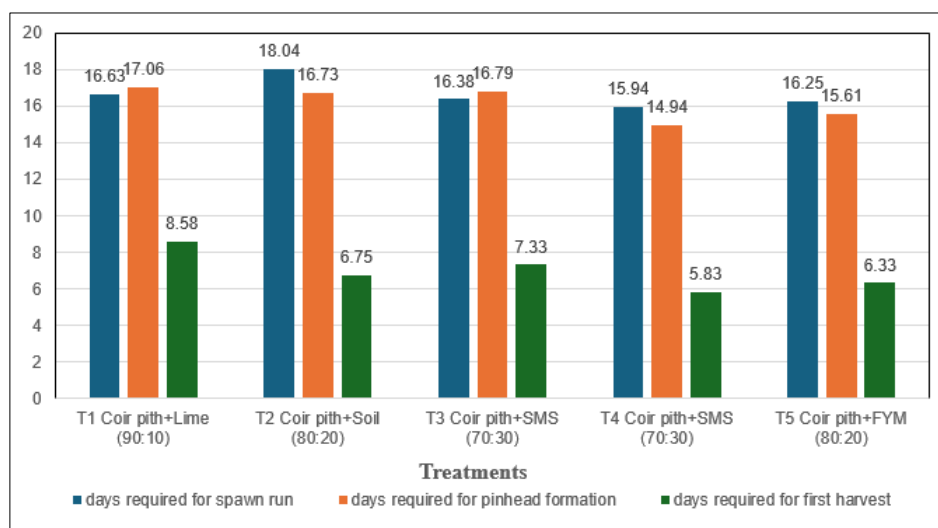


Fig 2: Growth observations of *Macrocybe gigantea* mushroom on different casing material

Conclusion: The results revealed that different casing materials significantly influenced the growth, development, and morphological characters of *Macrocybe gigantea*. Among the treatments, T₄ (coir pith + soil) was found to be the most effective, recording the maximum pileus diameter (7.37 cm), stipe length (12.48 cm), and stipe girth (9.82 cm), along with the minimum time required for spawn run (15.94

days), pinhead formation (14.94 days), and first harvest after pinhead formation (5.83 days). The lowest values for morphological parameters were recorded in T₁ (coir pith + lime), while the control treatment T₅ (coir pith + FYM) ranked second in morphological growth. Thus, T₄ (coir pith + soil) was identified as the most suitable casing material for promoting early growth, improving morphological

development, and enhancing the overall cropping performance of *Macrocybe gigantea*.

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