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**Research Article****Standardization of compost and casing for milky mushroom production****<sup>1</sup>Hammad Qasim, <sup>1</sup>Gulshan Irshad, <sup>1</sup>Amar Mehmood, <sup>1</sup>Rahim Uddin, <sup>2</sup>Syed Zulfiqar Ali, <sup>3</sup>Muhammad Azam Khan, <sup>1</sup>Inam-ul-Haq**<sup>1</sup>Department of Plant Pathology, PMAS Arid Agriculture University, Rawalpindi, Punjab, Pakistan.<sup>2</sup>Department of Plant Pathology, Balochistan Agriculture College, Quetta, Baluchistan, Pakistan.<sup>3</sup>Department of Horticulture, PMAS Arid Agriculture University, Rawalpindi, Punjab, Pakistan.**ABSTRACT**

Mushroom is a fungus that includes various species of the Tricholomataceae family and belongs to the phylum Basidiomycetes. After button and oyster mushrooms, milky mushroom (*Calocybe indica*) has high protein value with low fat content. *C. indica* contains a significant number of essential amino acids, protein (28%), vitamins (49%), fiber (14%), and low-fat (4%) food. *C. indica* has long been valued as a nutritional supplement, rich in thiamine, riboflavin, nicotinic acid, biotin, pyridoxine, and ascorbic acid. The current study aimed to determine the most suitable substrate and casing material for the successful cultivation of milky mushrooms. Three substrates, viz., wheat straw substrate, paddy straw substrate, and sugarcane bagasse substrate, were used for the production of *C. indica* and these substrates were obtained from the University Research Farm, Koont. Two types of methods (steam pasteurization and chemical pasteurization) were used for the pasteurization of compost. However, heat steam pasteurization showed highly significant results in the mycelial growth after the 7th, 14th and 21st days. Four casing (peat moss casing, coconut moss casing, rice husk casing, and clay soil casing (control)) were used for this experiment. Steam-pasteurized of wheat straw with peat moss casing was found to be the most effective combination for optimal growth and yield of *C. indica*.

**Keywords:** Standardization; compost; casing; *Calocybe Indica*.**Correspondence**Gulshan Irshad  
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**INTRODUCTION**

Mushroom is a fungus which includes various species of the Tricholomataceae family and belongs to the phylum Basidiomycetes. It is present where the soil is rich in organic matter and moist wood and in animal waste, these mushrooms quickly disappear and leave their mycelium after heavy rain or changes of temperature (Zied et al., 2011). According to researchers 70,000 fungi species are present in the world which only 2000 species (31 genera) are edible and others are poisonous mushrooms and small numbers are lethal (Waktola, G., & Temesgen, T. 2018). In all these fungi species the milky mushroom is most acceptable due to its nutritional value and worldwide production. Due to its medicinal and nutritional benefits, mushrooms have recently become more and more popular on a global scale. From a total of 495127 metric tons in 1961 to 10378163 metric tons in 2016, production of farmed edible mushrooms increased. More than 73% of the world's mushroom harvest is produced in China, with Italy ranking in second and the United States coming in third (Asif et al., 2023).

Mushrooms are popular ingredients in many cuisines around the world due to their unique taste, texture, and nutritional value. Among the various types of mushrooms, the milky mushroom (*Calocybe indica*) is gaining popularity due to its high protein content and low fat content. However, the cultivation of milky mushrooms requires a

specific substrate for its growth. In 2017 the milky mushroom was the first cultivated in Pakistan (Agrihunt, 2017). The cultivation of mushrooms has been gaining popularity in recent years, due to their nutritional values and medicinal properties. *Calocybe indica* contains a significant number of essential amino acids, is high in protein at 28%, lipids, fibre 14%, carbohydrates at 49%, and vitamins, and is also a low-fat 4% food. As evidenced by history, *C. indica* has long been utilized as a nutritional supplement due to its abundance in thiamine, riboflavin, and nicotinic acid. Biotin, pyridoxine, and ascorbic acid According to scientific literature, *C. indica* is used to treat vicus illnesses and disorders. the nutritional benefits of mushrooms, challenges in cultivation, and the prospects of integrating mushroom farming into sustainable agricultural practices to improve livelihoods and food availability (Asif Khan et al., 2024). After button and oyster mushrooms, it is now India's third commercially produced fungus. Due to this mushroom's poor yield, Indians are currently expressing less interest in its cultivation (Roy, A., & Prasad, P. 2014). Among the various types of mushrooms, the Milky Mushroom (*Calocybe Indica*) is a highly valued edible mushroom due to its taste, texture, and nutritional value. However, the cultivation of milky mushrooms is challenging due to a lack of standardization in the production of spawn and compost. to combat these challenges in this study different substrates evaluated for the effective mushroom compost and mushroom casing for effective growth of milky mushroom.

## MATERIALS AND METHODS

### Site Study

The research was conducted at the PMAS Arid Agriculture University Research Farm, Koont. Milky mushroom spawn provided by Fungal Plant Pathology Laboratory PMAS Arid Agriculture University Rawalpindi for the cultivation of mushrooms.

### Mushroom Cultivation

#### Room preparation

The mushroom cultivation room (MCR) was set at a temperature of 25 to 35°C monitored by using a hygrometer. The humidity of the Mushroom Cultivation room was maintained by a Humidifier and observed by using a Hygrometer. An exhaust Fan was used on different time interval 02 times in a day to maintain the level of Oxygen and Carbon dioxide. The floors of the MCR were paved so that excess water could be drained.

#### Compost preparation

Milky mushrooms were usually cultivated on different straws like wheat straw, rice straw and sugarcane baggase. Straw was cut into small pieces of 3-4 cm. Then the dry straw sacks were placed in the drum. Then the moisture sacks of straw were in the container and fired below the steam drum. Which heated the water in the drum and took the form of steam. The bags were steamed inside the drum for two to three hours, which wets the straw inside the sacks with hot steam so that this moisture is sufficient for spawning. The bags were removed from the drum and placed on the floor to be cooled.

#### Compost preparation by using diff. Straw for milky mushroom cultivation

Three substrates—wheat straw, rice straw, and sugarcane bagasse were selected for the cultivation of milky mushrooms. These substrates were chopped into 2–3 inch pieces and soaked for three days in a large drum containing a 2% (w/v) lime and 4% gypsum solution. The substrate was picked up leaving behind the lime-water solution. The substrate was heaped on a cemented floor and covered with a plastic sheet for 3 days. The pH of the substrate was 7. After soaking, each substrate was removed from the solution and allowed to drain for 2–3 hours to eliminate excess water. Prior to spawning, the moisture content of the wet substrate was adjusted to approximately 60%, as measured using a hygrometer. The substrate was filled in autoclavable clear bags (5 Kg/bag) sealed and sterilized into two different methods like Heat stream sterilization and chemical sterilization.

#### Pasteurization of different compost for milky mushroom cultivation (MMC)

##### Pasteurization of MMC compost by using the heat stream method

Steam pasteurization or the heat steam method was an additional efficient method for pasteurizing milky mushroom compost (*Calocybe indica*). Using steam, the temperature of the mushroom compost was raised to a point where hazardous microbes were killed or rendered inactive all the while maintaining the quality of the mushrooms.

A net bag was filled with various substrates that had been cut to a suitable length of 2.5 to 5 cm. The bag was then treated with hot water at 60°C in a drum for two to three hours, and the surplus water was allowed to drain out by hanging the bag for 20 hours. By spreading them out on the plastic sheet, the hot water-treated substrate's moisture content was allowed to evaporate, resulting in a moisture content of 55–60%.

Mycelial completely spread on substrates within 18-21 Days. Mycelial Growth of three substrates Wheat Straw Substrate WSS, Rice Straw Substrate RSS and Sugarcane Bagasse Substrate SBS were observed 3 times with the interval of 7 days (7<sup>th</sup> day, 14<sup>th</sup> Day and 21<sup>st</sup> Day).

#### **Pasteurization of compost by using the chemical method**

Formalin (formaldehyde) preservative and disinfectant chemical. Owing to formaldehyde's toxicity and possible health risks, safety precautions had to be taken when making a 3-4% formalin solution, 2% Mancozeb with 95% distilled water. The compost was completely covered for 2 days after chemical pasteurization. After 02 days placed in the cultivation room for further process.

#### **Spawning of milky mushrooms by different compost MMC**

Freshly prepared spawn that was 10 to 15 days old was used for the spawning. Prior to spawning, the wet substrate was kept at a moisture level of around 65%. The ready substrate was spawned at a rate of 5% using a wet weight basis in 60 x 40 cm polypropylene bags that were 100 gauge thick.

For different substrates pasteurized was filled into polythene bags. Spawn was added in layers while filling. The spawn is usually laid in three layers in such a way that the spawn will fall round towards the edges of the straw. Some of the spawn was also placed on the surface of the straw. The amount of spawn was about 20 to 30 grams per polythene bag. A sterilized needle was used to deceive the spawn sacks into letting air flow. The bags were moved into the cropping room after spawning and kept in a dark area with a temperature of 25–30°C and a relative humidity of 85–90% until the mycelium colonized the substrates, at which point the substrates were prepared for casing.

#### **Evaluation of different casing methods by MMC**

As the propagation of the spawn in the media was completed, a layer of soil was laid on it as a casing. The casing material helps the physical support, moisture absorption, and gas escape from the substrates. Different types of casing materials were used in milky mushroom cultivation. One of casing Peat Moss Casing has PMC of 50% peat moss, 25% sand and 25% soil and the other one Coconut Moss Casing CMC has 50% coconut moss, 25% sand and 25% soil. The third casing material Rice Husk Casing RHC has 50% Rice husk, 50% clay soil and the 4<sup>th</sup> casing was made with only 100% clay soil as control. The casing was prepared by thoroughly mixing the chosen ingredients in the appropriate ratio and then each one was chemically sterilized at 65°C with 4% formalin and 2% Mancozeb in Distilled water solution and covered with a plastic sheet for 48 hours. The Compost bags uniformly roughed top surface was covered with a layer of casing material that was about 3–4 cm thick and gently compressed. The temperature was kept at 25–30°C and 85–90% relative humidity until mycelium running in casing. After 10 days, the mycelium appeared on the casing soil. The cropping room's atmosphere was then altered by bringing in fresh air through exhaust and lighting for 6-8 hours. The relative humidity was kept between 90-95% percent by humidifier in the area three times a day.

#### **Parameters**

These parameters were observed during the growth of milky mushroom.

#### **Mycelial growth %**

Mycelial Growth of three substrates Wheat Straw Substrate WSS, Rice Straw Substrate RSS and Sugarcane Bagasse Substrate SBS were observed 3 times with the interval of 7 days, (7<sup>th</sup> day, 14<sup>th</sup> Day and 21<sup>st</sup> Day).

#### **Pinhead Formation days**

Pinhead formation on Peat Moss Casing PMC, Coconut Moss Casing CMC, Rice Husk Casing RHC and Clay Soil was visually observed 02 times with the interval of 4 days, (4<sup>th</sup> day and 8<sup>th</sup> Day)

#### **Fruiting body/bag**

Fruiting Bodies on Peat Moss Casing PMC, Coconut Moss Casing CMC, Rice Husk Casing RHC and Clay Soil were visually observed 02 times with the interval of 3 days, (3<sup>rd</sup> day and 6<sup>th</sup> Day)

#### **Statistical Analysis**

Data for the characters in compost like, mycelial growth percentage, pinhead formation days, fruiting bodies per bag and in casing material pinhead formation days, fruiting bodies per bag were statistically analyzed using two factorial designs, Analysis of Variance (ANOVA) through standard statistical tools, procedures, software Statistics 8.1 and the difference between means were studied by Least Significant Different (LSD) at probability level 0.05 (Steel and Torrie, 1997).

## **RESULTS AND DISCUSSION**

### **Evaluation of Different Compost Materials for Milky Mushroom Cultivation**

Three substrates (Wheat Straw Substrate WSS, Paddy Straw Substrate PSS and Sugarcane Bagasse Substrate SBS)

were observed for the cultivation of milky mushrooms.

### Mycelial Growth of Different Substrates by Steam and Chemical Pasteurization

Mycelial completely spread on substrates within 18-21 Days. Mycelial Growth of three substrates Wheat Straw Substrate WSS, Paddy Straw Substrate PSS and Sugarcane Bagasse Substrate SBS were observed 3 times with intervals of 7 days, (7th day, 14th Day and 21st Day).

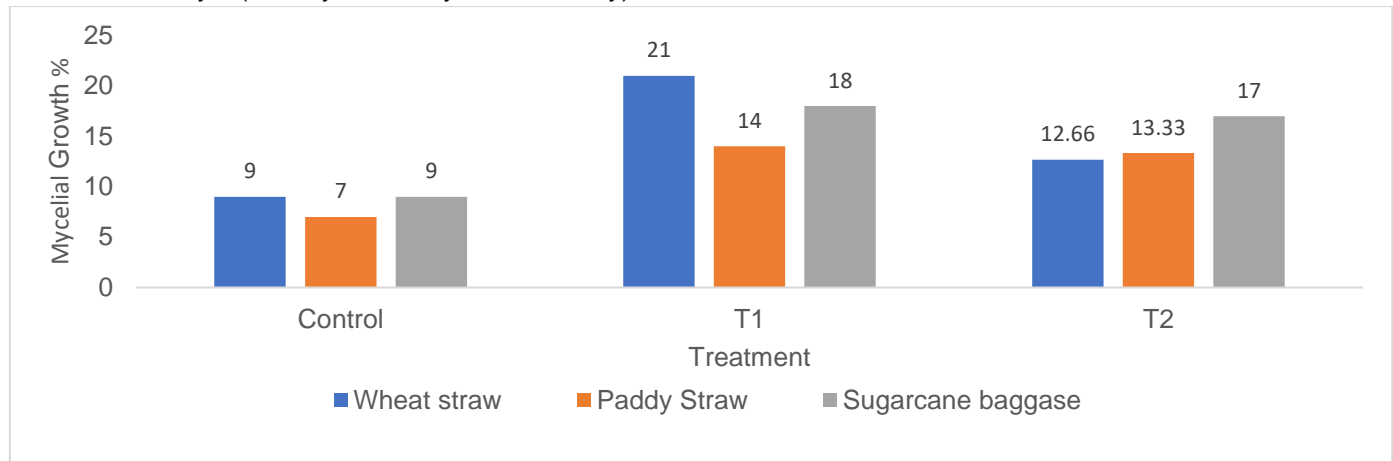


Figure 1. Mycelial growth of different substrates by steam and chemical pasteurization of 7th day.

The Graph in figure (1) shows the Mycelial Growth of Different Substrates on Compost. From three different substrates Wheat Straw with Steam Pasteurization T1 was effective Treatment after 7th Day.

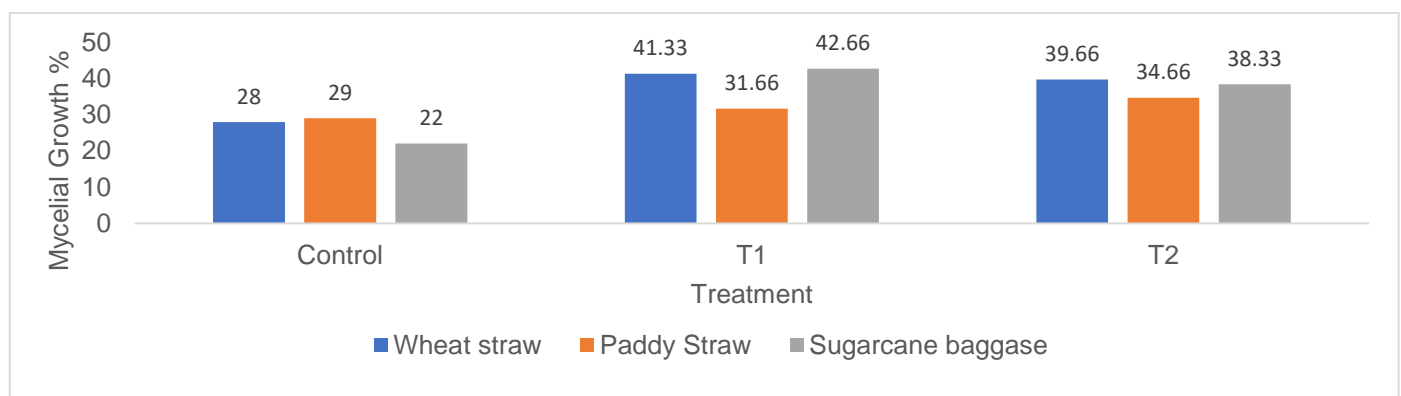


Figure 2. Mycelial growth of different substrates by steam and chemical pasteurization 14th day.

The Graph in figure (2) shows the Mycelial Growth of Different Substrates on Compost. From three different substrates, Sugarcane baggase with Steam Pasteurization T1 was an effective Treatment after the 14th Day.

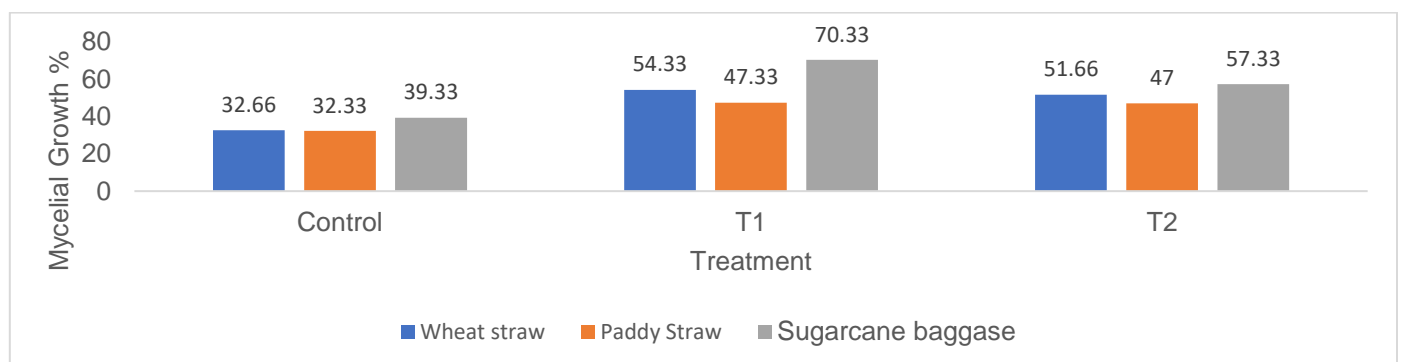


Figure 3. Mycelial growth of different substrates by steam and chemical pasteurization of 21st day.

The Graph in figure (3) shows the Mycelial Growth of Different Substrates on Compost. From three different substrates, Sugarcane bagasse with Steam Pasteurization T1 was effective Treatment after 21st Day. Variations in the ecological, humid, and climatic circumstances that predominated over the two study years are the cause of this fluctuation in spawn-run during the two summer seasons. Himani et al. (2003) have also observed similar spawn run outcomes.

#### Evaluation of Different Casing Material for *Calotype Indica* Production

Three different casing materials Peat Moss Casing PMC, Coconut Moss Casing CMC, Rice Husk Casing RHC and one control Clay Soil evaluated for milky mushroom cultivation by observing Pinhead Formation and fruiting bodies.

#### Evaluation of Different Casing Material for Pinhead Formation

Pinhead formation on Peat Moss Casing PMC, Coconut Moss Casing CMC, Rice Husk Casing RHC and Clay Soil was visually observed 02 times with the intervals of 4 days. (4th day and 8th Day).

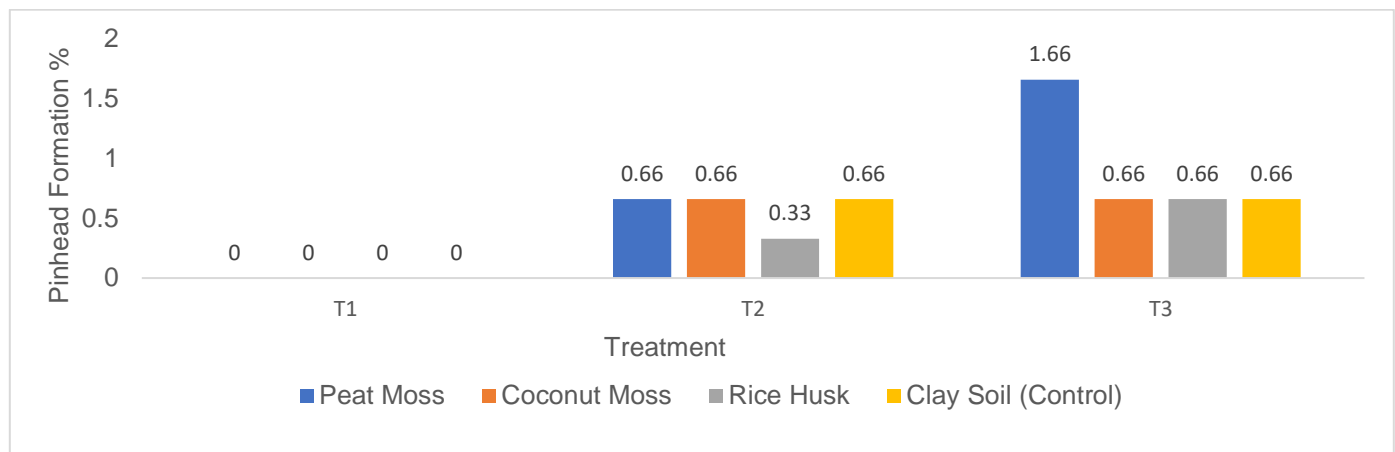


Figure 4. Evaluation of different casing material for pinhead formation of 4th day.

The Graph in figure (4) is showing the Pinhead formation on different casing material. From four different casing material Peat Moss on Sugarcane Baggase T3 was effective Treatment after 4th Day.

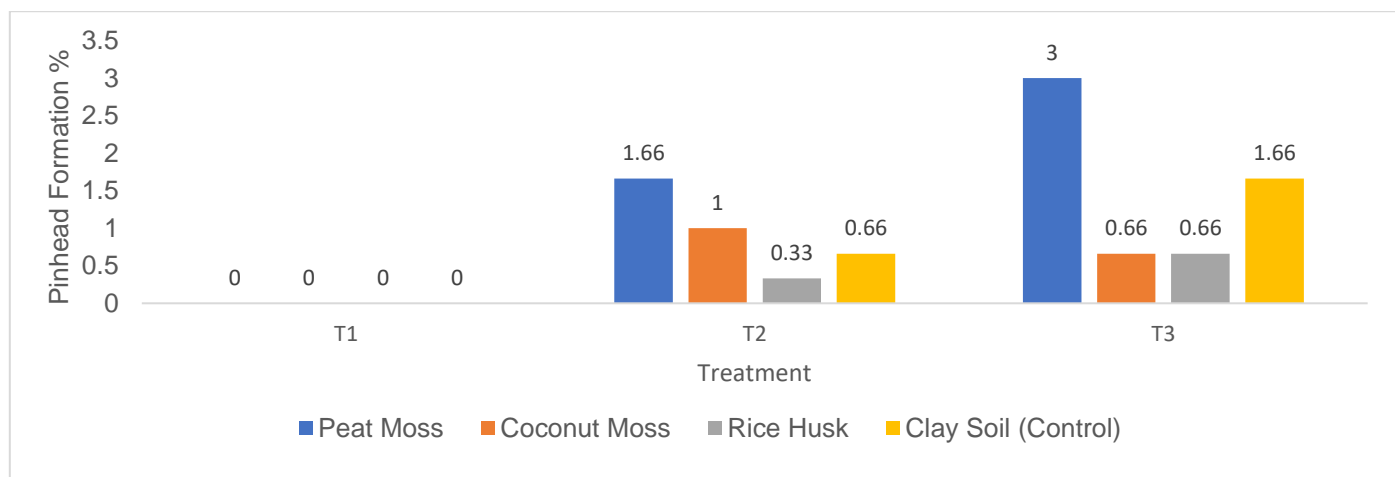


Figure 5. Evaluation of different casing material for pinhead formation of 8th day.

The Graph in figure (5) is showing the Pinhead formation on different casing material. From four different casing material Peat Moss on Sugarcane Baggase T3 was effective Treatment after 8th Day.

There was a noticeable variance in the total number of pinheads that were started across all milky mushroom treatments. because of the climate, temperature, and humidity. The cellulose, fiber, and lignin in the straw used in different treatments caused variations in the initiation of the pin heads. Calcium sulphate was added to maintain the pH of the substrate, while calcium carbonates helped to lessen the stickiness of the spawn. Because the lignin and cell were polysaccharide, the alkali treatment broke the ester bonds, increasing the availability of the carbohydrates and improving the digestion of straw. Sharma et al. (2021) have observed similar findings.

### Evaluation of Different Casing Material for Fruiting Bodies

Fruiting Bodies on Peat Moss Casing PMC, Coconut Moss Casing CMC, Rice Husk Casing RHC and Clay Soil was visually observed 02 time with the interval of 3 days. (3rd day and 6th Day).

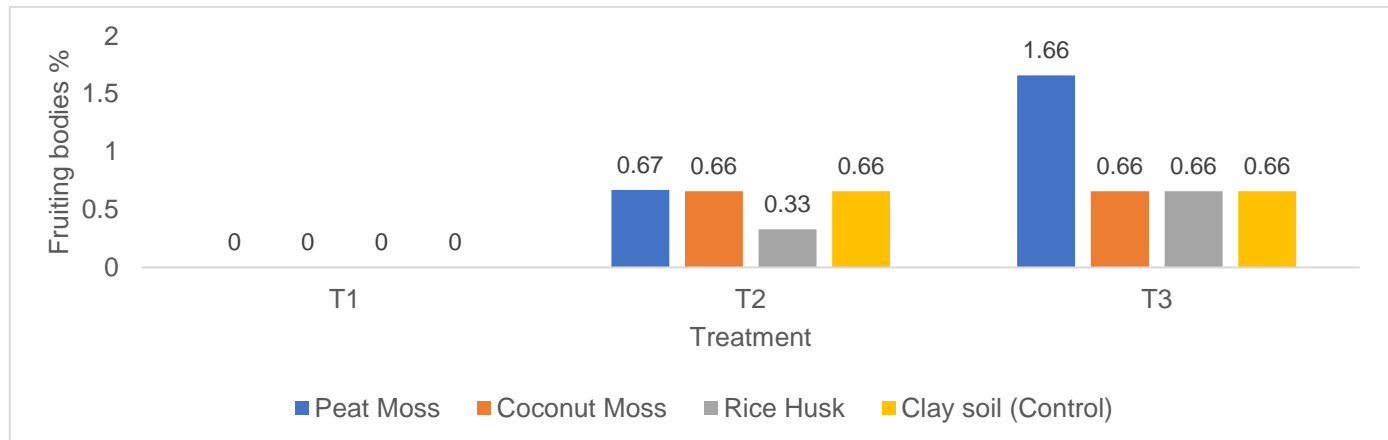


Figure 6. Evaluation of different casing material for fruiting bodies of 3rd day.

The Graph in figure (6) is showing the Fruiting bodies on different casing material. From four different casing material Peat Moss on Sugarcane Bagasse T3 was effective Treatment after 3rd Day.

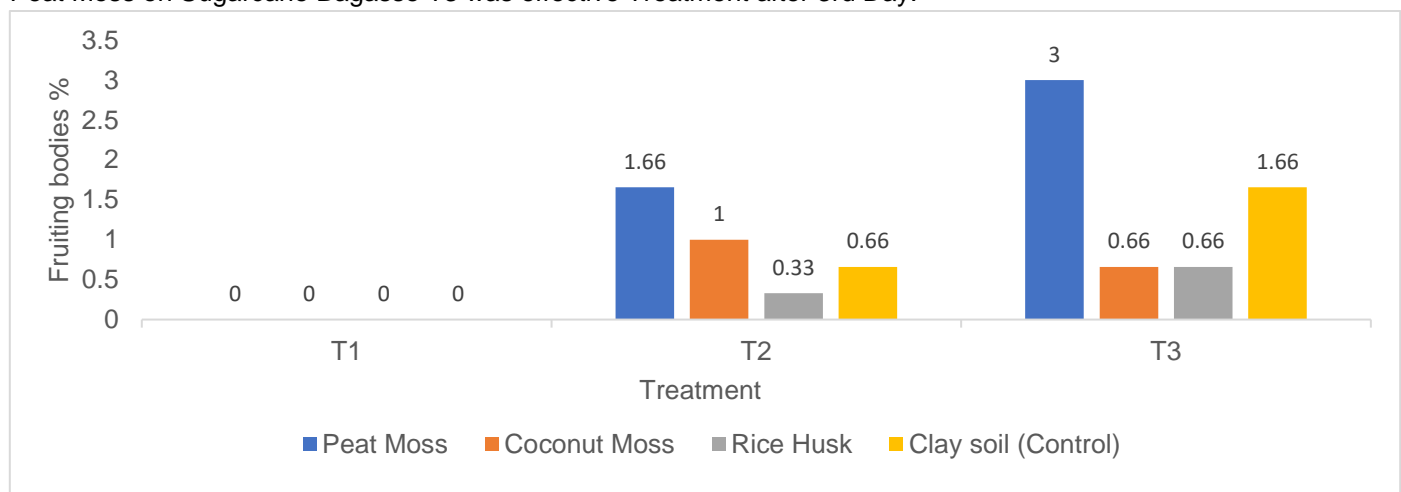


Figure 7. Evaluation of different casing material for fruiting bodies of 6th day.

The Graph in figure (7) is showing the fruiting bodies on different casing material. From four different casing material Peat Moss on Sugarcane Bagasse T3 was effective Treatment after 6th Day. Due to the temperature humidity, environmental conditions.

The straw used in different treatments comprises cellulose, fiber, and lignin, and calcium sulphate is added to maintain the pH of the substrate while calcium carbonates aid to lessen the stickiness of the spawn. This might be the cause of the variance in the quantity of harvested fruiting bodies. Because the lignin and cell were polysaccharide, the alkali treatment broke the ester bonds, increasing the availability of the carbohydrates and improving the digestion of straw. This might be the cause of the rise in fruiting body harvests (Straatsma et al., 2013).

Milky mushroom (*Calocybe indica*) is increasingly cultivated in tropical and subtropical areas due to its high nutritional value. Optimizing the substrate and casing is essential for raising biological efficiency and production. Wheat and paddy straw are proven to be very useful substrates. While Chakraborty et al. (2016) showed 196.12% biological efficiency using paddy straw, Singh et al. (2010) and Karnale (2020) found wheat straw to be more productive. Although less prevalent, sugarcane bagasse performed well when steam-pasteurized, supporting the findings of Sharma et al. (2021), who noted that steam treatment increased the digestibility of lignocellulosic materials.

Pinhead development and moisture retention are influenced by casing materials. Soil-sand combinations were determined to be better than clay by Tandon et al. (2006). Elangovan et al. (2024) found the maximum yield and early primordia with a 1-inch layer of sandy soil plus coir pith, whereas Chandrashekara et al. (2024) reported the best yield utilizing Arka Fermented Cocopeat (AFC) with sandy loam at a 90:10 ratio. These results corroborate the current study's findings that early pinning and production were enhanced by using peat and coconut moss instead of sugarcane bagasse. Elangovan et al. (2024) and Shukla (2007) both suggested 1.0 to 1.5 inches of casing thickness to balance aeration and moisture. Within ten days, 3–4 cm casing produced good mycelial spread in this investigation. Results are further enhanced by supplementation; Kumar (2020) discovered that rice powder raised productivity, while Singh et al. (2007) reported yield increases with soybean meal. FYM-soil casing for quicker mycelial development was also advocated by Karnale (2020).

## CONCLUSION

The selection of substrates and casing materials is essential to the success of *Calocybe indica* production. Paddy straw and wheat performed better than the other substrates, while steam-pasteurized sugarcane bagasse also produced positive outcomes. When adequately pasteurized and placed at the ideal thickness, casing materials such as peat moss and coconut coir promoted increased fruiting body growth and early pinhead initiation. The commercial standardization of milky mushroom growing in tropical agroclimatic zones can benefit from these findings.

## AUTHOR'S CONTRIBUTION

Under the direction of postgraduate scholar Hammad Qasim, the study benefited from the academic expertise of Dr. Gulshan Irshad, who is renowned for his important contributions. Amar Mehmood, Rahim Uddin, and S. Zulfiqar Ali conducted the experiments and evaluated the results. Prominent academics Prof. Dr. M. Inam ul Haq and Prof. Dr. M. Azam rigorously reviewed the work, adding to its overall quality with their insightful comments and careful assessments.

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## AVAILABILITY OF DATA AND MATERIAL

On reasonable request, the corresponding author will provide the datasets created and/or examined during the current work. To guarantee the findings' repeatability and openness, any further information, including raw and processed files, can be provided upon request.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

There are no human subjects or animal models in the study that need ethical approval. Every experimental method and protocol complied with national and institutional regulations.

## CONSENT FOR PUBLICATION

Consent for publishing is not necessary because this work does not contain any personal information about any individual, including pictures, videos, or other personal data.

## CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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