CULTIVATION OF *Pleurotus flabellatus* AND ITS STANDARDIZATION UNDER WEST BENGAL CONDITIONS

A Thesis
Submitted to
Bidhan Chandra Krishi Viswavidyalaya
in partial fulfilment of the requirements for the award of
the Degree of Master of Science (Agriculture)
in

Plant Pathology

By

NANDIPAM SURYA TEJA

Reg. No. 11A11P2324

DEPARTMENT OF PLANT PATHOLOGY
FACULTY OF AGRICULTURE
BIDHAN CHANDRA KRISHI VISWAVIDYALAYA
Mohanpur, Nadia, West Bengal-741252, India
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2025

Dedicated

to My

Beloved

Parents

BIDHAN CHANDRA KRISHI VISWAVIDYALAYA

Approval of Examiners for the award of the Degree of Master of Science (Agriculture) in Plant Pathology of Bidhan Chandra Krishi Viswavidyalaya

We, the undersigned, having been satisfied with the performance of **Mr. NANDIPAM SURYA TEJA,** a student of M.Sc. (Ag), Department of Plant Pathology, Faculty of Agriculture,Bidhan Chandra Krishi in the Viva-Voice examination, conducted today, 19th August 2025, recommended that the thesis entitled **"Cultivation of *Pleurotus flabellatus* and its standardization under West Bengal conditions"** be accepted for the award of the Degree of Master of Science (Agriculture) in Plant Pathology of Bidhan Chandra Krishi Viswavidyalaya.

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CERTIFICATE

This is to certify that the work recorded in the thesis entitled "**Cultivation of *Pleurotus flabellatus* and its standardization under West Bengal conditions**" submitted by **Mr. Nandipam Surya Teja** in partial fulfilment of the requirements for the award of the degree of Master of Science in **Plant Pathology**, Faculty of Agriculture of the Bidhan Chandra Krishi Viswavidyalaya, is the faithful and bonafide research work carried out under my supervision and guidance. The results of the investigation reported in thesis have not so far been submitted for any other Degree or Diploma. The content of the thesis has been checked for plagiarism and the similarity level is within the permissible limit (10%) as per the university rules. The soft copy of the thesis which has been checked for similarity and the hard copy of the thesis which has been submitted for the degree are identical. The assistance/ help received during the course of investigations have been duly acknowledged.

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Place: BCKV, Mohanpur

N. Surya Teja

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Abstract

Pleurotus species, commonly known as "oyster mushrooms" and referred to as "Dhingri" in India, are considered among the finest edible mushrooms. *Pleurotus* is a genus with significant medical, biotechnological properties and environmental applications. The *Pleurotus* species are commonly cultivated across temperate and subtropical parts of the world. They are found in woods where they attack the cellulose and lignin components of wood. *Pleurotus* species are more valuable due to their adaptability and simplicity of production. Oyster mushrooms grow on a variety of agricultural waste materials used as substrates at moderate temperatures, ranging from 20ºC to 30ºC, and at a humidity of 70-95%. *Pleurotus flabellatus* is an edible species of fungi with a white, lung-shaped to semicircular pileus. It is a member of the *Pleurotaceae* family and the order *Agaricales* of the class *Agaricomycetes* Although West Bengal has highly favourable agro-climatic conditions for oyster mushroom cultivation, this particular Pleurotus species remains under-researched, and its production is still in the early, slow-growing phase. The goal of the current study is to cultivate and standardize *Pleurotus flabellatus* under West Bengal conditions. For the study, cultural, morphological and post-harvest factors were examined in a laboratory and under mushroom house conditions. After seven days of inoculation, a temperature of 26°C was shown to be ideal for *Pleurotus flabellatus* mycelial growth (71.20 mm); however, mycelial growth declined to 53.27 mm when the temperature exceeded this level. The growth was at its slowest (18.05 mm) at 20°C. Mycelial growth was observed to be most favourable within a pH range of 7 to 8, with the maximum expansion (89.59 mm) occurring at pH 7.5. Substituting with starch in the PDA medium led to improved mycelial development, with growth extending up to 57.40 mm, making it the most effective carbon source in the study. Among the nitrogen sources evaluated at different concentrations, 0.3% L-asparagine promoted the highest mycelial expansion, reaching 46.54 mm after seven days of incubation. Of the media evaluated, Malt Extract Agar (MEA) proved most conducive to mycelial proliferation, recording a growth of 88.49 mm surpassing both Potato Dextrose Agar (PDA) and V8 Juice Agar (V8 JA). Regarding spawn production, Bajra grain supported the quickest colonization, completing the spawn run in 15.33 days, followed by Jowar at 16.33 days, while Wheat required 19.66 days. Cultivation experiments conducted in mushroom house conditions

across three substrate types identified paddy straw as the most suitable for growing *Pleurotus flabellatus* Paddy straw resulted in significantly higher average yield (232.84 g) and biological efficiency (72.66%) compared to the other substrates tested, with banana leaves yielding 175.25 g at 31.54% efficiency, and sawdust producing the lowest values at 151.83 g and 23.20% efficiency. Other morphological characters such as number of basidiocarp, individual weight of basidiocarp, stipe length and diameter of pileus, was found to better in paddy straw substrate.

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PDA	**Potato Dextrose agar**
MEA	**Malt Extract Agar**
V8 JA	**V8 Juice Agar**
RH	**Relative humidity**
CD	**Critical difference**
SE(m)	**Standard error of means**
gm	**gram**
cm	**centimetre**
mm	**millimetre**
^{o}C	**Degree Celsius**
Max	**Maximum**
Min	**Minimum**
pH	**Pouvoir hydrogene/Power of hydrogen**
Temp.	**Temperature**
Spp.	**Species**
et al.	**And others**
viz.	**namely**
etc.	**Etcetera**
i.e.	**That is**
@	**At the rate of**
BE	**Biological Efficiency**
Fig.	**Figure**
DAI	**Days after inoculation**

Chapter 1: Introduction

Mushrooms are the visible fruiting bodies of large filamentous fungi that develop above the ground. They have been consumed as food and valued for their medicinal properties for hundreds of years. In mushrooms, the pileus, or cap, is the broad, umbrella-shaped part that protects the spore-producing surface. The stipe, or stem, supports the cap and raises it to aid in spore dispersal. Beneath the cap are thin, blade-like structures called lamellae, or gills, which produce and release spores in most species. In some mushrooms, these gills are replaced by small pores that perform the same function.

Mushrooms have been known and valued since ancient times, with records indicating that the Egyptians regarded them as the "plant of immortality." Hieroglyphics dating back roughly 4,600 years depict them as a sacred gift reserved for royalty, while ordinary people were prohibited from eating them. In Asia, mushroom cultivation was practiced as early as 600 AD in China. In Europe, France was the first to develop methods for growing them.

Asia dominates global mushroom production, with China leading the industry through advanced cultivation techniques and strong domestic demand. India, ranked sixth worldwide, is experiencing rapid growth in its mushroom sector.

Globally, the most widely cultivated edible mushroom genera include *Lentinula*, accounting for about 22% of total production, followed by *Pleurotus* at 19%, *Auricularia* at 18%, *Agaricus* at 15%, *Flammulina* at 11%, *Volvariella* at 5%, and other varieties making up the remaining 10% (Royse et al., 2017). In India, commercial mushroom farming primarily focuses on five main types: white button (*Agaricus bisporus*), oyster (*Pleurotus* spp.), paddy straw (*Volvariella volvacea*), milky (*Calocybe indica*), and shiitake (*Lentinula edodes*).

In India, button mushrooms hold the largest share of production at about 73%, followed by oyster mushrooms, which account for roughly 16% (Sharma et al., 2017).

Pleurotus species, commonly known as oyster mushrooms, are among the most widely cultivated edible fungi worldwide. Their fan or oyster shell-shaped fruiting bodies come in various colours such as white, cream, grey, yellow, pink, and light brown (Rajarathnam et al., 1987). These mushrooms belong to the order Agaricales and family Pleurotaceae, and are mostly saprophytic, growing on lignocellulosic agro-wastes. They help to recycle

agricultural residues into nutritious mushrooms and useful by-products like fertilizer, animal feed, and biogas (Kakon et al., 2012).

In India, the leading producers of this mushroom include Odisha, Karnataka, Maharashtra, Andhra Pradesh, Madhya Pradesh, West Bengal, and several states in the North Eastern hill region.

Pleurotus flabellatus typically produces dull white fruiting bodies, occasionally with darker shades, which is pure white under controlled lighting in mushroom houses. Its gills are moderately spaced, with clavate to mucronate cheilocystidia. The cap measures between 8 to 11 cm, starting convex with rolled margins and later expanding into a fan or shell shape. Mature fruit bodies display ivory coloration, often without a stalk. If present, the stalk is short, solid, tough, eccentric, and white. The gills connect at the base, forming a network down the stem, and the spore print is white. This species is widely distributed across temperate and subtropical forest regions around the globe. It thrives in warm climates and typically emerges in late summer. Known for its delicious taste, attractive appearance, rich nutritional and medicinal properties, *P. flabellatus* has gained increasing popularity in recent years, particularly in India, where it is also considered promising for commercial cultivation.

Pleurotus flabellatus is increasingly gaining attention for its notable health-promoting properties. Its popularity in commercial cultivation is growing worldwide due to its ease of production, high yield potential, and impressive nutritional composition. This mushroom is especially abundant in folic acid (vitamin B_9), contains fat levels ranging from 0.9% to 7.5% (Lavelli et al.,2018) and is composed of 25% to 76% carbohydrates. Protein content varies from approximately 9.3 g to 37.4 g per 100 g of fresh weight (Raman et.al 2021). Additionally, it serves as a rich reservoir of essential minerals like sodium, calcium, phosphorus, iron, and potassium, and is packed with vitamin C and various B vitamins. Apart from their nutritional appeal, these mushrooms have been linked to several therapeutic properties, including anticancer, heart-protective, blood sugar-regulating, antioxidant, and liver-supportive effects.

Mushroom cultivation can support the local economy by reducing poverty risk, improving livelihoods through job creation and income generation from local, regional, and international markets, and offering opportunities for processing industries (Bose, 2016). Contributing economically, nutritionally, and medicinally, mushroom farming

plays an important role in improving the health and quality of life of rural communities (Prakasam, 2012). It is a simple, low-cost activity well-suited to rural areas, requiring substantial labour and providing employment opportunities in both rural and semi-rural regions (Nagaraj et al., 2017).

Although oyster mushrooms hold a significant place in India's mushroom sector and West Bengal ranks sixth in their production, there is still a lack of comprehensive research on the optimized cultivation of *Pleurotus flabellatus*. Hence, this study aims to establish standardized cultivation methods for *P. flabellatus* under mushroom house conditions in West Bengal. The primary objectives of the study are as follows:

1. Collection, maintenance and studies on cultural characteristics of *Pleurotus flabellatus*.
2. To study the morphological characteristics of *Pleurotus flabellatus*.
3. To conduct fructification trials on *Pleurotus flabellatus*

Fruitbodies of *Pleurotus flabellatus*

Chapter 2: Review of Literature

Pleurotus flabellatus, is an edible species of fungi that has a broad, fan or oyster-shaped cap which is in white or ivory colour. The cap margin is in rolled when young, and the flesh is white and firm belongs to family *Pleurotaceae*, order *Agaricales* of class *Agaricomycetes*.

Pleurotus species are found in natural environments as lignocellulosic fungi, growing on tree trunks in subtropical and temperate forests and causing white rot of wood. According to Bhattacharjya et al. (2014), *Pleurotus* species can secrete both hydrolytic and oxidative enzymes, enabling them to utilize complex organic compounds found in agricultural residues and industrial byproducts.

They possess highly efficient enzyme systems capable of breaking down various low-cost substrates such as lignin, cellulose, hemicellulose, pectin, and other industrial wastes allowing them to produce protein-rich food at minimal cost (Subramanian and Shanmugasundaram, 2015).

Oyster mushrooms can be grown on a variety of substrates, including paddy straw, maize stalks or cobs, vegetable plant residues, and bagasse. For optimal growth, however, an ideal substrate should be rich in carbohydrates and supplemented with nitrogen (Ashraf et al., 2013) Adding supplements boosts mushroom production by supplying vital nutrients necessary for mycelial growth, leading to improved health and increased yield (Josephine, 2015).

Pleurotus flabellatus, a widely cultivated species of oyster mushroom, is grown extensively around the world, particularly in regions of Asia, Europe, and North America. China stands as the top producer, while countries such as Italy, Thailand, Korea, Philippines, Japan, and Taiwan also play major roles in its worldwide cultivation.

In India, this mushroom is commonly grown in states such as Odisha, Karnataka, Maharashtra, and West Bengal.

Due to its saprophytic characteristics, *P. flabellatus* can easily thrive in various climatic conditions, making it suitable for cultivation across various agroclimatic zones. Additionally, it plays a crucial ecological role by breaking down lignocellulosic waste,

supporting organic matter recycling, and showing promise in environmental cleanup through bioremediation.

Oyster mushrooms grow best within a moderate temperature range of 20°C to 30°C. During the initial stage, known as mycelial growth or spawn run, the ideal temperature is between 25°C and 30°C, which helps the mycelium spread through the substrate. For the next stage, when the mushrooms begin to form fruiting bodies, a slightly lower temperature of 22°C is required. A temperature of 26°C has been found to be ideal, as it supports the initiation of pinheads as well as the development of fully matured mushrooms. One of the notable advantages of oyster mushrooms is their capacity to break down various types of agricultural waste, converting lignocellulosic biomass into nutritious, flavourful, and high-quality food products (Quimio, 1978).

Mushroom growth is highly affected by environmental conditions such as pH, temperature, and the type of medium used (Bugarski et al., 2002). In light of this, the present investigation focused on determining the best locally available substrate, optimal spawn quantity, suitable culture medium, favourable pH and temperature ranges, and the most effective carbon and nitrogen sources. Additionally, various grains were evaluated for their potential in spawn production of these mushrooms. The goal was to develop the most effective cultivation strategies tailored to the conditions of West Bengal.

2.1 Cultural Characteristics:

According to Calam et al. (1971), mycelial growth is affected by several factors, including the type of culture medium, pH, temperature, nutrient levels, and environmental conditions. The culture medium is particularly important as it delivers the nutrients essential for the mycelium's development. Chang and Miles (2001) emphasized that selecting the right medium can speed up mycelial growth and ensure consistent, high-quality production throughout the year.

2.1.1 Effect of temperature on mycelial growth

Temperature plays a crucial role as an environmental factor influencing the mycelial growth of fungi. Oyster mushrooms are capable of growing well in moderate temperature conditions, typically between 18°C and 30°C (Mejía and Albertó, 2013).

Lovkesh et al. (2006) investigated how temperature and pH influenced the interaction between *P. sajor-caju*, *P. florida*, *P. flabellatus* and *T. viride*. Their study focused on how deviations from 30°C affected the percentage of growth inhibition in the *Pleurotus* species.

According to Hoa and Wang (2015), a temperature of 28 °C is ideal for the growth of *P. ostreatus* and *P. cystidiosus*. These temperature preferences indicate that *Pleurotus* species grow best during the warmer months—particularly summer and autumn—in tropical and subtropical climates. This seasonal adaptability presents a valuable opportunity to expand oyster mushroom farming in low-income and developing regions (Oei, 1991; Kashangura, 2008)

Sardar et al. (2015) also noted that when *Pleurotus* species were grown aseptically on PDA across various temperature ranges, most of them showed optimal growth at 25°C.

In a study by Naresh Kumar Bhadana et al. (2020), the radial mycelial growth of four *Pleurotus* species—*P. florida*, *P. flabellatus*, *P. eryngii*, and *P. djamor*—was examined under temperatures ranging from 20°C to 35°C. The results showed that *P. djamor* and *P. eryngii* achieved peak growth (90 mm) at 29°C, while *P. florida* followed closely with 83.5 mm at the same temperature. *P. flabellatus*, however, reached its maximum growth (78 mm) at 26°C and did not grow at 35°C. The poorest performance was recorded at 32°C for *P. flabellatus* (40.5 mm) and at 20°C for *P. djamor* (42 mm).

2.1.2 Effect of different pH on mycelial growth

According to Mukherjee and Nandi (2000), the most favorable starting pH for submerged culture was found to be 6.4 for *P. florida* and 6.2 for *P. citrinopileatus*.

Singh et al. (2000) explored the influence of different starting pH levels in the nutrient medium on the growth of *P. flabellatus* and *P. ostreatus*. Their findings indicated that *Pleurotus* species showed the best mycelial development when the pH was maintained between 5 and 8.

Ram and Pant (2001) found that *Pleurotus sajor-caju* and *P. flabellatus* achieved optimal mycelial colonization at a pH of 6.0 across all three substrate types tested. In a related study, Han et al. (2015) noted that *P. flabellatus* thrived within a pH range of 4.0 to 7.0, with the most favourable growth observed between pH 5.5 and 6.0. These results

highlight the importance of maintaining pH levels tailored to specific species to enhance mycelial growth in oyster mushroom cultivation.

pH is a key factor in the effective cultivation of oyster mushrooms. According to Khan et al. (2013), most mushroom species grow best in conditions that are neutral to slightly alkaline. In line with this, Miles and Chang (1997) highlighted that pH influences metabolic activities, which in turn play a vital role in how effectively mushrooms absorb and utilize nutrients.

Khan et al. (2013) studied oyster mushroom (*Pleurotus* spp.) cultivation on cotton waste, adjusting lime levels to 0%, 2%, 4%, and 6% of the substrate's weight. These treatments resulted in corresponding pH values of 7.2, 7.8, 8.2, and 8.7. The results indicated that slightly alkaline conditions favoured mushroom growth, with the highest yields recorded at pH levels of 7.2 and 7.8, both producing equally strong results.

According to Singh and Singh (2018), the fastest and most extensive mycelial growth occurred at a pH of 7.5 after eight days of incubation. They also observed that deviations above 7.5 or below 6.5 in pH levels resulted in a steady reduction in growth.

Pant et al. (2020) evaluated mycelial development across five pH levels and observed the highest growth at pH 7. Beyond this point, as the pH increased to 8 and 9, a gradual reduction in growth was noted.

2.1.3 Effect of different carbon sources on mycelial growth

In a study by M. K. Bhattacharjee and N. Samajpati (1989), the impact of various carbon sources on mycelial development in *Pleurotus* species was investigated, using 2.5% glucose as the baseline. The results showed that complex sugars like starch and dextrin, along with D-xylose, supported better growth than glucose alone. *P. ostreatus* exhibited optimal growth on starch, glucose, and maltose, whereas *P. flabellatus* favored mannose, fructose, and glucose. For *P. tuber-regium*, fructose led to the highest growth, followed by mannose and cellobiose. These differences in carbon source preference were linked to inherent physiological variations among species and strains.

Hoa and Wang (2014) investigated how different carbon sources influenced the mycelial growth of two oyster mushroom species, *Pleurotus ostreatus* and *Pleurotus cystidiosus*, using potato agar (PA) as the base medium. They supplemented the medium with 2%

concentrations of various sugars, including glucose, dextrose, fructose, maltose, sucrose, and molasses. Additionally, they evaluated the effects of varying sucrose levels, ranging from 1% to 10%, on fungal growth.

Ha Thi Hoa and Chun-Li Wang (2015) evaluated how various carbon sources affected the mycelial growth of two oyster mushroom species, *Pleurotus ostreatus* and *Pleurotus cystidiosus*. Their study revealed that *P. ostreatus* exhibited the highest growth on glucose (8.60 cm), followed closely by sucrose (8.54 cm) and molasses (8.34 cm). In contrast, *P. cystidiosus* grew best with glucose (3.50 cm), sucrose (3.54 cm), and dextrose (3.44 cm). These observations highlight the necessity of understanding each species' specific carbon source preferences to improve cultivation practices.

Kumar et al. (2018) assessed the impact of eight different carbon sources—glucose, dextrose, fructose, maltose, mannitol, lactose, sucrose, and starch—on the radial mycelial growth of *Pleurotus florida* and *Pleurotus sajor-caju*. Their study revealed that *P. florida* exhibited the most pronounced growth when fructose was used, followed by starch, sucrose, and mannitol. In contrast, *P. sajor-caju* showed maximum growth with starch, while sucrose, mannitol, and fructose also contributed notably to its development.

2.1.4 Effect of different nitrogen sources on mycelial growth

H. C. Srivastava and Zakia Bano (1969) highlighted the crucial role of nitrogen sources in the growth of *Pleurotus flabellatus*. Among the compounds evaluated, ammonium citrate stood out as the most beneficial, producing the greatest mycelial biomass and protein content, particularly at a concentration of 2 g/L. In contrast, other nitrogen forms—such as ammonium sulphate, nitrate, and chloride—were less effective, likely due to their tendency to lower the medium's pH. Asparagine allowed for moderate growth, whereas ammonium tartrate resulted in limited development, also linked to increased acidity. Overall, ammonium citrate proved to be the most suitable nitrogen source for enhancing both growth and protein synthesis.

Nitrogen plays a crucial role in the formation of essential biological molecules like proteins, nucleic acids, purines, pyrimidines, and polysaccharides (Drozdowski et al., 2010; Abdullah et al., 2015). It also contributes structurally, as it is a fundamental element of fungal cell walls, which are primarily made up of β-(1–4)-linked N-acetylglucosamine units (Miles and Chang, 1997). This necessary nutrient can be provided through sources

such as ammonium nitrate or various organic nitrogen compounds (Chang and Miles, 2004; Gil-Ramírez et al., 2013).

Hesami et al. (2014) investigated how asparagine, glutamine and glycine influenced the growth of *Agaricus bisporus*. Their study revealed that supplementing the growth medium with 0.15% asparagine led to a 117% increase in mycelial growth. According to Kumar et al. (2018), the use of ammonium chloride, ammonium nitrate, and ammonium phosphate significantly promoted the growth of *Pleurotus florida* and *Pleurotus sajor-caju*. In a related study, Upadhyay et al. (2002) found that the presence of readily absorbable amino acids or proteins greatly enhanced fungal mycelial growth.

2.1.5 Effect of different culture media on mycelial growth

Nasim et al. (2001) evaluated the effects of three different culture media on the mycelial growth of *P. ostreatus*, *P. sajor-caju*, *P. cystidiosus*, and *V. volvacea*. Among the tested media, malt extract agar proved to be the most favorable for growth, while potato dextrose agar (PDA) showed the least support for mycelial expansion.

In a study conducted by Baliyan (2008), the mycelial development of several *Pleurotus* species—*P. florida*, *P. flabellatus*, *P. sajor-caju*, *P. fossulatus*, and *P. sapidus*—was assessed across different media. The results showed that malt extract agar (MEA) significantly outperformed both potato dextrose agar (PDA) and wheat straw extract agar (WSEA) in supporting fungal growth.

Rawte and Diwan (2011) investigated biomass production in five *Pleurotus* species—*P. florida*, *P. sajor-caju*, *P. eous*, *P. flabellatus*, and *P. sapidus*—using various culture media, including Richard's broth, Asthana Hawker's medium, Czapek's Dox, and potato dextrose medium. Among the species tested, *P. florida* recorded the highest biomass output at 1.86 g, whereas the lowest yield (1.15 g) was noted for both *P. florida* and *P. eous* in Asthana Hawker's medium. Despite the lower yields, Asthana Hawker's medium still proved more effective than PEA in supporting fungal growth.

Bhadana (2014) conducted a study on four Pleurotus species—*P. florida*, *P. djamor*, *P. flabellatus*, and *P. eryngii*—using eight different culture media. The findings showed that the highest radial growth (90.00 mm) was achieved in *P. djamor* on oat extract agar, *P. florida* on potato dextrose agar, *P. eryngii* on pearl millet extract agar, and *P. flabellatus* also on pearl millet extract agar.

According to Yadav (2014), *P. flabellatus* achieved the greatest radial growth of 90.00 mm when grown on barley extract agar. Slightly lower growth was recorded on oat agar (88.50 mm) and pearl millet extract agar (82.50 mm). The poorest growth occurred on potato dextrose agar, where the radial spread was limited to 76.00 mm.

According to Kumar (2015), *P. florida* exhibited the highest radial growth rate on black gram extract agar medium (11.25 mm/day), with pigeon pea extract agar medium showing slightly lower growth (10.78 mm/day). In contrast, for *P. flabellatus*, the greatest radial growth was recorded on pigeon pea extract agar (11.25 mm/day), followed closely by black gram extract agar (10.90 mm/day).

2.1.6 Effect of different grains on spawn preparation

Thulasi et al. (2010) observed that for spawn production, sorghum and paddy grains were tested, with *P. florida* achieving complete colonization by day 16. Among the two, sorghum proved to be the more effective substrate.

Mbogo et al. (2011) investigated the effect of various cereal grains on the spawn development of *Pleurotus* species. In their study, maize, wheat, and millet were each used in four replicates as substrates for cultivating mother spawn of *P. ostreatus*, with mycelial growth monitored every three days. The results showed clear differences among the grains—maize supported the most vigorous growth, wheat resulted in moderate mycelial expansion, while millet produced the least growth.

According to Sahu et al. (2014), sorghum and wheat grains facilitate rapid spawn development in *Pleurotus eous*, whereas maize results in the highest mushroom yield. Paddy provides moderate performance, while lathyrus and soybean were found to be unsuitable. In summary, sorghum and wheat are optimal for quick spawn growth, and maize is the most effective for achieving maximum yield.

Saurabh et al. (2014) evaluated the mycelial growth of *Pleurotus flabellatus* on various grain substrates using bottle cultures. Among the grains tested, oat supported the most robust growth, followed by barley. Maize facilitated moderate mycelial development, while wheat resulted in the least growth performance.

Md Mijan Hossain (2018) study assessed the effect of five grains on *P. sajor-caju* spawn development. Ragi showed the highest mycelial growth (5.5 cm on day 7 and 12.25 cm

on day 14), followed by sorghum, wheat, and paddy, while maize showed the least. These results are consistent with previous studies by Shah et al. (2004) and Pathmasini et al. (2008), who also reported faster spawn growth in ragi (kurakkan) compared to other grains.

Varsha et.al (2019) examined that both sorghum and wheat grains were effective for spawn production of *Pleurotus* spp., but sorghum grains often led to faster spawn development and higher yields, making it slightly more favourable.

2.2 Yield attributes

Rajarathnam et al. (1986) observed that paddy straw serves as a suitable substrate for cultivating *Pleurotus flabellatus*. Supplementing with organic nitrogen compounds — such as cotton seed, wheat bran, or casein—after the spawn run notably enhanced yield and protein content. Conversely, applying acid or alkali treatments to the straw and supplementing with glucose showed no positive effect. Ferrous sulfate had a mild yield boosting effect, while early nitrogen additions and the use of urea negatively impacted mushroom growth.

Islam et al. (2009) study found that mango and shiris sawdust were the best substrates for growing *Pleurotus flabellatus*, yielding the highest mushroom production and best cost returns. Mango sawdust produced the largest and heaviest mushrooms, while coconut sawdust performed the worst. Overall, substrate type had a significant impact on growth, yield, and profitability.

Thomas et al. (2014) assessed the yield potential of *Pleurotus pulmonarius* and *Pleurotus eous* grown on various lignocellulosic materials, including paddy straw, banana leaves, sawdust, and coffee husk. Their findings revealed that *P. eous* recorded its highest yield and biological efficiency on sawdust (885.6 g and 88.53%), whereas *P. pulmonarius* showed optimal performance on paddy straw, producing 866.9 g with a biological efficiency of 87.13%.

Hoa et al. (2015) study found that corncob (CC) and sugarcane bagasse (SB) were more effective substrates than sawdust (SD) for growing *Pleurotus ostreatus* and *P. cystidiosus*. Substrates with 100% CC or SB produced higher yields, larger mushrooms, and better nutritional content, including more protein and minerals. These benefits were linked to their lower carbon-to-nitrogen (C/N) ratios. In contrast, 100% SD had the poorest results.

While CC delayed the first harvest slightly, it gave the highest mushroom weight and protein content. Overall, CC and SB are better alternatives to sawdust for oyster mushroom cultivation.

According to Sitaula et al. (2018), paddy straw by itself proved to be the most effective substrate for cultivating *Pleurotus ostreatus*, resulting in the quickest growth, highest biological efficiency (96.3%), and largest mushroom size. Although the combination with maize cob produced a greater fresh yield, it had a lower efficiency. Substrates mixed with sawdust and sugarcane bagasse showed the poorest performance overall.

Maheswari et al. (2020) assessed the impact of three agro-waste substrates—corn mixture (T1), paddy + ragi straw (T2), and sugarcane bagasse (T3)—on *Pleurotus ostreatus* cultivation. Among them, sugarcane bagasse delivered the highest yield (761.5 g), while paddy and ragi straw achieved the greatest biological efficiency (92.08%) and fruiting body count. In contrast, the corn-based mix showed the weakest performance. The study highlights paddy–ragi straw as ideal for efficiency and sugarcane bagasse for yield, presenting both as cost-effective, locally available options for sustainable mushroom farming.

Muswati et al. (2021) investigated the effects of various substrate combinations on *Pleurotus ostreatus* production. Among the seven treatments, cotton waste (Trt1) yielded the best results, with the highest yield (1.29 kg) and biological efficiency (86.15%). A close second was the mix of cotton husk, wheat straw, and baobab fruit shells (Trt4), which also supported fast mycelial growth, strong water retention, and superior mushroom quality. In contrast, wheat straw and baobab shells alone resulted in significantly lower performance

Chapter 3: Materials and Methods

This chapter provides a comprehensive summary of the materials, methodologies, and experimental techniques employed to achieve the objectives of the planned laboratory study. The research was conducted during the 2024–25 academic session at the Department of Plant Pathology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur.

3.1 Experimental site

All research activities involving the *Pleurotus flabellatus* were carried out in the laboratory and mushroom cultivation room of the Department of Plant Pathology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya. The institution is situated at the Mohanpur campus in West Bengal, around 53 kilometres away from Kolkata**.**

3.2 Cleaning, washing and sterilization

Glassware such as petri dishes, conical flasks, test tubes and other items manufactured by 'Borosil' were used throughout the experiments. Initially, these were soaked for 24 to 48 hrs in a cleaning solution containing sulfuric acid and potassium dichromate, followed by thorough scrubbing, washing with detergent, and rinsing with tap water. The cleaned glassware was then dried at 60°C and sterilized using a hot air oven at 180°C for two hours. Instruments including the cork borer, scalpel, blades, scissors, and inoculation needle were disinfected with 95% alcohol and sterilized by flaming. The PDA media were autoclaved at 121°C and 15 psi for 20 minutes to ensure sterilization.

3.3 Mother culture

The mother culture of *Pleurotus flabellatus* was sourced from ICAR-DMR, Solan. For experimental use, the pure culture was preserved at 4°C. To maintain its viability and prevent contamination, the culture was routinely sub-cultured on slants in test tubes and on Petri plates after every 15 days interval.

3.4 Experimental design and statistical data analysis

For cultural studies, 5, 7, and 10 treatments were laid out for carbon sources, pH, and temperature, respectively, each replicated 5 times. For optimum media selection and nitrogen sources, 3 treatments were designed, each replicated 7 times.

In case of morphological studies and post-harvest studies for 3 substrates, with 5treatments, each were replicated 7 times.

Online agriculture data analysis tool (OPSTAT) was used to analyse the statistical data through one-factor, two-factor, and three-factor data analysis system. The standard methodology for CRD was used to examine all of the experimental data. Calculating the critical difference at a 5% probability level.

3.5 Materials

3.5.1 Glass wares

Borosilicate glassware was immersed overnight in a cleaning solution containing potassium dichromate and sulfuric acid, followed by sterilization in a hot air oven at 180°C for two hours. To ensure aseptic conditions, culture media and substrate were autoclaved at 15 psi for 20 minutes

3.5.2 Polypropylene bags

Polypropylene bags measuring 30 × 45 cm were used to prepare mushroom beds. After spawning, the bags were hung at equal distances on horizontal bamboo poles to ensure proper spacing and aeration.

3.5.3 Substrate for spawning

The experiment was conducted by using three different substrates viz paddy straw and banana leaves which was procured from local farmers and vendors which were chopped into 2–3 cm pieces, and sawdust was obtained from nearby wood shop suppliers.

3.5.4 Equipment and apparatus

The cultures tubes, were incubated in a BOD incubator maintained at 25 ± 2°C. After sub culturing, the culture tubes were kept in a refrigerator at 4°C for long term storage. All necessary materials were weighed using an electronic balance. Prior to starting the experiments, the equipment was calibrated based on the requirements of the study. Inoculation procedures for cultural examinations were conducted inside a laminar airflow cabinet, and sterilization of glassware, grain spawn, and substrates was conducted using an autoclave.

3.5.5 Meteorological data

A room thermometer was employed to monitor the daily maximum and minimum temperatures, along with morning and evening humidity levels, within the mushroom cultivation room. These climatic observations were evaluated to determine their effect on yield and associated growth parameters, and conclusions were based on the collected data.

3.5.6 Source of materials

Grains such as jowar, bajra, and wheat, used for spawn preparation, were procured from local suppliers. The main substrates utilized for mushroom cultivation including paddy straw, banana leaves, and sawdust were collected from nearby farms and vendors. Laboratory chemicals like ethanol, formalin, calcium carbonate ($CaCO_3$), calcium sulphate ($CaSO_4$), PDA, MEA, V8 juice agar, NaOH, HCl, L-asparagine, ammonium chloride, ammonium nitrate, starch, glucose, sucrose, maltose, and mannitol, as well as essential materials such as polypropylene bags for spawn production, ropes, scissors, and labelling tags, were made available by the Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya.

3.5.7 Preparation of media

For cultural studies, three cultural media viz. Potato Dextrose Agar (PDA), V8 Juice Agar (V8 JA) and Malt Extract Agar (MEA) were prepared, along with Potato Dextrose Broth (PDB).

1.Potato Dextrose Agar (PDA)

Materials	Amount (gm)
Potato	200.0 gm
Agar Agar	20 gm
Dextrose	20 gm

2.Malt Extract Agar (MEA)

Materials	Amount (gm)
Malt Extract	25.0 gm
Peptone	5.0 gm
Agar Agar	20.0 gm
Distilled water	1000 ml

3.V8 Juice Agar (V8 JA)

Materials	Amount (gm)
V8 Juice Agar Powder	44.3gm
Distilled water	1000 ml

4.Potato Dextrose Broth (PDB)

Materials	Amount (gm)
Potato	200.0 gm
Dextrose	20.0 gm
Distilled water	1000 ml

3.6 Methods

3.6.1 Culture Procurement

The pure culture of (*Pleurotus flabellatus*) was acquired from ICAR-DMR, Solan, and stored at 4°C for future experimental use. To maintain its viability and prevent contamination, the culture was periodically sub cultured into petri plates and test tube slants and routinely propagated in bulk**.**

3.6.2 Cultural Studies

3.6.2.1 Effect of different temperatures on mycelial growth

Mycelial discs measuring 8 mm in diameter were taken from a seven-day-old culture using a cork borer and placed onto sterilized Petri plates (90 mm in diameter) containing PDA medium. The plates were maintained at temperatures of 20°C, 22°C, 24°C, 26°C, 28°C, 30°C and 32°C. Radial growth of the mycelium was recorded on the 3rd, 5th, and 7th days following inoculation.

3.6.2.2 Effect of different pH on mycelial growth

Potato Dextrose Agar (PDA) was prepared with pH levels ranging from 3.5 to 8.0 in 0.5 increments, with adjustments made using NaOH to increase and HCl to decrease the pH, verified using a digital pH meter. After pH adjustment, the medium was sterilized in an autoclave at 121°C and 15 psi for 15 minutes. Once cooled, 20 ml of the sterilized medium was poured into sterile Petri plates. A mycelial disc (8 mm in diameter) was taken from the actively growing a 7-day-old fungal culture and carefully placed at the center of each plate. The cultures were incubated at a temperature of 25 ± 2°C, and the extent of radial mycelial growth was measured on the 3rd, 5th, and 7th day after inoculation.

3.6.2.2 Effect of different carbon sources on mycelial growth

Five types of carbon sources viz dextrose, maltose, sucrose, starch, and mannitol were tested to study their effect on mycelial growth of *Pleurotus flabellatus*. Each source was incorporated into the medium at a 2% concentration by mixing 2 grams of the carbon compound with 2 grams of agar-agar and 20 grams of potato extract in 100 ml of water.

Mycelial discs measuring 8 mm, taken from a 7-day-old culture of *Pleurotus flabellatus*, were inoculated onto the prepared media. Observations on radial growth were taken on the 3rd, 5th, and 7th days after inoculation.

3.6.2.4 Effect of different nitrogen sources on mycelial growth

Three types of nitrogen compounds L-asparagine, ammonium chloride, and ammonium nitrate were selected. Each was precisely measured using a digital balance and added to the base Potato Dextrose Agar (PDA) at five different concentrations: 0.1%, 0.3%, 0.5%, 0.7%, and 0.9%. After preparation, the media were sterilized in an autoclave at 121°C under 15 psi pressure for 15 minutes. Once sterilized, the media were dispensed into clean Petri dishes. A fungal disc, 8 mm in diameter and cut from the edge of a week-old actively growing culture, was placed at the centre of each plate. The cultures were incubated at a temperature of 25 ± 2°C, and the expansion of mycelium was documented on days 3, 5, and 7 post-inoculation.

3.6.2.5 Effect of different culture media on mycelial growth

Three distinct culture media potato dextrose agar (PDA), V8 juice agar (V8) and malt extract agar (MEA) were used to test the best media The media were distributed into sterilized 90 mm Petri dishes and inoculated with 8 mm discs of *Pleurotus flabellatus* mycelium, sourced from an actively growing culture. Radial growth of the mycelium was recorded on the 3rd, 5th, and 7th days after inoculation to assess the growth performance across different media.

3.6.3 Morphological studies

3.6.3.1 Preparation of spawn

The optimum grain for spawn preparation were used viz Jowar, Bajra, and Wheat grains in spawn preparation, following methodologies were followed. The grains were first sieved using a 40-mesh screen to eliminate any unwanted particles. They were then rinsed multiple times with tap water to ensure cleanliness. After washing, the grains were boiled for 15 to 20 minutes, followed by an additional soaking period in hot water lasting approximately 12 to 15 minutes. Once this process was complete, excess water was removed, and the grains were spread over a wire mesh surface to allow air-drying for 1 to 2 hours.

For substrate preparation, 1 kg of wheat grains was thoroughly combined with calcium carbonate and calcium sulphate in a 1:3 ratio. The treated mixture was then portioned into polypropylene bags, each containing 200 grams of grain. These bags were sealed with non-absorbent cotton and sterilized in an autoclave at 121°C and 15 psi for 1.5 hours. After sterilization, the bags were kept undisturbed for 24 hours to allow excess moisture to dissipate and then left to cool overnight. Before inoculation, the bags were exposed to UV light for 15 minutes.

Under sterile conditions, the grains were inoculated with mycelium previously cultured in PDA broth and then maintained at a temperature of 25 ± 2°C. To ensure uniform fungal growth throughout the substrate, the bags were lightly agitated on a weekly basis until the grains were entirely colonized. Throughout the incubation period, observations were made regarding both the time required for colonization and the physical appearance of the developing mycelium. Once full colonization was achieved, the prepared grains were utilized as spawn for inoculating the main cultivation substrates. Separate spawn batches were developed for each treatment to maintain consistency across experimental setups.

3.6.3.2 Substrates preparation and Spawning

To conduct fruiting trials on different substrates, fresh paddy straw, banana leaves, and sawdust free from visible contaminants were selected for mushroom cultivation. The paddy straw, banana leaves were finely chopped into small pieces of 2–3 cm length before being used in the substrate preparation. All three substrates were then soaked in water overnight, followed by pasteurization in hot water at a temperature of 65–70°C. After that, the substrates underwent sterilization in an autoclave at 128°C and 20 psi.

After sterilization, the substrates were evenly spread on a clean polythene sheet and allowed to cool. Once they reached room temperature, the cooled materials were firmly filled into perforated polypropylene bags to ensure adequate air circulation. Inoculation was carried out using the layer spawning technique, applying spawn at varying rates of 1%, 2%, 3%, 4%, and 5%, depending on the treatment group. Each level of spawn application was replicated across seven bags. The tops of the bags were tightly sealed, appropriately tagged, and placed on bamboo shelves for incubation.

3.6.3.3 Preparation of mushroom bed

The spawned bags were placed in a mushroom cultivation room, where the temperature was maintained between 22–25°C and relative humidity between 80–85% to facilitate the spawn run. After the mycelium had fully colonized the substrate, the bags were opened and hung on a bamboo structure using plastic ropes to promote fruiting. Observations were made on several aspects, including the duration of the spawn run, initiation of pinheads, fruiting patterns, and total yield. These observations were then subjected to statistical analysis to evaluate the effects of different treatment levels.

3.6.3.4 Harvesting of the mushrooms

Harvesting was done once the initial primordia developed into fully grown mushrooms. Throughout the cropping period, the cultivation beds were kept moist by watering three times daily. After cutting open the bags, visible pinheads started forming on the surface within one to two days, and the first flush was typically ready for collection within three to five days. Mature mushrooms were picked by gently twisting and pulling them with the fingers before the caps began to upturn, and were placed in polythene bags for collection. Additional flushes usually two to three emerged at intervals of 7 to 10 days. Each cultivation bed remained productive for approximately 45 to 50 days after inoculation.

3.6.4 Post harvesting studies

3.6.4.1 Weighing of Mushroom

Freshly harvested mushrooms were immediately weighed using a single-pan balance. Measurements of pileus diameter and stipe length were recorded, and moisture levels were measured using standard protocols outlined by Ashraf et al. (2013).

3.6.4.2 Yield and biological efficiency (B.E.%)

The total mushroom production was calculated by adding the weights of all fruiting bodies obtained across both flushes. To assess biological efficiency—defined as the quantity of mushrooms produced per unit weight of dry substrate the following calculation method, originally outlined by Chang et al. (1981), was applied:

Biological efficiency (%) $= \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$

This calculation provided a clear measure of how effectively the substrate was converted into mushroom biomass.

3.6.4.3 Moisture content of mushroom

To determine the moisture content, the fruiting bodies were sun-dried for several days until a constant weight was achieved. The drying process continued until no further change in weight was observed, indicating the removal of moisture from the samples.

Moisture content (%) $= \frac{\text{Weight of fresh sample} - \text{weight of dry sample}}{\text{Weight of fresh sample}} \times 100$

3.7 Record of observations

Throughout the entire research period, a range of observations were systematically recorded. These included the radial mycelial growth on different solid media, pH levels and temperature, duration required for spawn development on various grains, time taken for spawn run, along with morphological characteristics such as pileus colour, surface texture, margin shape, and gill type. Additional data collected involved measurements of pileus length and breadth, stipe length, total number of fruiting bodies, and both fresh and dry weights of mushrooms for each replication.

Plate.1 Preparation of Substrate a) Sterilized Paddy Straw b) Sterilized Banana Leaves c) Sterilized Saw Dust d) Adding of Spawn to Substrate

Plate 2. Colonized Mushroom Bags-a) Paddy Straw b) Banana leaves c) Sawdust before mycelial colonisation. d) Paddy Straw e) Banana leaves f) Saw dust after mycelial colonisation g) Pin head Stage h) Watering to Colonised Bag

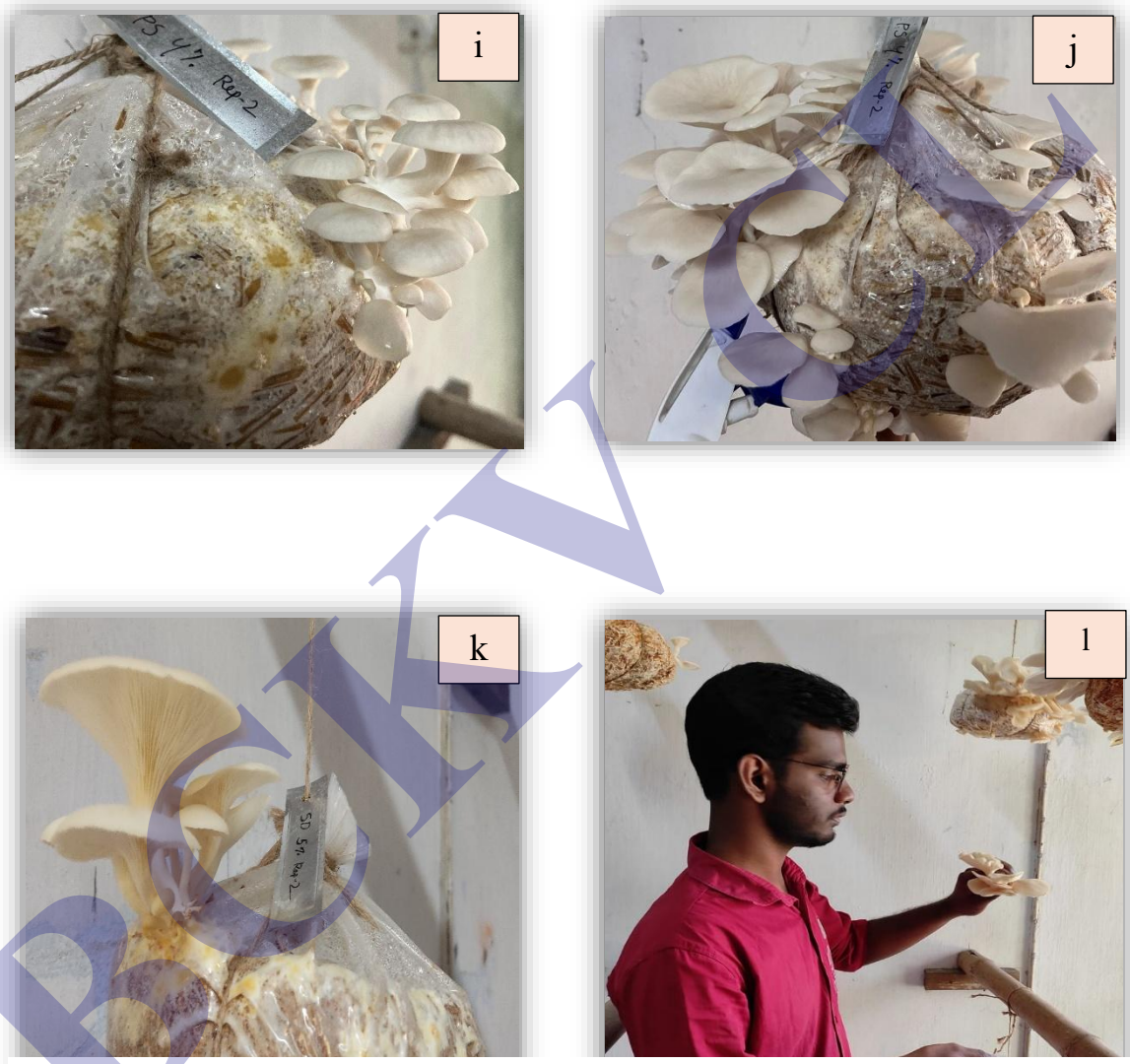


Plate 3. i)Initial Stage Fruit bodies j) & k) Fully matured basidiocarps

l) Harvesting of mushroom

Chapter 4: Results and Discussion

The outcomes of the present research have been systematically categorized and are discussed under the following sections:

4.1 Cultural studies

4.2 Morphological studies and yield attributes

4.3 Post harvest characters

4.1 Cultural studies

A pure culture of *Pleurotus flabellatus* was obtained from ICAR-DMR, Solan. To ensure its continued viability for future use, the culture was regularly sub-cultured every fifteen days. Various cultural traits of the fungus were studied under regulated laboratory conditions, and the results are outlined in the subsequent sections.

4.1.1 Effect of different temperatures on mycelial growth

The radial mycelial growth was examined at seven different temperatures i.e., 20ºC, 22ºC, 24ºC, 26ºC, 28ºC, 30ºC and 32°C.The mycelium of *Pleurotus flabellalatus* grows well at temperature between 24°C and 28°C.Its development was observed to be most favourable at a temperature of 26°C.After seven days of inoculation ,it produced the highest radial growth (71.20 mm).With the rise in temperature from 20°C to 26°C radial mycelium's growth rate gradually increased. Although beyond 26°C, growth was also seen to slow down, however radial growth was less at 20°C (18.mm) compared to 32°C (35mm) at 7 DAI. The temperature 26°C showed significantly higher radial growth i.e. 71.20mm when compared to other temperatures

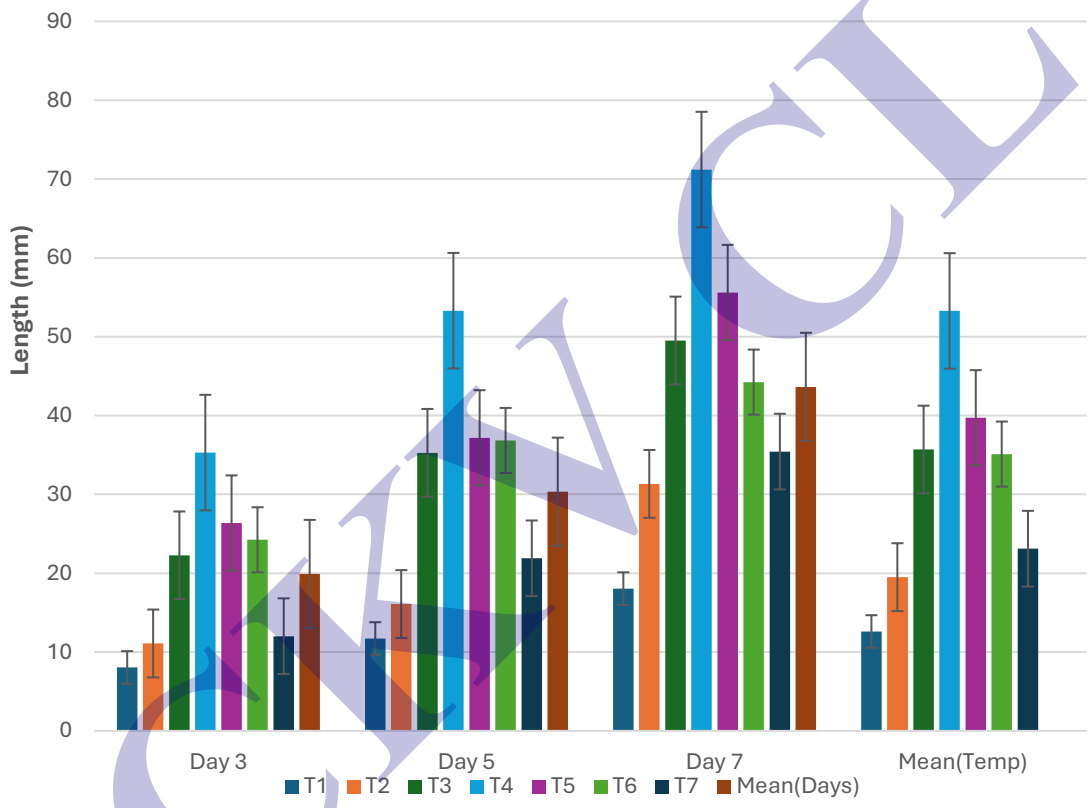
The observations from the present work collaborate with those of Bhadana et al. (2020), who also observed that *Pleurotus flabellatus* achieved its maximum radial mycelial growth at 26 °C. Rout et al. (2015) and Wei et al. (2002) also observed that *P. flabellatus* exhibits optimal growth at a similar temperature range. Neelam et al. (2013) similarly reported that the mycelial growth of *Pleurotus florida*, a species within the oyster mushroom group, was optimal within the temperature range of 25°C to 30°C. According to Chang and Miles (2004), *Pleurotus ostreatus* grows well at elevated temperatures, with

28°C being particularly favourable. The species shows optimal development within the temperature range of 25°C to 30°C.

Table 1. Mycelial growth (mm) of *Pleurotus flabellatus at* different temperatures (3, 5 and 7 DAI)

Temperatures	Mycelial growth (mm) Day 3	Mycelial growth (mm) Day 5	Mycelial growth (mm) Day 7	Mean (Temp°C)
20°C	8.05	11.72	18.05	12.61[f]
22°C	11.09	16.09	31.33	19.50[e]
24°C	22.27	35.27	49.52	35.69[c]
26°C	35.30	53.30	71.20	53.27[a]
28°C	26.37	37.18	55.61	39.72[b]
30°C	24.24	36.84	44.24	35.11[c]
32°C	12.01	21.89	35.43	23.11[d]
Mean (Days)	19.90	30.33	43.63	
	Temperature	**Days**	**Temp × Days**	
SE(m)±	0.22	0.15	0.39	
CD	0.64	0.42	1.11	

Fig-1.Bar diagram representing mycelial growth of *Pleurotus flabellatus* at different temperature on 3,5 & 7 DAI



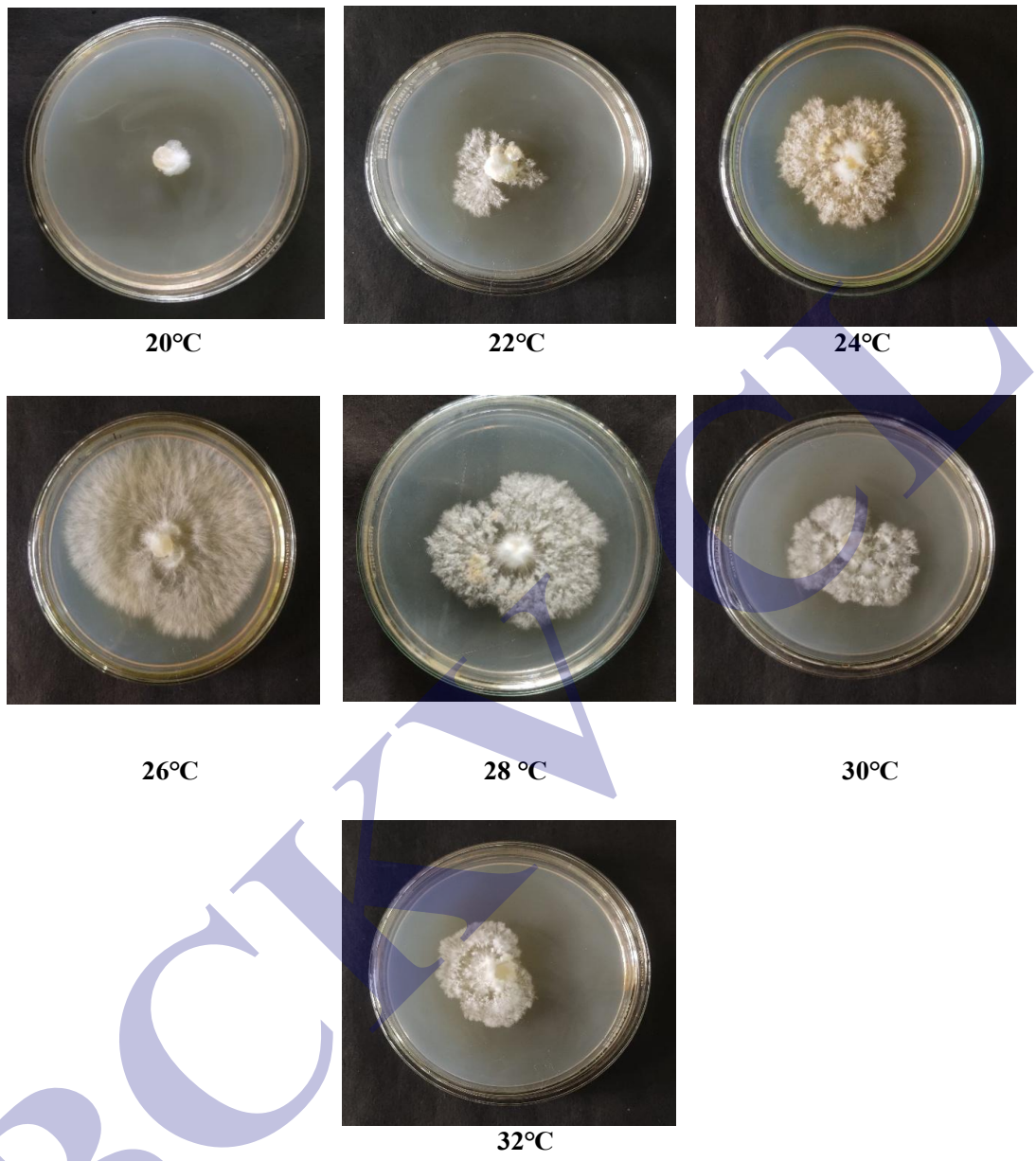


Plate 4: Mycelial growth of *Pleurotus flabellatus* at different temperatures (7 DAI)

4.1.2 Effect of different pH on mycelial growth of *Pleurotus flabellatus*

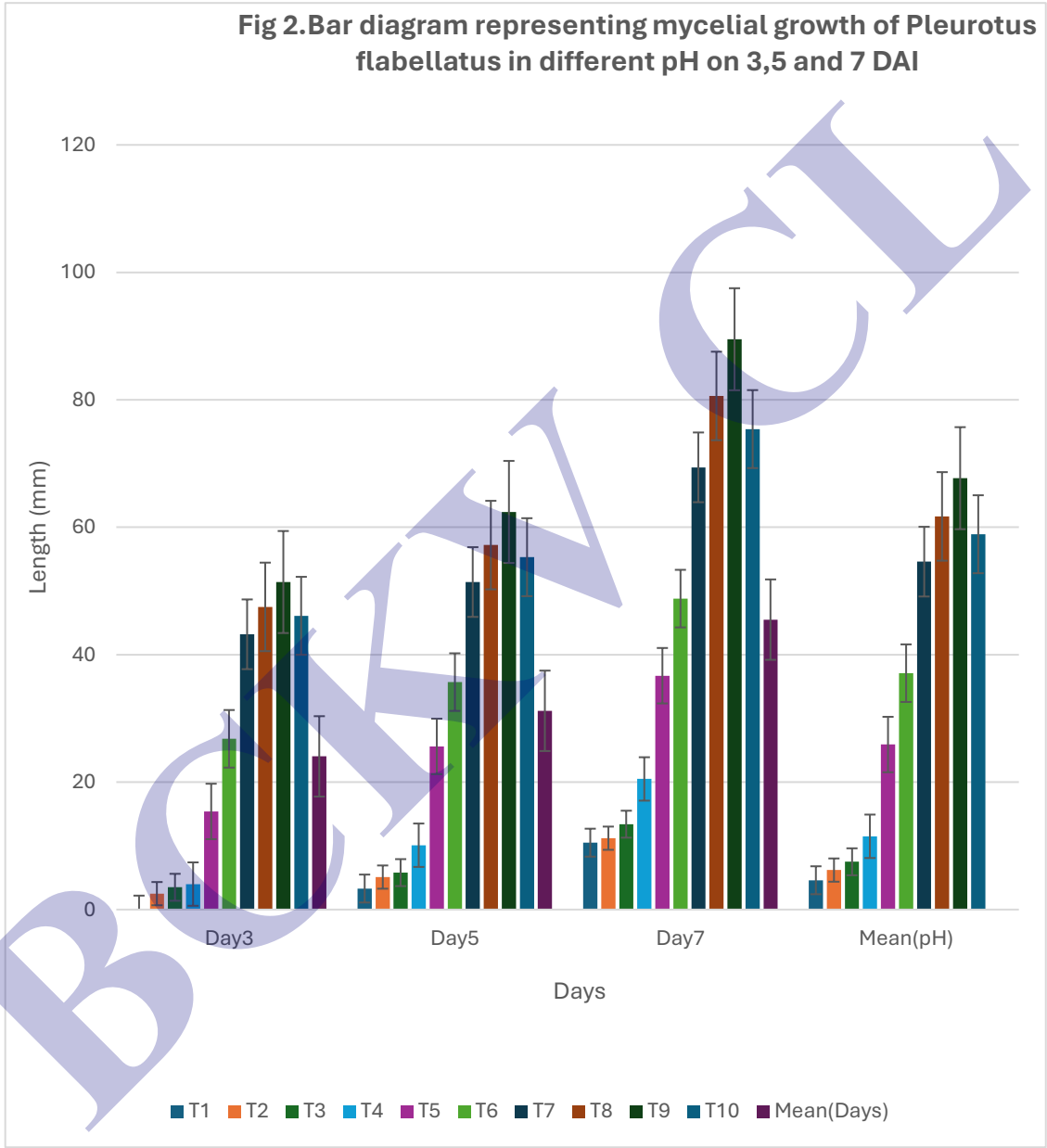
The radial mycelial growth of *Pleurotus flabellatus* was assessed on potato dextrose agar (PDA) medium under ten different pH conditions: 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, and 8.0. Findings revealed that the fungus achieved its maximum growth at pH 7.5, with a radial spread of 89.5mm after seven days of incubation. Growth increased progressively from strongly acidic to neutral pH, with the minimal growth (10.5 mm) observed at pH 3.5. Moderate development occurred at pH values of 6.0 and 6.5, while the most favourable growth was noted at pH 7.0 and 7.5. A reduction in growth was observed when the pH exceeded 7.5.

These results correspond with earlier studies of Singh and Singh (2018), who found that optimal mycelial expansion occurred at pH 7.5 after eight days of incubation, while deviations above or below this value led to a noticeable reduction in growth. Similarly, Pant et al. (2020) evaluated five pH levels and reported that pH 7.0 yielded the most substantial mycelial development, whereas elevated pH values such as 8.0 and 9.0 adversely affected growth. Additional findings by researchers like Aziz et al. (2018), Ahmed et al. (2019) also highlighted pH 7.0 as the most suitable for the growth of various *Pleurotus* species. Furthermore, Gorai and Sharma (2018) emphasized that the ideal pH range for mycelial growth lies between 6.5 and 7.5. Altogether, the data support the idea that *P. flabellatus* performs best in environments that are slightly acidic to neutral.

Table 2: Mycelial growth (mm) of *Pleurotus flabellatus* at different pH (3, 5 and 7 DAI)

pH	Mycelial growth (mm) Day 3	Mycelial growth(mm) Day 5	Mycelial growth (mm) Day 7	Mean (pH)
3.5	No growth	3.3	10.5	4.6^{j}
4.0	2.5	5.1	11.2	6.2^{i}
4.5	3.5	5.8	13.4	7.5^{h}
5.0	4.0	10.1	20.5	11.5^{g}
5.5	15.4	25.6	36.7	25.9^{f}
6.0	26.8	35.7	48.8	37.1^{e}
6.5	43.2	51.4	69.4	54.6^{d}
7.0	47.5	57.2	80.6	61.7^{b}
7.5	51.4	62.4	89.5	67.7^{a}
8.0	46.1	55.3	75.4	58.9^{c}
Mean	24.04	31.19	45.50	
	pH	**Days**	**pH X Days**	
SE(m) ±	0.260	0.142	0.450	
CD	0.728	0.399	1.261	

Fig 2.Bar diagram representing mycelial growth of Pleurotus flabellatus in different pH on 3,5 and 7 DAI



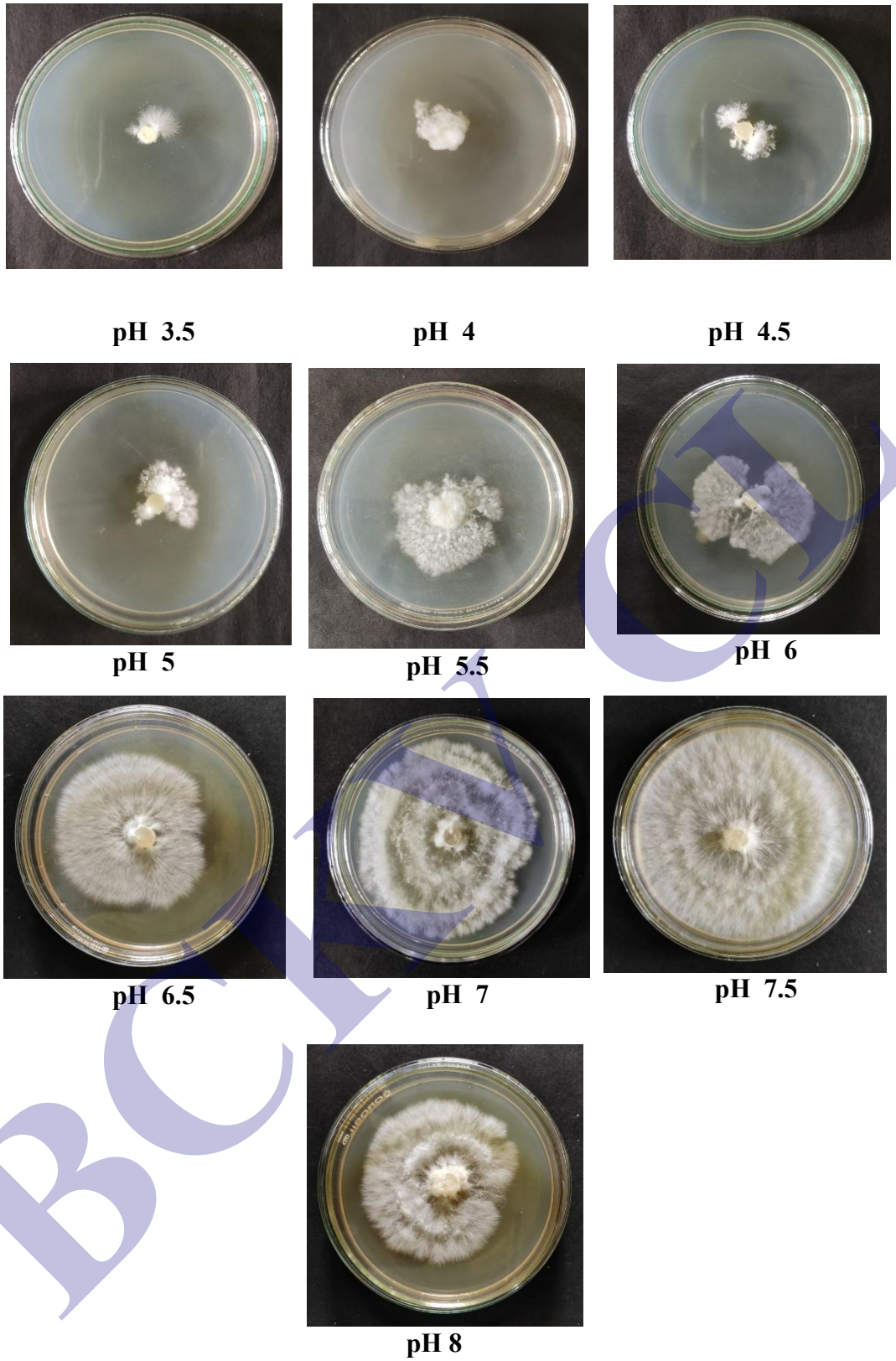


Plate 5: Mycelial growth of *Pleurotus flabellatus* at different pH (7 DAI)

4.1.3 Effect of different carbon sources on mycelial growth of *Pleurotus flabellatus*

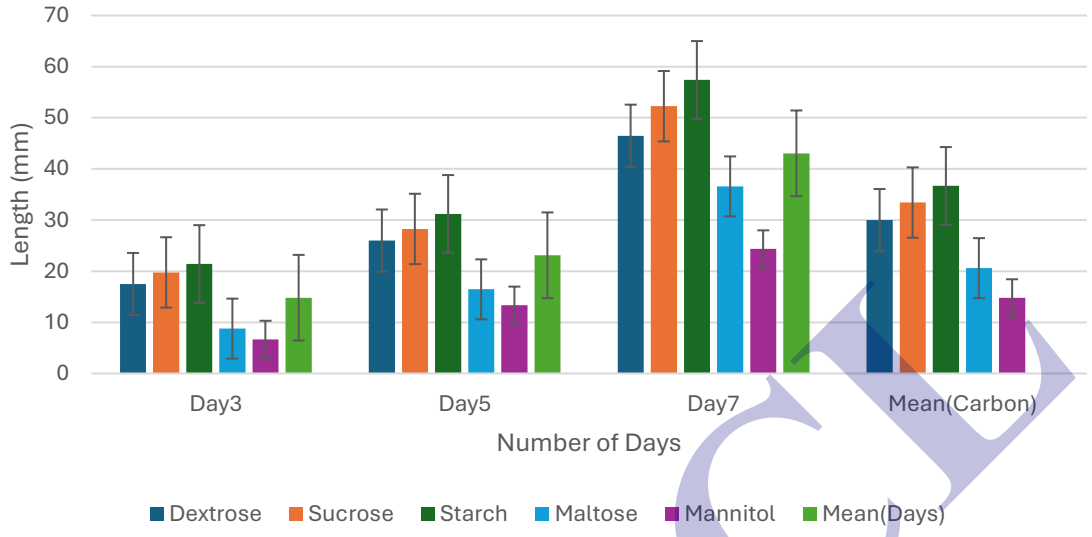
The radial mycelial growth of Pleurotus *flabellatus* responds to various carbon sources, five types—dextrose, sucrose, starch, maltose, and mannitol—were incorporated into the culture medium. The fungal mycelium exhibited distinct growth behaviours depending on the carbon source used. Uniform mycelial growth was observed using different carbon sources, starch exhibited most expansive growth after seven days of incubation (57.40 mm). This was followed by sucrose (52.26 mm), dextrose (46.48 mm), and maltose (36.58 mm), while mannitol proved the least radial expansion, resulting in the lowest radial expansion (24.34 mm).

Carbon substrates significantly influence mycelial biomass formation and the synthesis of functional metabolites during mushroom cultivation (Kupradit et al., 2020). The present observations are consistent with those reported by Debnath et al. (2021), who identified starch as the most effective carbon source for enhancing the biomass of *Pleurotus flabellatus*. Earlier studies by Sakamoto et al. (1978) and Nikon (1979) also emphasized starch as the optimal energy source for *Pleurotus* growth. However, the current results contrast with the findings of Eswaran and Ramabadran (2000) and Hoa and Wang (2015), who reported that glucose and sorbitol were more efficient in promoting biomass accumulation in *Pleurotus* species

Table 03. Mycelial growth (mm) of *Pleurotus flabellatus* at different carbon sources

C-sources	Mycelial growth(mm) Day 3	Mycelial growth(mm) Day 5	Mycelial growth(mm) Day 7	Mean
Dextrose	17.48	25.98	46.48	29.98^{c}
Sucrose	19.76	28.26	52.26	33.42^{b}
Starch	21.40	31.20	57 .40	36.67^{a}
Maltose	8.77	16.45	36.58	20.60^{d}
Mannitol	6.66	13.34	24.34	14.78^{e}
Mean (Days)	14.81	23.11	43.05	
	C-source	**Days**	**Media X Days**	
SE(m) ±	0.204	0.158	0.353	
CD	0.578	0.223	1.001	

Fig 3.Bar diagram representing the mycelial growth of Pleurotus flabellatus in different carbon sources on 3,5 and 7 DAI



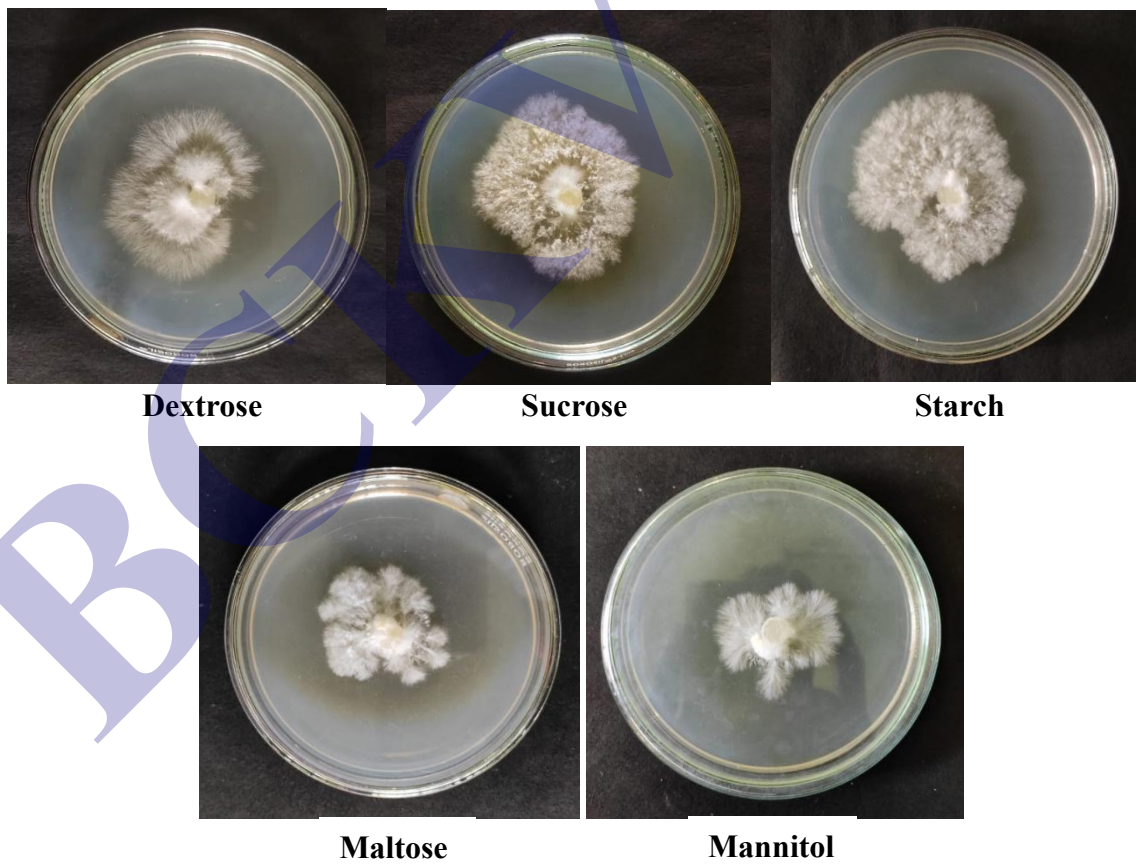


Plate 6: Mycelial growth of *Pleurotus flabellatus* at different Carbon sources (7 DAI)

4.1.4 Effect of different Nitrogen sources on mycelial growth

This study evaluated the effects of three nitrogen sources L-asparagine, ammonium chloride, and ammonium nitrate on mycelial growth. The findings showed that growth responses differed significantly with varying concentrations of these compounds. L-asparagine, in particular, promoted greater mycelial development at a 0.3% concentration compared to 0.1%. In contrast, both ammonium chloride and ammonium nitrate demonstrated a reduction in mycelial growth as their concentrations were increased.

The maximum mycelial development was recorded on PDA medium enriched with L-asparagine, achieving 46.54 mm of radial growth after seven days of incubation followed by ammonium chloride (43.17mm) and ammonium nitrate (31.45mm). Among the concentrations tested, 0.3% L-asparagine promoted the highest growth (46.54 mm), while 0.1% resulted in 42.12 mm and 0.5% decreased significantly to 19.55 mm, showing a downward trend with increasing concentration. For ammonium chloride, the optimal growth occurred at 0.1% (43 mm), followed by 0.3% (34 mm) and 0.5% (18 mm), indicating a similar reduction at higher levels. This declining pattern was also evident with ammonium nitrate.

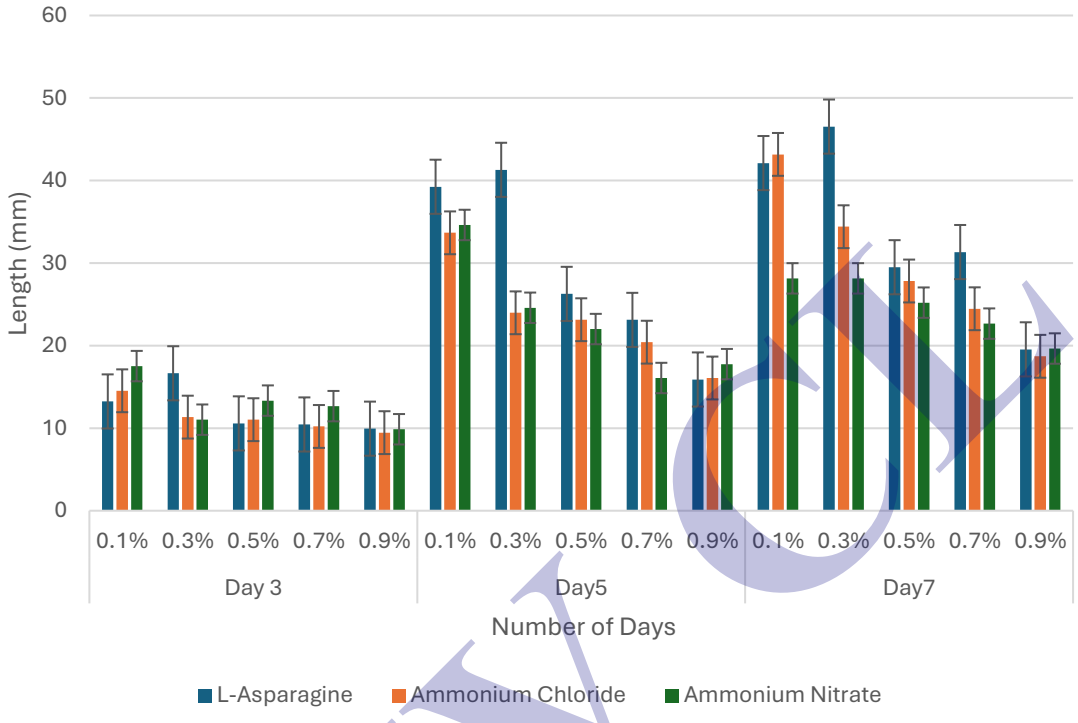
Therefore, it can be inferred that 0.3% L-asparagine and 0.1% ammonium chloride were more efficient in supporting robust mycelial development in the species, whereas 0.1% ammonium nitrate was comparatively less effective in promoting fungal growth.

This study demonstrated that both asparagine and ammonium chloride significantly enhanced the mycelial biomass production of *Pleurotus flabellatus*. The improved growth may be linked to the availability of easily absorbable amino acids or protein compounds, as suggested by Upadhyay et al. (2002). These results align with those of Eswaran and Ramabadran (2000), who identified asparagine as the most beneficial nitrogen source for boosting mycelial development in *Pleurotus* species. However, Debnath et al. (2021) reported differing results, highlighting peptone as the most favourable nitrogen supplement for increasing biomass in *P. flabellatus*

Table 4: Effect of different Nitrogen sources on mycelial growth of *Pleurotus flabellatus* (3, 5 and 7 DAI)

	Mycelial growth (mm) Day 3					Mycelial growth(mm) Day 5					Mycelial growth (mm) Day 7				
N-sources	**0.1%**	**0.3%**	**0.5%**	**0.7%**	**0.9%**	**0.1%**	**0.3%**	**0.5%**	**0.7%**	**0.9%**	**0.1%**	**0.3%**	**0.5%**	**0.7%**	**0.9%**
L-Asparagine	13.24	16.65	10.58	10.45	9.94	39.25	41.30	26.27	23.12	15.90	42.12	46.54	29.50	31.34	19.55
Ammonium chloride	14.53	11.34	11.03	10.21	9.46	33.67	23.98	23.14	20.42	16.08	43.17	34.41	27.83	24.47	18.71
Ammonium nitrate	17.52	16.42	13.34	12.67	9.87	34.62	24.59	22.01	20.80	17.76	31.45	28.15	25.21	22.67	19.65
	Days		**Concentration**		**Days X Conc.**	**N-source**			**Days X N-source**		**Conc. X N-source**		**Days X Conc. X N-source**		
SE(m)±	0.028		0.058		0.101	0.078			0.135		0.174		0.301		
CD	0.093		0.165		0.286	0.218			0.377		0.487		0.843		

Fig 4. Bar diagram representing the mycelial growth of *Pleurotus flabellatus* on different nitrogen sources at different cocentration media on 3,5 and 7 DAI



L-Asparagine **Ammonium Chloride** **Ammonium Nitrate**

Plate 7: Mycelial growth of *Pleurotus flabellatus* at different Nitrogen sources (7 DAI)

4.1.5 Effect of different culture media on mycelial growth

Three different culture media potato dextrose agar (PDA), malt extract agar (MEA), and V8 juice agar (V8 JA) were used to determine the mycelial and pattern of *Pleurotus flabellatus*. After seven days of incubation, malt extract agar supported the greatest radial growth (88 mm), followed by V8 agar (75 mm), while potato dextrose agar exhibited the least growth (54 mm). In general, the mycelium of *P. flabellatus* demonstrated satisfactory growth across all media tested.

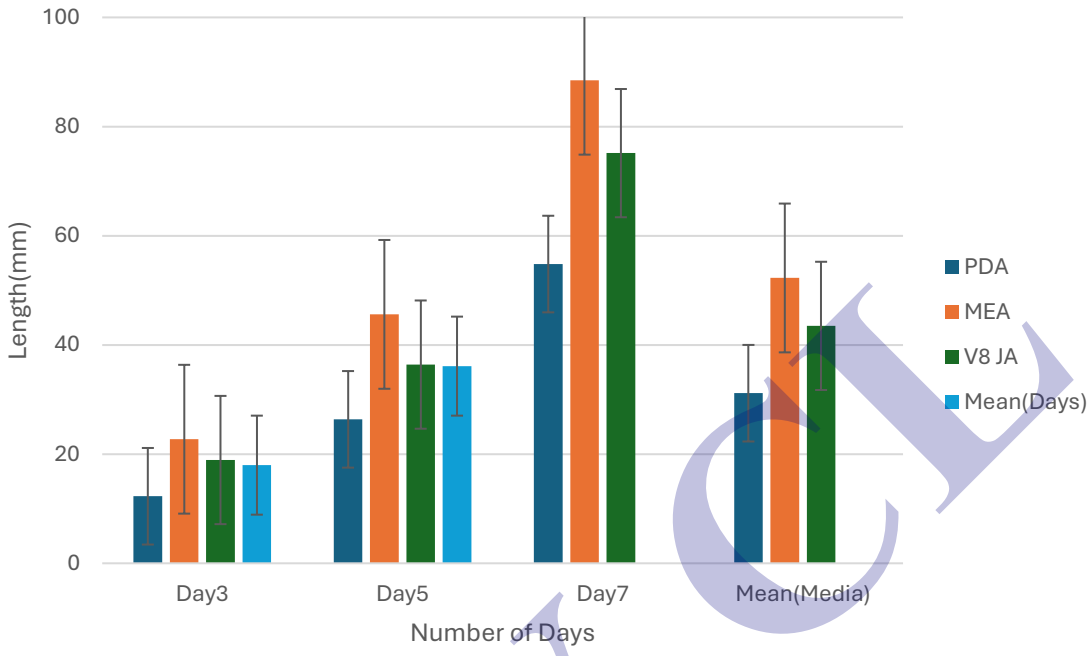
Baral et al. (2018) investigated the mycelial growth of various strains of *Pleurotus flabellatus* and *Pleurotus sajor-caju* using five different culture media: Among them, MEA was found to be the most effective in supporting rapid radial mycelial growth, especially in monokaryotic isolates of *P. flabellatus*, and was thus chosen as the optimal medium for further dikaryotization experiments.

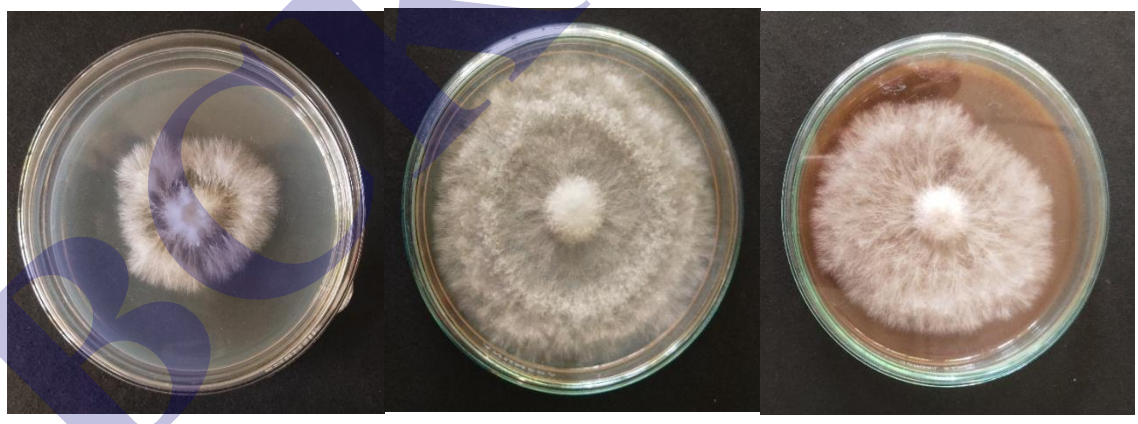
Sharma (2022) observed that Potato Dextrose Agar (PDA) resulted in the greatest radial growth (54.23 mm) and biomass production of *Pleurotus flabellatus*. In contrast, Mishra (2016) reported that barley extract agar supported the maximum mycelial expansion (~90 mm), while PDA broth was the least effective. Our results align with those of Mohd (2012), who tested six different media, identified Malt Extract Agar (MEA) as the most supportive of radial mycelial growth, whereas PDA and water agar showing the lowest growth. Consistent with these observations, Mahadevan and Shanmugasundaram (2018) studied *Pleurotus sapidus* on six media types and concluded that both MEA and PDA were optimal for robust mycelial development.

Table 5. Mycelial growth (mm) of *Pleurotus flabellatus* at different culture media

Culture Media	Mycelial growth(mm) Day 3	Mycelial growth(mm) Day 5	Mycelial growth(mm) Day 7	Mean (Media)
PDA	12.28	26.37	54.82	31.16[c]
MEA	22.73	45.60	88.49	52.27[a]
V8 JA	18.92	36.40	75.14	43.49[b]
Mean (Days)	**17.98**	**36.12**	**72.82**	
	Media	**Days**	**Media X Days**	
SE(m)±	0.324	0.324	0.561	
CD	0.933	0.933	1.617	

Fig 05. Bar diagam representing the mycelial growth of Pleurotus flabellatus on different culture media on 3,5 and 7DAI





PDA **MEA** **V8 JA**

Plate 8: Mycelial growth of *Pleurotus flabellatus* at different culture media (7 DAI)

4.2 Morphological studies and Yield attributes

Fructification trial of *Pleurotus flabellatus* using three different substrates viz Paddy Straw, Banana leaves, saw dust was conducted in a mushroom house where the temperature was kept between 25–28°C and relative humidity ranged from 75% to 85%. The experiment compared the performance of *Pleurotus flabellatus* on three different substrates paddy straw, banana leaves, and sawdust using three types of grain-based spawn: wheat, jowar, and bajra. The layer spawning method was employed, with spawn introduced at different concentrations (1% to 5%). The aim of the study was to assess and optimize the cultivation parameters by monitoring the mushroom's morphological characteristics under these standardized conditions.

4.2.1 Effect of different grains on spawn run

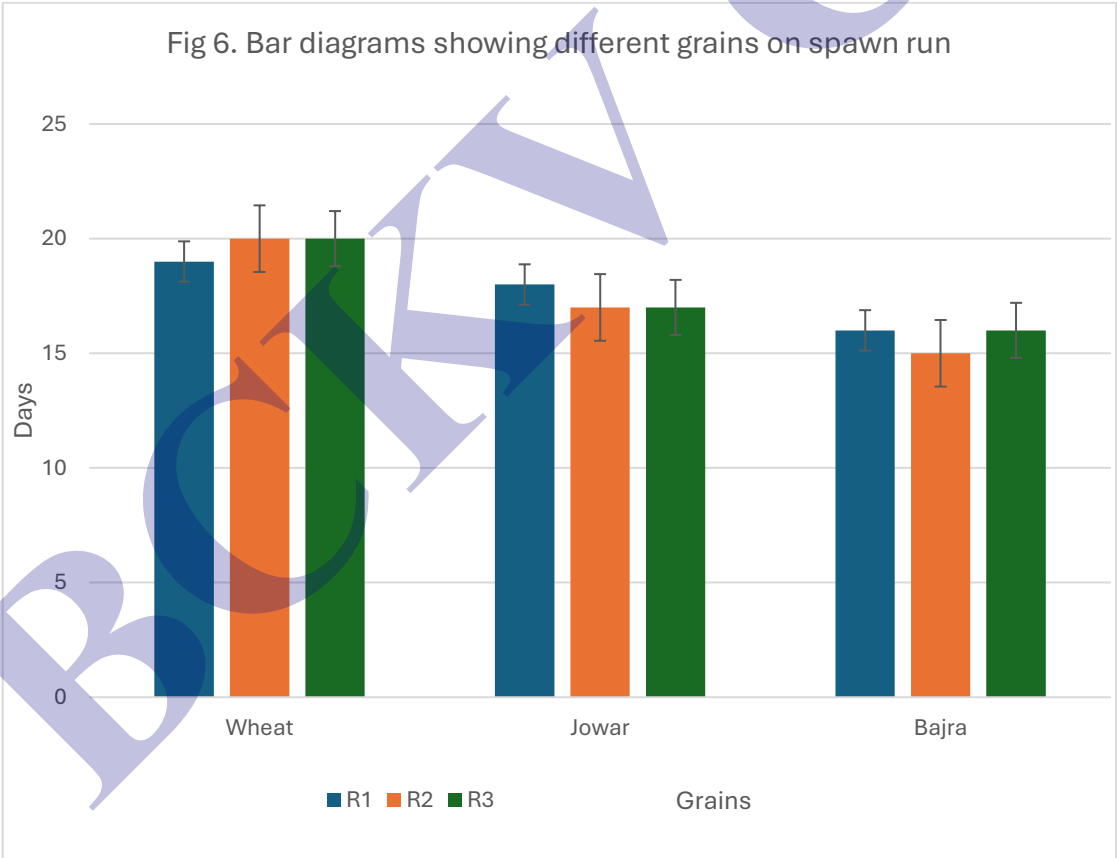
Spawn serves as the most vital component in mushroom cultivation, functioning as the primary inoculum. To assess which cereal grain supports the fastest mycelial colonization, three grains wheat, jowar, and bajra were tested. Among these, bajra exhibited the quickest colonization at 15.33 days, followed by jowar at 16.33 days, and wheat at 19.66 days.

A study by Bhadana et al. (2022) investigated the spawn development of *Pleurotus florida* and *Pleurotus djamor* using six grain types: wheat, bajra, maize, barley, jowar, and oat. For *P. florida*, the fastest growth was observed on jowar (15 days after inoculation), followed by barley (16 DAI) and wheat (19 DAI). In the case of *P. djamor*, bajra supported the fastest colonization (14 DAI), with wheat following at 17 DAI findings that align with the current observations.

Similarly, D. L. Narh et al. (2011) assessed jowar and bajra grains both individually and in combinations (3:1, 1:1, 1:3 ratios). The most rapid mycelial spread occurred with the 3:1 jowar-to-bajra mix (75% J + 25% B), completing colonization in 16 days. Jowar alone took 18 days, which closely matches the results observed in this study.

Table 6: Effect of different grains on spawn run (days) of *Pleurotus flabellatus*

Spawn grain	R1	R2	R3	Mean	SE(m)±	CD
Wheat	18	20	21	19.66[a]		
Jowar	17	16	16	16.33[b]	**0.189**	**0.588**
Bajra	15	16	15	15.33[c]		



4.2.2 Spawn run days in different substrates

The spawn run is the initial stage in mushroom cultivation, during which the fungal mycelium spreads throughout and colonizes the substrate completely. The time taken from inoculation until the substrate is fully covered with white, thread-like mycelial growth is referred to as the spawn run duration. This period can vary based on several factors, including the mushroom species, the nature of the substrate, environmental parameters, and the spawn dosage. In this study, the objective was to examine how different substrate types and spawn concentrations affect the time required for full colonization

For this purpose, spawn was applied at five different concentrations: 1%, 2%, 3%, 4%, and 5%. The observations were recorded and, on an average, *Pleurotus flabellatus* took 13 days in paddy straw substrate followed by banana leaves (18 days) and saw dust (27 days). For all the substrates, as the spawn rate increased, spawn run days was gradually decreased. The difference between three substrate is significant. The lowest spawn run days (11.90 days) was recorded from paddy straw substrate @ 5% and the highest spawn run days (32.50 days) was recorded from saw dust @ 1% spawn rate.

The present findings align with those of Arunesh Kumar et al. (2021), who reported that increasing spawn doses (1% to 5%) significantly reduced spawn run duration of *Pleurotus cornucopiae* on agroforestry residues. The fastest colonization occurred on wheat straw (14 days) and paddy straw (15.33 days) at 5% spawn, while sawdust showed the slowest growth (up to 34.33 days at 1%). Overall, higher spawn rates led to quicker mycelial colonization, with wheat and paddy straw being the most efficient substrates.

The results of this study align with earlier research, indicating that paddy straw remains one of the most effective materials for cultivating *Pleurotus flabellatus*. Prior investigations, including those by Swamydhas and Lakshmanan (2019), also recognized paddy straw as a highly favourable substrate for mushroom growth. Supporting evidence comes from Kumar et al. (2019), who found that *Pleurotus ostreatus* thrived best on paddy straw. Additionally, findings by Jegadeesh et al. (2018) and Sharma et al. (2013) emphasized that rice straw consistently produced superior yields and biological efficiency across several *Pleurotus* varieties.

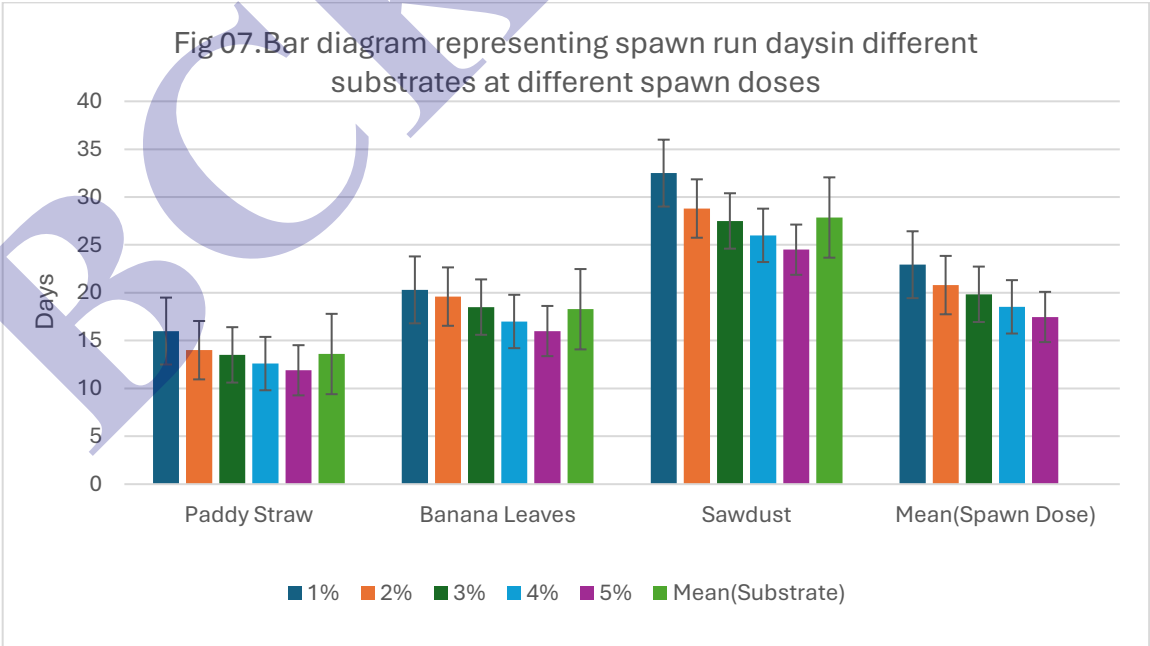
Taken together, these findings indicate that paddy straw provides a favourable nutrient profile Verma et.al (2024) especially in terms of cellulose and hemicellulose content that

encourages strong mycelial development and mushroom production. Its richness in these components appears to play a key role in improving biological efficiency, establishing paddy straw as a preferred substrate for the commercial growth of *Pleurotus flabellatus*.

Table 7: Spawn run days on different substrates and at different spawn dose (%)

Substrates	Spawn Dose					Mean (Substrate)
	1%	2%	3%	4%	5%	
Paddy straw	16.00	14.00	13.51	12.60	11.90	13.60^{c}
Banana leaves	20.30	19.60	18.50	17.00	16.00	18.28^{b}
Saw dust	32.50	28.80	27.50	26.00	24.50	27.86^{a}
Mean (Spawn Dose)	22.93	20.80	19.84	18.53	17.47	

	Substrate	Spawn dose	Substrate X Spawn Dose
SE(m)±	0.145	0.187	0.324
CD	0.421	0.543	0.941



4.2.3 Fresh weight

Following complete colonization of the substrate, pinheads emerged within 6 to 8 days. These pinheads developed into mature basidiocarps within the next 3 to 4 days. Upon the appearance of pinheads, the cultivation bags were opened and the substrates were regularly moistened by spraying water three times daily to maintain adequate moisture levels until harvesting. The harvested mushrooms were then weighed immediately using a digital weighing balance.

Variations in fresh mushroom yield were observed among the three tested substrates when different spawn concentrations were applied. A notable statistical difference was observed among the three substrates, with paddy straw significantly outperforming the others. It yielded the highest average production of 232.84 g, followed by banana leaves at 175.25 g, and sawdust at 151.83 g. Correspondingly, the biological efficiency was highest for paddy straw (72.66%), compared to 31.54% for banana leaves and 23.20% for sawdust. These findings clearly demonstrate the superior performance of paddy straw in terms of both mushroom yield and biological efficiency.

The maximum yield on paddy straw was recorded at a 5% spawn rate (262.85g) followed by the 4% spawn rate (245.56 g), (table 8) with minimum significant difference between the two. The highest biological efficiencies 82.03 % and 76.6% (table 9) were also recorded at these respective spawn rates.

For sawdust, the 5% spawn rate resulted in the greatest yield (168.22 g) and the highest biological efficiency (25.71%), with minimal variation observed across other spawn concentrations.

In the case of banana leaves, the most substantial yield was achieved at the 5% spawn rate (198.92g), followed by the 4% rate (186.84g), again with minimum statistical difference. The peak biological efficiency (35.81%) was also noted at the 5% spawn level.

The current outcomes are in agreement with previous research by Chanakya et al. (2015), who reported that supplementing paddy straw with biogas digester residue significantly improved biological efficiency, reaching up to 209.22% in *Pleurotus flabellatus*. In a similar vein, Kumar et al. (2019) identified paddy straw as the most effective base material for cultivating *Pleurotus ostreatus*. Supporting evidence from earlier investigations by Jegadeesh et al. (2018) and Sharma et al. (2013) further emphasized

that rice straw consistently delivered superior yields and efficiency across multiple *Pleurotus* varieties.

The current results align with the observations of Arunesh Kumar et al. (2021), who found that increasing the spawn rate from 1% to 5% led to a significant rise in the fresh yield of *Pleurotus cornucopiae* grown on different agroforestry residues. Among the tested substrates, wheat straw delivered the highest yield of 517.33 g per 2 kg at the 5% spawn rate, followed closely by paddy straw with 498.33 g. Conversely, sawdust consistently produced the lowest yields, with a maximum of only 205.33 g at the same spawn rate. On average, yields more than doubled as the spawn rate increased, rising from 210.86 g at 1% to 428.73 g at 5%. These findings underscore the significance of using an appropriate spawn rate and selecting effective substrates—particularly wheat and paddy straw—to enhance mushroom production.

Table 8: Fresh weight (gm) on different substrates and at different spawn doses

Substrates	Spawn dose					Mean (Substrate)
	1%	2%	3%	4%	5%	
Paddy straw	202.04	220.53	233.24	245.56	262.85	232.84[a]
Banana leaves	154.89	163.80	171.84	186.84	198.92	175.25[b]
Saw dust	136.34	144.22	150.79	159.60	168.22	151.83[c]
Mean (Spawn dose)	164.42	176.18	185.29	197.33	209.99	

	Substrate	Spawn dose	Substrate X Spawn dose
SE(m)±	1.403	1.812	3.138
CD	4.073	5.258	9.108

Fig 8. Bar graph representing fresh weight of mushroom in different substrates with different spawn doses

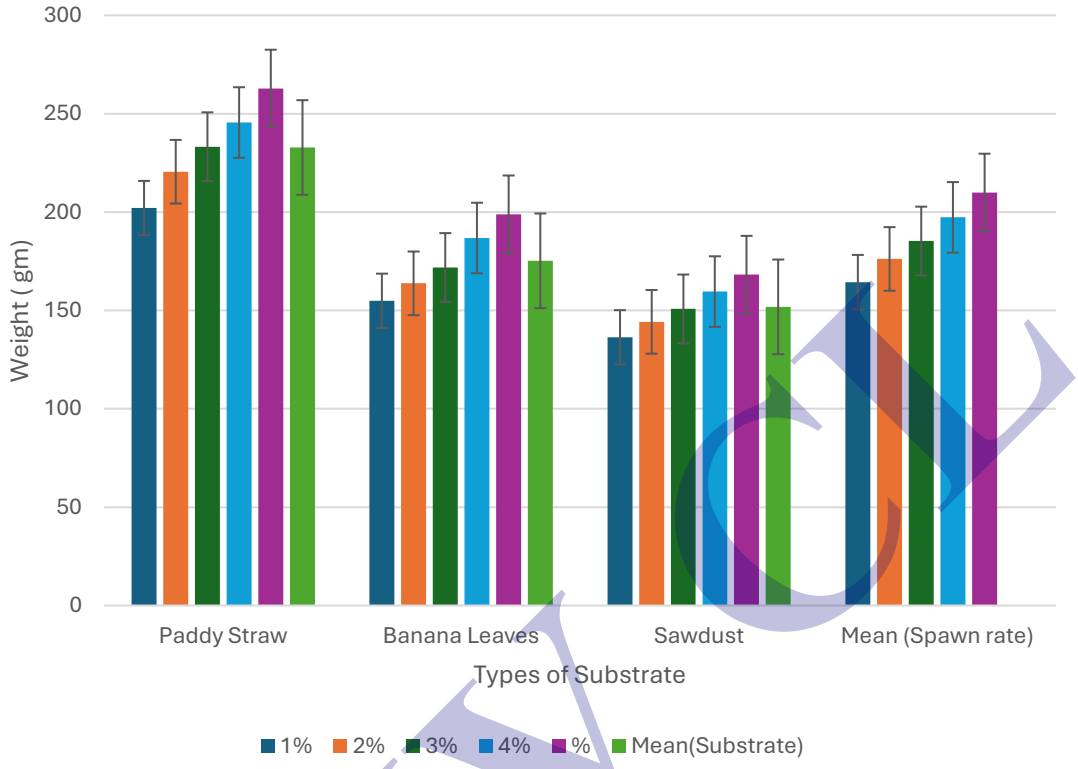


Table 09: Biological efficiency (%) on different substrate at different spawn doses

Substrates	Spawn dose					Mean (substrate)
	1%	2%	3%	4%	5%	
Paddy straw	63.05	68.82	72.79	76.63	82.03	72.66[a]
Banana leaves	27.88	29.48	30.93	33.63	35.81	31.54[b]
Saw dust	20.84	22.04	23.04	24.39	25.71	23.20[c]
Mean (Spawn dose)	37.26	40.11	42.25	44.88	47.85	

	Substrate	Spawn dose	Substrate X Spawn dose
SE(m)±	0.342	0.442	0.766
CD	0.994	1.283	2.222

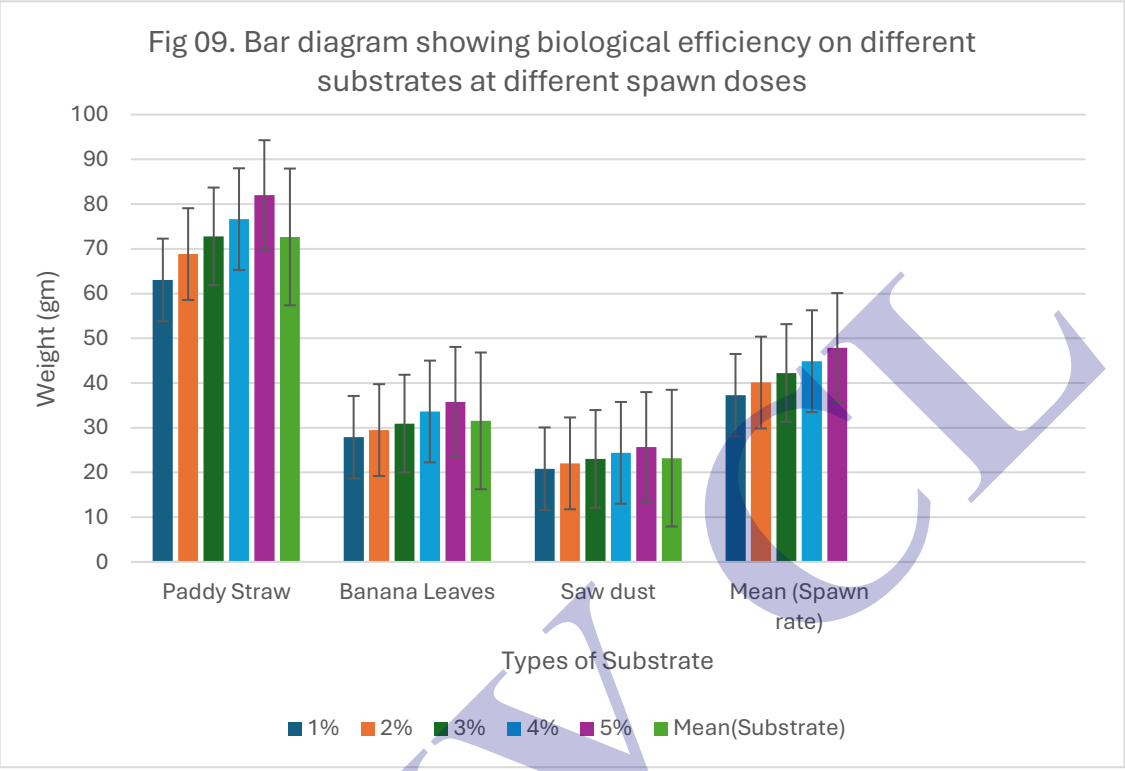


Fig. 9 Fresh Weight of the Fruit Bodies

4.2.4 Number of basidiocarps

The number of basidiocarps varied across the three substrates and different spawn rates. While the differences were not highly pronounced, the maximum number of basidiocarps was recorded on paddy straw at a 5% spawn rate (32), closely followed by the 4% rate (30). For banana leaves, there was a gradual increase in basidiocarp count with higher spawn rates, reaching its peak at the 5% level (30 basidiocarps). In the case of sawdust, the greatest number of basidiocarps (27) was observed at the 5% spawn rate.

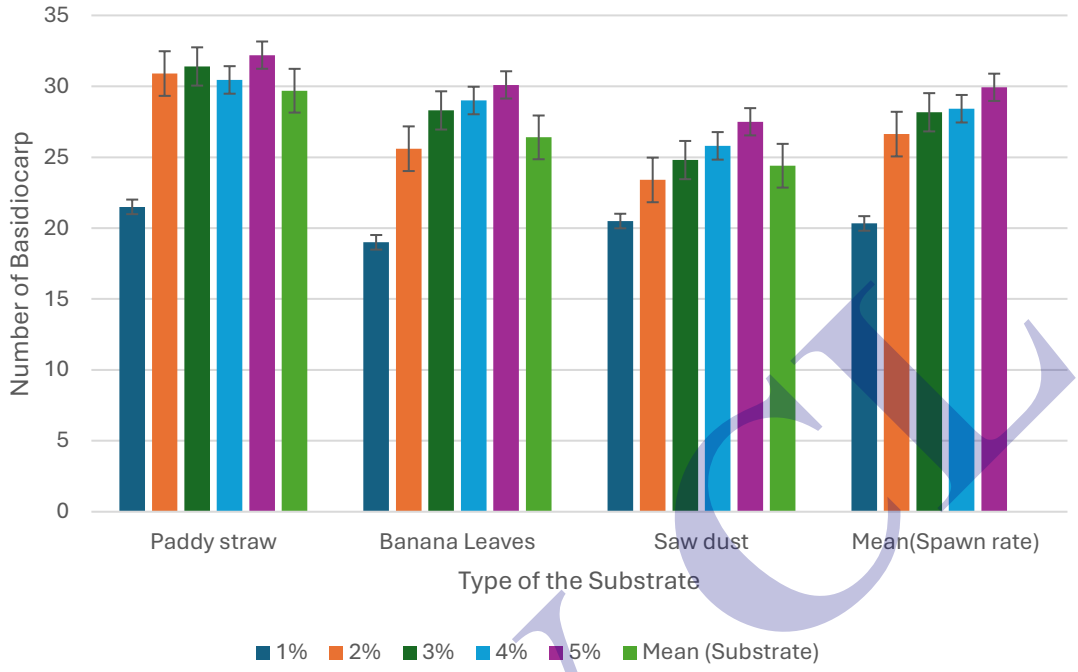
Despite noticeable differences among the substrates, altering the spawn rates to 3%, 4%, or 5% had minimal effect and did not significantly alter the total number of basidiocarps produced.

Table 10: Number of basidiocarps on different substrates at different spawn doses

Substrates	Spawn dose					Mean (Substrate)
	1%	2%	3%	4%	5%	
Paddy straw	21.50	30.90	31.40	30.45	32.20	29.6[a]
Banana leaves	19.00	25.60	28.30	29.00	30.10	26.4[b]
Saw dust	20.50	23.40	24.80	25.80	27.50	24.4[c]
Mean (Spawn dose)	20.33	26.63	28.17	28.42	29.93	

	Substrate	Spawn dose	Substrate X Spawn dose
SE(m)±	0.299	0.386	0.668
CD	0.868	1.120	1.940

Fig10.Bar diagram showing number of basidiocarps on different substrates at different spawn doses



4.2.5 Length of the stipe

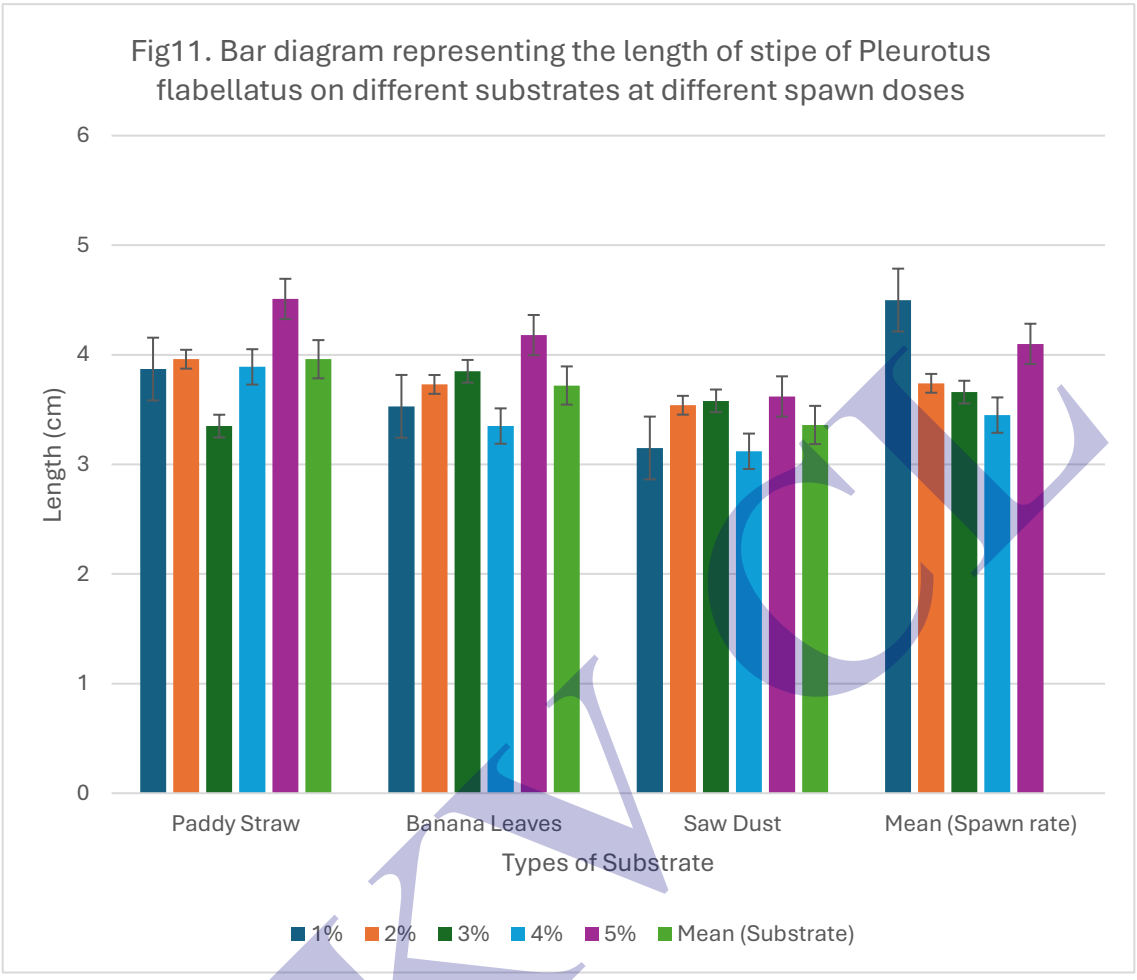
After harvesting, the stipe length of the mushrooms was measured. Although some variation in length was observed across different spawn rates, the differences were relatively minor. Among the tested substrates, paddy straw produced the longest average stipe length (3.96 cm). The maximum stipe length (4.51 cm) was recorded at a 5% spawn rate on paddy straw, followed by 4.18 cm on banana leaves at the same spawn rate.

The shortest stipe length (3.12 cm) was observed on sawdust at a 4% spawn rate. These findings are consistent with the results of Patel and Trivedi (2016), who found that wheat straw and sugarcane trash had minimal influence on the stipe length of *Pleurotus sajor-caju*, while paddy straw led to relatively longer stipes. However, it is important to mention that extended stipe length is typically regarded as an undesirable trait.

Table 11: Length of the stipes on different substrates at different spawn doses

Substrates	Spawn dose					Mean (Substrate)
	1%	2%	3%	4%	5%	
Paddy straw	3.87	3.96	3.55	3.89	4.51	3.96[a]
Banana leaves	3.53	3.73	3.85	3.35	4.18	3.72[b]
Saw dust	3.15	3.54	3.58	3.12	3.62	3.36[c]
Mean (Spawn dose)	3.52	3.74	3.66	3.45	4.10	

	Substrate	Spawn dose	Substrate X Spawn dose
SE(m)±	0.031	0.040	0.070
CD	0.090	0.117	0.202



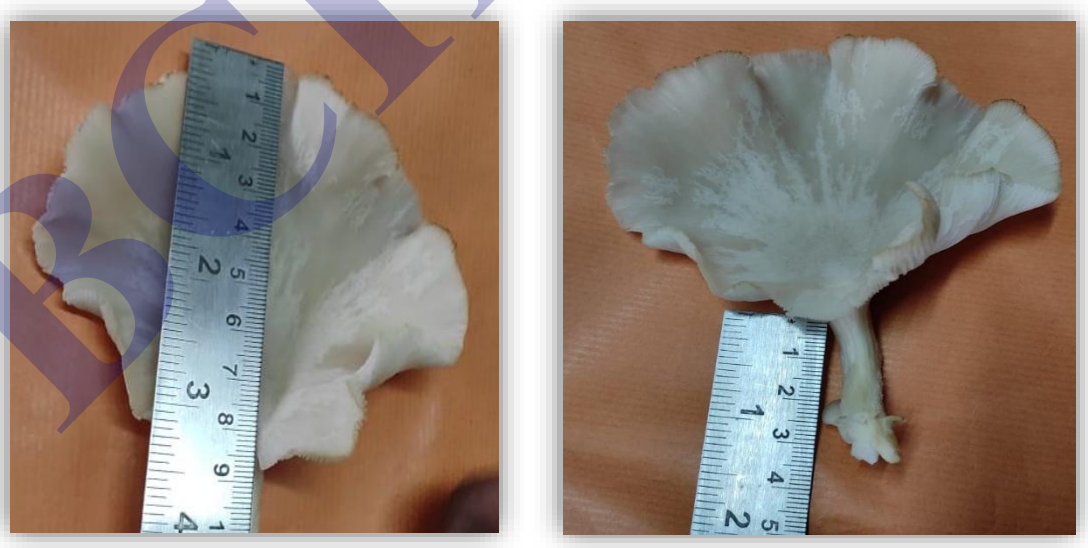


Plate.10 Measuring of length of the Pileus & length of the Stipe

4.2.6 Diameter of pileus on different substrates at different spawn dose

The diameter of the pileus, including both length and breadth, was measured during the study. As shown in Table 12, no significant variation in pileus length was observed across the different spawn rates. Although there were slight differences among the substrates, the pileus length between paddy straw and saw dust did not show any statistically significant variation. The maximum pileus size (7.60 cm) was noted on sawdust at a 4% spawn rate.

However, when examining the breadth, a clear distinction was evident among the three substrates. Paddy straw produced the widest pileus (10.93 cm) at a 1% spawn rate. Interestingly, across all substrates, the largest pileus size in terms of breadth was consistently associated with the 1% spawn rate, with the size tending to decrease as the spawn rate increased. These findings are supported by the study of Stanley et al. (2011), who observed that pileus diameters of 5.50 cm, 4.07 cm, and 3.20 cm were produced at spawn rates of 1%, 2%, and 3% respectively, highlighting a similar trend

Table 12: Length (cm) of pileus on different substrates at different spawn doses

Substrates	Spawn rate					Mean (Substrate)
	1%	2%	3%	4%	5%	
Paddy straw	6.97	6.78	6.46	6.10	6.24	6.51[b]
Banana leaves	5.83	5.52	5.32	5.48	5.90	5.61[c]
Saw dust	6.40	6.80	7.13	7.60	6.45	6.87[a]
Mean (Spawn dose)	6.40	6.37	6.30	6.39	6.20	
	Substrate	Spawn dose	Substrate X Spawn dose			
SE(m)±	0.046	0.059	0.103			
CD	N/A	0.173	0.299			

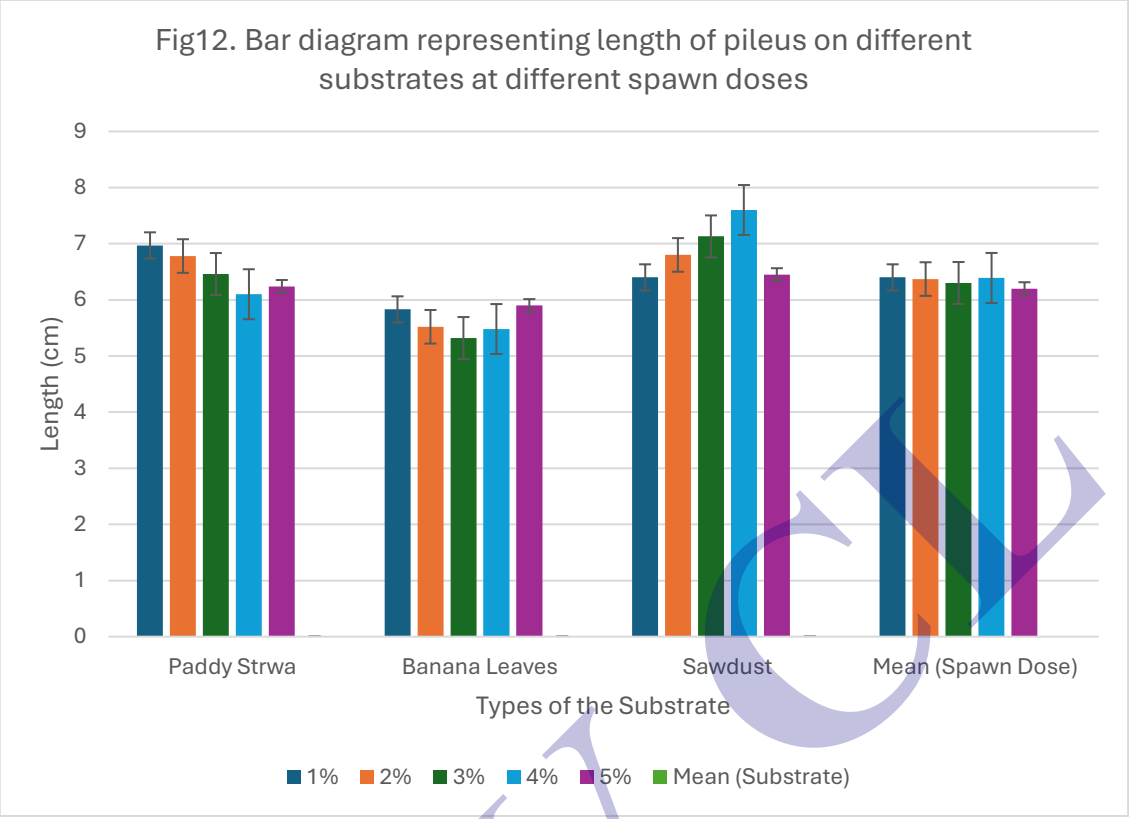
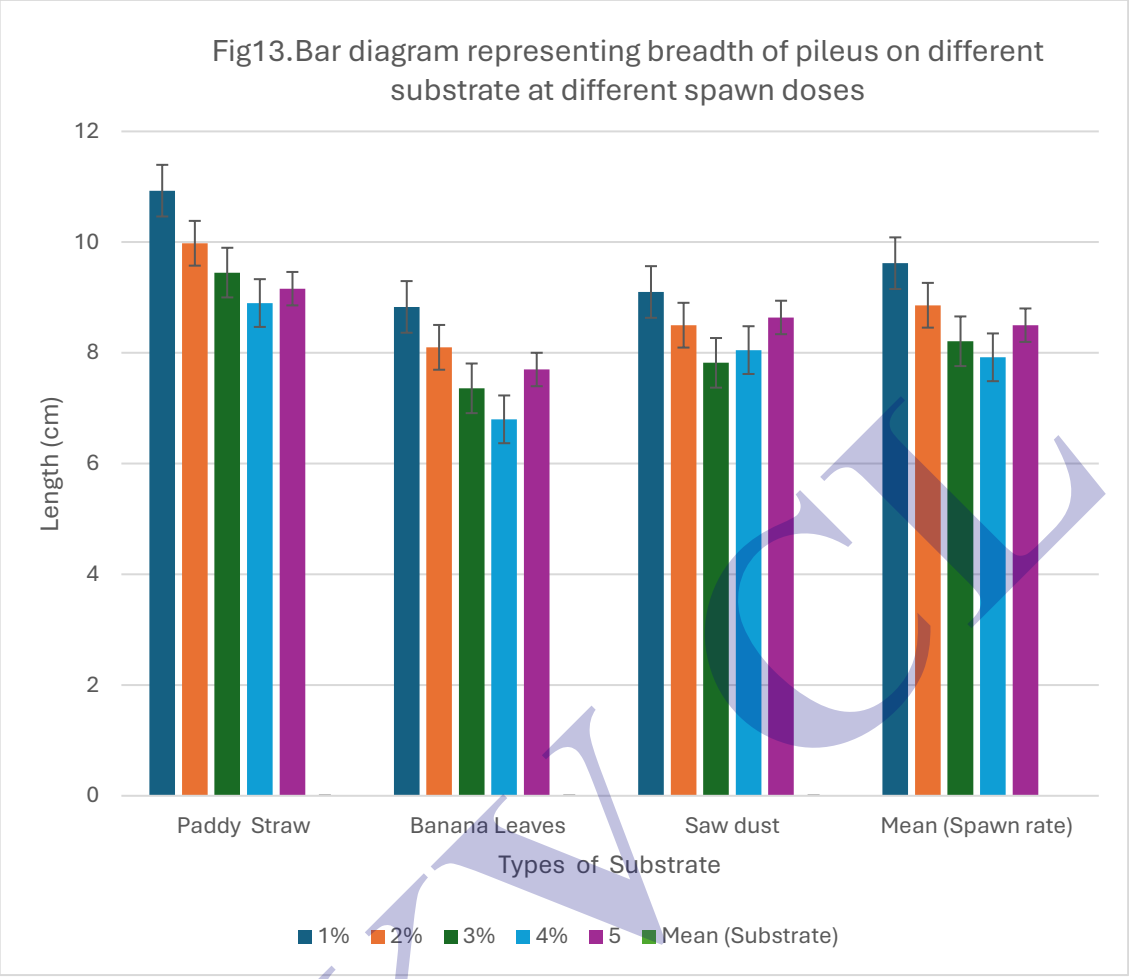


Table 13: Breadth (cm) of pileus on different substrates at different spawn doses

Substrates	Spawn dose					Mean (Substrate)
	1%	2%	3%	4%	5%	
Paddy straw	10.93	9.98	9.45	8.90	9.16	9.67[a]
Banana leaves	8.83	8.10	7.36	6.80	7.70	7.75[c]
Saw dust	9.10	8.50	7.82	8.05	8.64	8.43[b]
Mean (Spawn dose)	9.62	8.86	8.21	7.92	8.50	
	Substrate	Spawn dose	Substrate X Spawn dose			
SE(m)±	0.063	0.081	0.141			
CD	0.183	N/A	0.409			



4.2.7 Average weight of individual basidiocarp

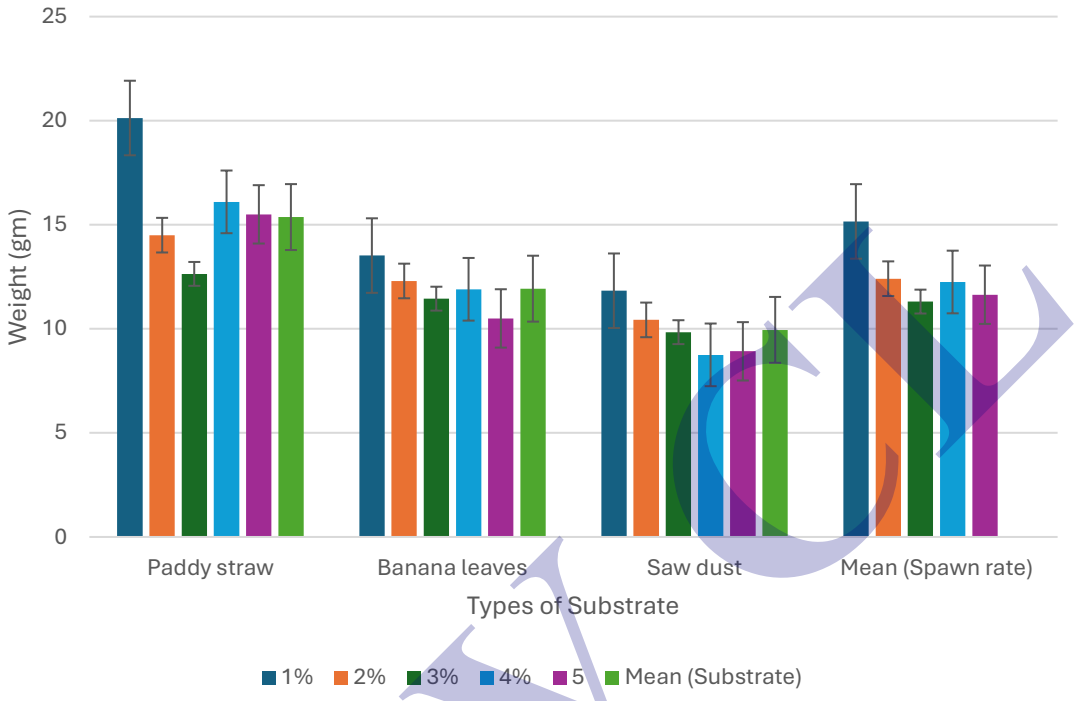
The experiment demonstrated significant differences in the average weight of individual fruiting bodies depending on the substrate used and the amount of spawn applied. An evident trend emerged where lower spawn levels were associated with larger basidiocarps. Among all tested substrates, paddy straw yielded the heaviest mushrooms, outperforming both banana leaves and sawdust. The greatest fruit body mass was achieved on paddy straw at a spawn application of 1%, registering 20.13 grams, while a 4% spawn level resulted in 16.10 grams. Likewise, the peak fruiting body weights for banana leaves and sawdust were noted at the 1% spawn rate, measuring 13.52 grams and 11.83 grams respectively.

Table 14: Average weight (gm) of individual basidiocarps on different substrates at different spawn doses

Substrates	Spawn dose					Mean (Substrate)
	1%	2%	3%	4%	5%	
Paddy straw	20.13	14.50	12.64	16.10	15.50	15.37[a]
Banana leaves	13.52	12.30	11.45	11.90	10.50	11.93[b]
Saw dust	11.83	10.43	9.84	8.75	8.92	9.95[c]
Mean (Spawn dose)	15.16	12.41	11.31	12.25	11.64	

	Substrate	Spawn dose	Substrate X Spawn dose
SE(m)±	0.096	0.123	0.214
CD	0.277	0.358	0.620

Fig 14 Bar diagram showing average weight of individual basidiocarps on different substrates at different spawn doses



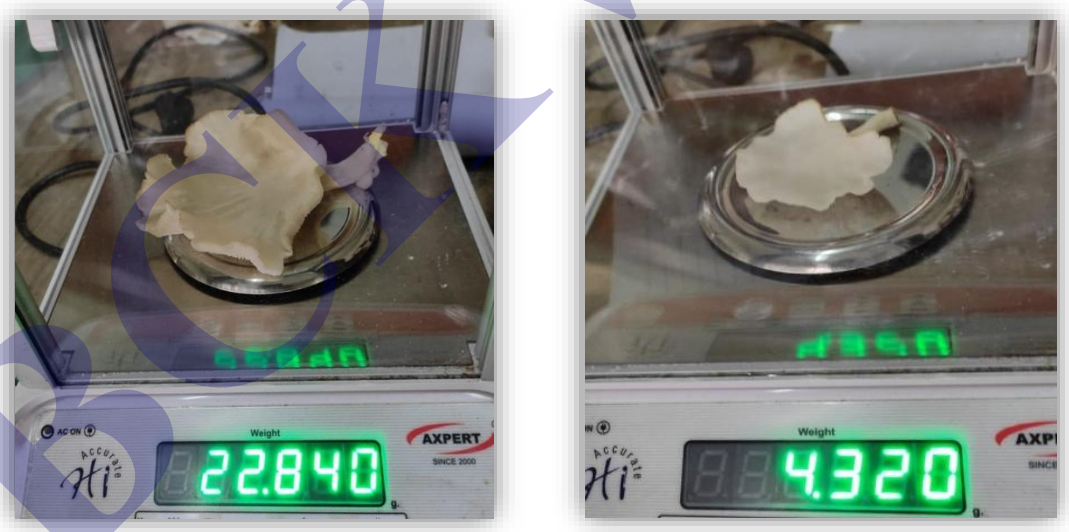


Plate.11 Weight of Individual Basidiocarp

4.3 Post harvest characters

This study focused on evaluating post-harvest characteristics of the fungus, specifically its dry weight and moisture content. The findings related to these post-harvest parameters are outlined below.

4.3.1 Dry weight of *Pleurotus flabellatus* on different substrates

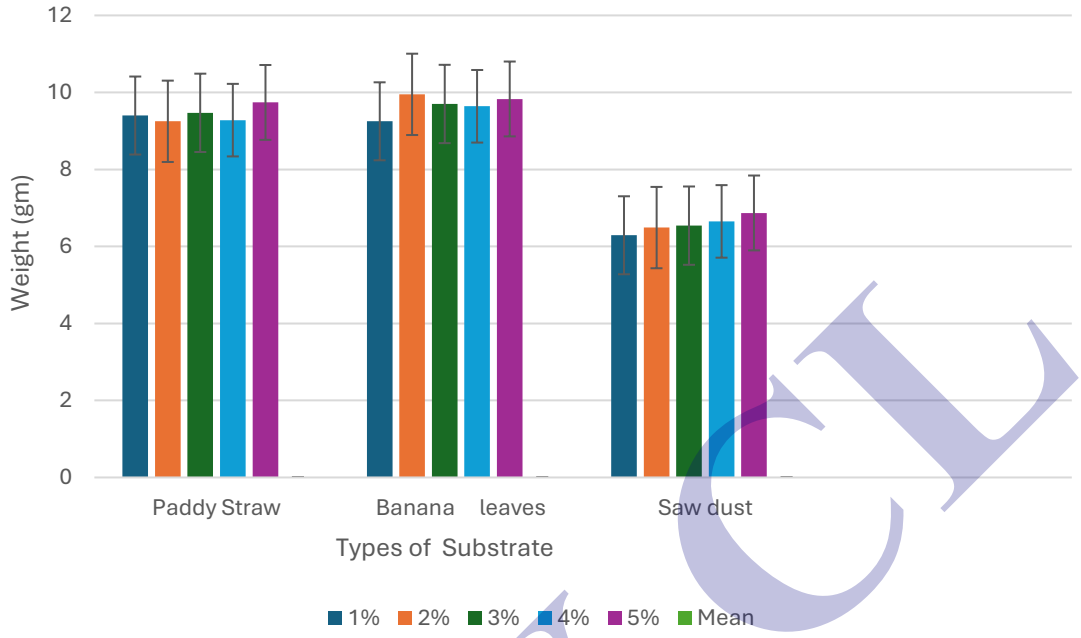
In this study, spawn rate was included only to compare its interaction with different substrates, as its overall impact was found to be negligible. Following harvest, mushrooms were weighed and all essential observations were recorded. The basidiocarps were then sun-dried over several days until their weight stabilized. Each substrate was tested using 100 grams per replication, with at different spawn doses conducted for evaluation.

The harvested fruiting bodies were sun-dried, revealing that resulted in the lowest dry weight saw dust (6.56 g per 100 g of fresh weight), while banana leaves produced the highest (9.67 g per 100 g of fresh weight). This variation in dry weight could be attributed to differences in moisture content of the basidiocarps developed on the various substrates. Royse (1999) and Islam et al. (2019) both demonstrated that sawdust is the least effective substrate for *Pleurotus* cultivation in terms of dry mushroom yield and that findings are identical to our one.

Table 15: Average dry weight (gm) of *Pleurotus flabellatus* on different substrates

Substrates	1%	2%	3%	4%	5%	Mean	SE(m)±	CD
Paddy Straw	9.40	9.25	9.47	9.28	9.74	9.428[a]	0.098	0.305
Banana leaves	9.25	9.95	9.70	9.64	9.83	9.674[a]		
Saw dust	6.29	6.49	6.54	6.65	6.87	6.568[b]		

Fig 15. Bar Diagram representing the dry weight of *Pleurotus flabellatus* on diferent substrates



4.3.2 Moisture content

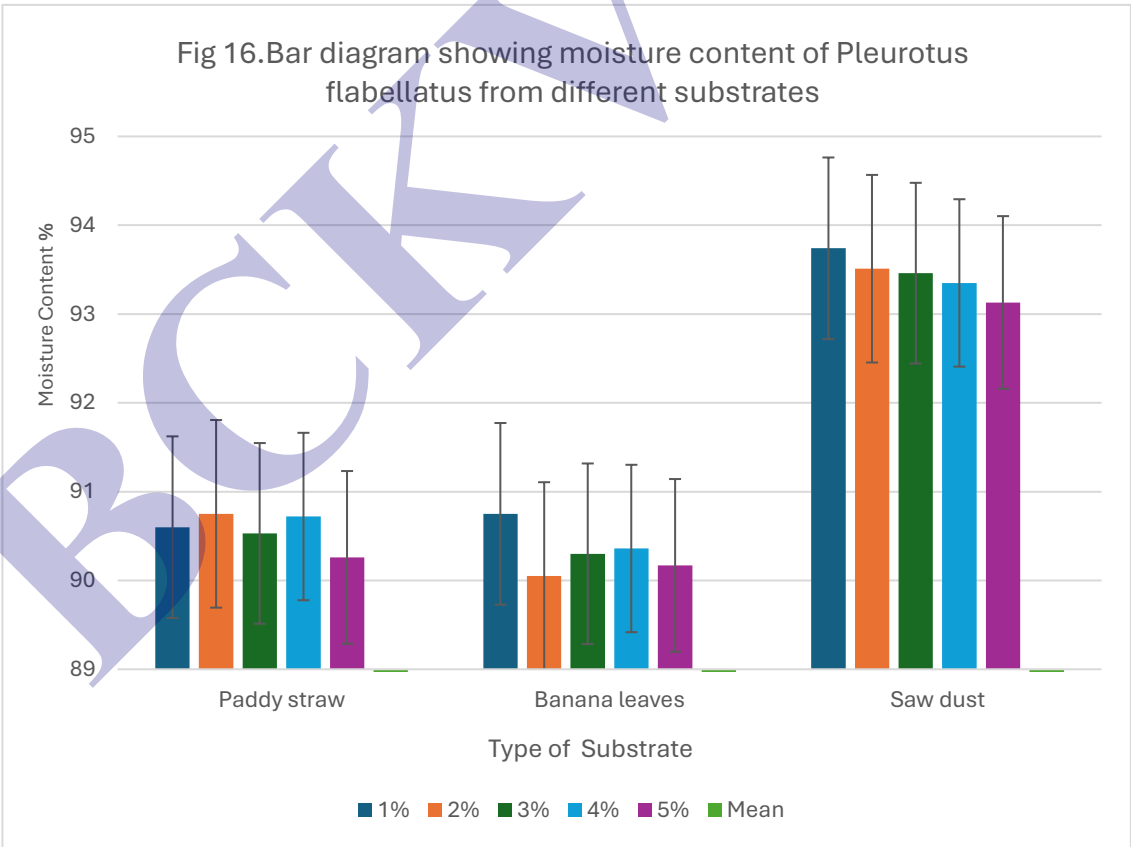
Following harvest, the basidiocarps were weighed in their fresh state, after which they were sun-dried until a stable dry weight was achieved. To estimate moisture content, at least five separate samples of 100 grams each were used for every substrate type. The moisture level in the mushrooms was subsequently calculated using a standard formula.

$$\textbf{Moisture content (\%)} = \frac{\text{Weight of fresh sample} - \text{weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

Based on the standard method for determining moisture content, the mushrooms cultivated on sawdust having the lowest dry mass naturally exhibited the highest moisture percentage. Among the three substrates tested, mushrooms from sawdust showed the greatest average moisture content at 93.43%, followed by those grown on at 90.57% paddy straw, while the least moisture content, 90.32%, was found in mushrooms cultivated on banana leaves.

Table 16: Average moisture content (%) of *Pleurotus flabellatus* on different substrates

Substrates	1%	2%	3%	4%	5%	Mean	SE(m)±	CD
Paddy straw	90.60	90.75	90.53	90.72	90.26	90.57[b]	1.026	3.196
Banana leaves	90.75	90.05	90.30	90.36	90.17	90.32[b]		
Saw dust	93.74	93.51	93.46	93.35	93.13	93.43[a]		



Chapter 5: Summary and Conclusion

The *Pleurotus flabellatus* is an edible fungus recognized for its ivory or white coloration and fan- or oyster-shaped cap. The cap typically measures between 8 and 11 cm in width, starting off convex with a rolled-in edge during early growth, and later flattening out. As it matures, the cap expands outward like a shell, with the broadest section extending away from the stalk. The edge of the cap eventually develops wavy and lobed features. Fully developed fruiting bodies retain a pale ivory hue. While a stem is often absent, when present it measures about 0.5 to 1 cm in length and 0.5 cm in thickness. It is solid, firm, off-centred, and white in colour. The gills are also white, and they connect at the base through cross-ridges, forming a noticeable raised network that extends down any existing stem. The spores produce a white print.

1. The present study aims to optimize the cultivation technique of *Pleurotus flabellatus* in West Bengal," a region where this species has not yet been extensively studied. Both laboratory and mushroom house conditions were used to evaluate various cultural, morphological, and post-harvest traits. The cultural experiments focused on identifying the most suitable growth medium, temperature, pH level, and nutritional supplements (including carbon and nitrogen sources) for effective mycelial development, along with selecting the best grains for spawn preparation. Morphological assessments were conducted to determine the most effective substrate and spawn rate for enhancing yield and dry matter content. *Pleurotus flabellatus* mycelium showed the most vigorous expansion when incubated at temperatures ranging from 24°C to 28°C, with 26°C proving to be the most conducive for consistent growth observed on the 3rd, 5th, and 7th days following inoculation on PDA medium.
2. The fungus demonstrated maximum mycelial advancement in media adjusted to pH values between 7.0 and 8.0, with the greatest radial extension (89.5 mm) recorded at pH 7.5 after a week of growth.
3. Among the three media tested, Malt Extract Agar (MEA) supported the fastest and most robust growth, followed by V8 Juice Agar, with Potato Dextrose Agar (PDA) showing the least effectiveness.
4. While all five carbon sources supported mycelial development, starch yielded the highest growth rate, followed by sucrose and dextrose. Mannitol showed the least growth after a seven-day incubation.

5. Regarding nitrogen supplementation, 0.3% L-asparagine significantly enhanced mycelial expansion, followed by 0.1% ammonium chloride and 0.1% ammonium nitrate, based on assessments on days 3, 5, and 7 post-inoculations.
6. Bajra *(Pennisetum glaucum)* emerged as the most suitable grain for spawn preparation, with colonization completed in 15.33 days, while Jowar and Wheat took 16.33 and 19.66 days, respectively.
7. Fruiting experiments revealed that higher spawn rates on different substrates led to faster spawn runs. On paddy straw, spawn run times were fastest at 5% (11.90 days) and slowest at 1% (16 days). For banana leaves, the spawn run ranged from 16 days at 5% to 20.30 days at 1%. Sawdust showed the slowest colonization, ranging from 24.50 days (5%) to 32 days (1%).
8. The highest yield was obtained with paddy straw at a 5% spawn rate, producing 262.85 grams of mushrooms and achieving the highest biological efficiency of 82.03%, outperforming the other substrates.
9. Although traits like number of basidiocarps, stipe length, pileus diameter, and individual basidiocarp weight showed no significant variation, the best outcomes for these characteristics were still observed in paddy straw at 3–5% spawn rates.
10. In terms of dry weight, banana leaves yielded the highest values and correspondingly lower moisture content, while sawdust showed the highest moisture content and the lowest dry weight, though the moisture levels across all substrates were relatively similar.
11. The harvested *Pleurotus flabellatus* mushrooms displayed their characteristic fanlike shape with a white to ivory coloration.

Based on the study findings, it can be concluded that *Pleurotus flabellatus* is well-suited for cultivation under the environmental conditions of West Bengal as well as in controlled mushroom cultivation rooms maintaining temperatures between 22°C and 28°C and relative humidity (RH) between 75% and 90%. To promote rapid mycelial development, incubation should be carried out at temperatures ranging from 24°C to 28°C, with the culture medium maintained at a neutral to slightly alkaline pH (7–7.5).

Instead of the commonly used Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) is recommended for better mycelial growth. For nutritional enrichment, starch can be

used as a carbon source in place of dextrose, and L-asparagine at 0.3% concentration is ideal as a nitrogen supplement. For spawn production, Bajra grains are the preferred substrate, as they support quicker colonization by the mycelium. Regarding fruiting substrates, paddy straw cut into 2–3 cm lengths is the most effective option for optimal growth and yield.

Chapter 6: Future Scopes of Research

Pleurotus species, or oyster mushrooms called "Dhingri" in India are among the most consumed edible fungi, valued not only for their rich nutritional profile but also for their medicinal, biotechnological, and environmental significance. They thrive across temperate and subtropical regions, offering non-starchy carbohydrates, moderate protein, fiber, essential minerals, amino acids, and vitamins, making them a highly wholesome food.

In 2013, global mushroom production was about 3,400 million tons, with *Pleurotus* contributing roughly 25%, making them the second most cultivated mushrooms worldwide. By 2016, India produced over 120,000 metric tons of mushrooms, 16% of which came from *Pleurotus*. West Bengal ranked sixth nationally in oyster mushroom production, yielding around 1,500 metric tons annually (7% of the national share).

Pleurotus flabellatus is especially notable for its adaptability to varied climates and its ability to grow on diverse agricultural residues with minimal inputs. Yet, despite West Bengal's favourable year-round growing conditions, production remains limited signalling a pressing need for targeted research and development to unlock its full commercial potential. Although West Bengal has favourable agroclimatic conditions that support year-round oyster mushroom cultivation, the state still lags in global mushroom production. This highlights the need for comprehensive and focused research in the following domains:

1. *Pleurotus flabellatus* has strong potential for future research. Scientists aim to develop high-yielding, stress-tolerant strains that grow better in various climates. Research also targets disease resistance, faster growth, and longer shelf life, improving large-scale farming. The mushroom produces enzymes like laccase and cellulase, useful in medicine, paper, and waste treatment, offering both farming and industrial benefits.

2. *Pleurotus flabellatus* can be cultivated on a wide range of low-cost, locally available agricultural wastes such as paddy straw, sugarcane bagasse, cotton stalks, and sawdust. This not only reduces the cost of production but also provides a sustainable way to recycle agro-waste that would otherwise be burned or discarded.

3. By converting these wastes into nutritious mushrooms, it promotes eco-friendly farming practices and supports organic and sustainable agriculture. Additionally, it plays a key role in solid waste management by turning organic waste into valuable food and compostable materials, making it an excellent tool for environmental conservation.

4. *Pleurotus flabellatus* farming needs low investment but gives good returns, making it ideal for rural, tribal, and urban areas. It creates job opportunities for women, youth, and self-help groups in growing, processing, and selling mushrooms. It also promotes small businesses and training centres in mushroom farming.

5. *Pleurotus flabellatus* is rich in protein, fiber, vitamins, and minerals, making it a healthy food. It is low in fat and cholesterol, so it's good for heart and diabetic patients. It can help fight malnutrition and improve food security, especially for poor or rural communities.

6. *Pleurotus flabellatus* can be made into products like pickles, powder, soups, snacks, and nuggets. It also has medicinal benefits like antioxidant and immune-boosting properties. These products can support small businesses and exports.

7. *Pleurotus flabellatus* needs less water and land than many other crops which grows without chemical fertilizers or pesticides, making it great for organic farming. After harvesting, the left-over material can improve soil health and help in reducing carbon in the environment.

8. Integration with vermicomposting, fish farming, and organic farming models. By-products (spent mushroom substrate) can be used in animal feed, compost, or biofuel production.

Bibliography

Ambili, S. and Nithya, T. P. (2014). Oyster mushroom cultivation – A study in Palakkad district, Kerala. *Int. J. Manage. Soc. Sci. Res. Rev.*, **1**:104–105.

Amuneke, E. H., Dike, K. S. and Ogbulie, J. N. (2011). Cultivation of *Pleurotus ostreatus*: An edible mushroom from agro base waste products. *J. Microbiol. Biotechnol. Res.*, **1(3)**:1–14.

Arunesh Kumar, V., et al. (2021). Optimization of spawn doses and agroforestry residues as substrates for *Pleurotus cornucopiae* production. *J. Pharmacogn. Phytochem.*, **10(1)**:1–8.

Ashraf, J., Ali, M. A., Ahmad, W., Ayyub, C. M. and Shafi, J. (2013). Effect of different substrate supplements on oyster mushroom (*Pleurotus* spp.) production. *Food Sci. Technol.*, **1(3)**:44–51.

Bhadana, N. K., Singh, G., Kumar, D. and Srivastava, S. (2020). Effect of some abiotic factors on the growth and development of different *Pleurotus* spp. *Int. J. Curr. Microbiol. Appl. Sci.*, **9(10)**:1079–1088

Bhattacharjee, M. K. and Samajpati, N. (1989). Optimization of mycelial growth of *Pleurotus sajor caju* (Fr.) Singer in relation to some physical and nutritional factors. *Indian J. Mycol. Res.*, **27(1)**:59–65.

Bugarski, D., Gvozdenović, D. J., Ćervenski, J. and Takač, A. (2002). Effect of major environmental conditions on the development of the mycelium and growth of the oyster mushroom (*Pleurotus ostreatus*). *Acta Hortic.*, **579**:53–58.

Calam, C. T. (1971). The evaluation of mycelial growth. In: Norris, J. R. and Ribbons, D. W. (Eds.), *Methods in Microbiol.*, **1**:567–591.

Chandran, R., Ravichandran, R., Radha, A. G. and Thulasi, C. (2010). Mycelial culture and spawn production of two oyster mushrooms (*Pleurotus florida* and *Pleurotus eous*) on different substrates including sorghum and paddy grains. *Int. J. Biol. Technol.*, **1(3)**:39–42.

Chang, S. T., Buswell, J. A. and Miles, P. G. (1981). Definition and evaluation of biological efficiency in the cultivation of edible mushrooms. *J. Sci. Technol. Food Agric.*, **32(12)**:119–123.

Chang, S. T. and Miles, P. G. (1992). Mushroom biology—a new discipline. *Mycologist*, **6**:64–65.

Chang, S. T. and Miles, P. G. (2004). Substrate and mycelial growth. In: *Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact* (2nd ed.).

Chang, S. T. and Wasser, S. P. (2017). The cultivation and environmental impact of mushrooms. In: *Oxford Res. Ency. Environ. Sci.* Oxford University Press.

Chiu, S. W., Ching, M. L., Fong, K. L. and Moore, D. (1998). Spent oyster mushroom substrate performs better than many mushroom mycelia in removing the biocide pentachlorophenol. *Mycol. Res.*, **102(12)**:1553–1562.

Debnath, S., Saha, R., Dutta, A., Das, P. and Saha, A. K. (2021). Production of exopolysaccharides (EPSs) and evaluation of biological properties of *Pleurotus flabellatus* (Berk. & Br.) Sacc. *Proc. Natl. Acad. Sci. India Sect. B Biol. Sci.*, **91**:581–591.

Deepalakshmi, K. and Mirunalini, S. (2014). *Pleurotus ostreatus*: An oyster mushroom with nutritional and medicinal properties. *J. Biochem. Technol.*, **5(2)**:718–726.

Eswaran, A. and Ramabadran, R. (2000). Studies on some physiological, cultural and post harvest aspects of oyster mushroom *Pleurotus eous*. *Trop. Agric. Res.*, **12**:360–374.

Galappaththi, M. C. A., Dauner, L., Madawala, S. and Karunarathna, S. C. (2021). Nutritional and medicinal benefits of oyster (*Pleurotus*) mushrooms: A review. *Fungal Biotec*, **1(2)**:65–87.

Gorai, B. and Sharma, R. (2018). Determination of optimum temperature and pH for mycelial growth of *Pleurotus* spp./strains. *Int. J. Microbiol. Res.*, **10(6)**:1287–1289.

Hesami, A., et al. (2014). The effects of three amino acids (asparagine, glutamine and glycine) on biological parameters of *Agaricus bisporus*. *Int. J. Food Agric. Sci.*, **2**:187–191.

Hoa, H. T. and Wang, C. L. (2015). The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, **43(1)**:14–23.

Hoa, H. T., Wang, C. L. and Wang, C. H. (2015). The effects of different substrates on the growth, yield, and nutritional composition of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, **43(4)**:423–434.

Hossain, M. M. (2018). Effect of different grains on mycelial growth and yield of *Pleurotus sajorcaju*. *Int. J. Curr. Microbiol. App. Sci.*, **7(9)**:9–13.

Islam, M. Z., Rahman, M. H. and Hafiz, F. (2009). Cultivation of oyster mushroom (*Pleurotus flabellatus*) on different substrates. *Int. J. Sustain. Crop Prod.*, **4(1)**:45–48.

Josephine, R. M. (2014). A review on oyster mushroom (*Pleurotus* spp.). *Int. J. Adv. Biotechnol. Res.*, **1**:7–9.

Khan, M. W., Ali, M. A., Khan, N. A., Aslam, M., Rehman, A. and Javed, N. (2013). Effect of different levels of lime and pH on mycelial growth and production efficiency of oyster mushroom (*Pleurotus* spp.). *Pak. J. Bot.*, **45(3)**:853–860.

Kumar, S., et al. (2018). Optimization of mycelial growth parameters for *Pleurotus florida* and *P. sajor-caju*. *Int. J. Curr. Microbiol. App. Sci.*, **Special Issue-7**:4818–4823.

Kumar, S., Singh, G., Singh, R., Kumar, P. and Bhatt, S. S. (2022). Assessment of grain extract media on mycelial growth of *Pleurotus* spp. (*P. sapidus* and *P. flabellatus*). *Asian J. Environ. Ecol.*, **17(1)**:10–16.

Lavelli, V., Proserpio, C. and Gallotti, F. (2018). Circular reuse of bio-resources: the role of *Pleurotus* spp. in the development of functional foods. *Food Funct.*, **9(3)**:1353–1372.

Lovkesh, Beniwal and Pahil. (2006). Physiological and fungistatic interaction studies between *Pleurotus* spp. and *Trichoderma viride*. *Crop Res. Hisar*, **32(3)**:499–503.

Mahadevan, K. and Shanmugasundaram, K. (2018). Comparative effect of different culture media on mycelial growth performance of *Pleurotus sapidus*. *J. Pharmacogn. Phytochem.*, **7(4)**:874–878.

Maheswari, S., Rajarajan, P., Pandian, P. M., Sheeba, E. and Bayineni, V. K. (2020). Influence of different substrates on growth and yield of *Pleurotus ostreatus*. *J. Appl. Hortic.*, **22(3)**:215–219.

Mane, V. P., Patil, S. S., Syed, A. A. and Baig, M. M. V. (2007). Bioconversion of low-quality lignocellulosic agricultural waste into edible protein by *Pleurotus sajor-caju* (Fr.) Singer. *J. Zhejiang Univ. Sci. B*, **8(10)**:745–751.

Mbogoh, J. M., Anjichi, V. E., Rotich, F. and Ahoya, N. K. (2011). Substrate effects of grain spawn production on mycelium growth of oyster mushroom. *Acta Hortic.*, **911**:469–471.

Miles, P. G. and Chang, S. T. (1997). *Mushroom biology: Concise basics and current developments*. World Scientific.

Mishra, S., Singh, V. K. and Yadav, D. (2016). Therapeutic properties of *Pleurotus* species (oyster mushrooms) for human food and medicine: An overview. *J. Food Chem. Toxicol.*, **52(2)**:123–145.

Mohamad, S. A., Awang, M. R., Rashid, R. A., Ling, L. S., Daud, F., Hamid, A. A., ... and Yusoff, W. M. W. (2015). Optimization of mycelial biomass production in submerged culture fermentation of *Pleurotus flabellatus* using response surface methodology. *Adv. Biosci. Biotechnol.*, **6(6)**:419–426.

Muswati, C., Simango, K., Tapfumaneyi, L., Mutetwa, M. and Ngezimana, W. (2021). The effects of different substrate combinations on growth and yield of oyster mushroom (*Pleurotus ostreatus*). *Int. J. Agron.*, **2021**, Article ID 9962285.

Nagaraj, R., Arunkumar, P., Hanumanthaswamy, B. C. and Rathod, J. M. (2017). Mushroom production for self-employment – an impact study. *Int. J. Curr. Microbiol. App. Sci.*, **6(9)**:2991–2997.

Nasim, G., Malik, S. H., Bajwa, R., Afzal, M. and Mian, S. W. (2001). Effect of three different culture media on mycelial growth of oyster and Chinese mushrooms. *J. Biol. Sci.*, **1(4)**:1130–1133.

Neelam, S., Chennupati, S. and Singh, S. (2013). Comparative studies on growth parameters and physio-chemical analysis of *Pleurotus ostreatus* and *Pleurotus florida*. *Asian J. Plant Sci. Res.*, **3(1)**:163–169.

Oei, P. and van Nieuwenhuijzen, B. (2005). *Small scale mushroom cultivation: Oyster, shiitake and wood ear mushrooms (Agrodok No. 40)*. Wageningen, The Netherlands: Agromisa & CTA.

Patel, P. and Trivedi, R. (2016). Effect of substrate type on stipe length and cap diameter of *Pleurotus sajor-caju*. *International Research Journal of Natural and Applied Sciences*, **3**(6): 40–44.

Peng, C.H. (1996). Improving yield of *Pleurotus eryngii* var. *eryngii* using supplemented sawdust substrates. *Bioresource Technology*, **58**(2): 87–90.

Prakasam, V. (2012). Current scenario of mushroom research in India. *Indian Phytopathology*, **65**(1): 1–11.

Quimio, T.H. (1978). Indoor cultivation of *Pleurotus ostreatus*. *Philippine Agriculturist*, **61**: 253–262.

Rajarathnam, S., Bano, Z. and Patwardhan, M.V. (1986). Nutrition of the mushroom *Pleurotus flabellatus* during its growth on paddy straw substrate. *Journal of Horticultural Science*, **61**(2): 223–232.

Raman, J., Jang, K.-Y., Oh, Y.-L., et al. (2021). Cultivation and nutritional value of prominent *Pleurotus* spp.: an overview. *Mycobiology*, **49**(1): 1–14.

Royse, D.J., Baars, J. and Tan, Q. (2017). Current overview of mushroom production in the world. In: Zied, D.C. and Giménez, A.P. (Eds.), *Edible and Medicinal Mushrooms: Technology and Applications*. Hoboken, NJ: Wiley, pp. 5–13.

Sahu, A., Singh, B.P., Singh, A.K. and Singh, V.K. (2014). Screening of suitable grain substrates for spawn development, growth and yield of *Pleurotus flabellatus*. *Research Journal of Agricultural Sciences*, **8**(2): 539–544.

Sakamoto, R., Niimi, T. and Takahashi, S. (1978). Submerged culture of edible fungi in high-consistency starch media. Studies on submerged culture of edible fungi. Part II. *Journal of the Agricultural Chemical Society of Japan*, **52**(2): 83–90.

Sardar, H., Ali, M.A., Ayyub, C.M. and Ahmed, R. (2015). Effects of different culture media, temperature and pH levels on the growth of wild and exotic *Pleurotus* species. *Pakistan Journal of Phytopathology*, **25**(1): 1–5.

Sharma, V.P., Annepu, S.K., Gautam, Y., Singh, M. and Kamal, S. (2017**).** Status of mushroom production in India. *Mushroom Research*, **26**(2): 111–120.

Singh, M., Kamal, S. and Sharma, V.P. (2017**).** Status and trends in world mushroom production I. *Mushroom Research*, **26**(1): 1–20.

Singh, S. and Singh, G. (2018). Effect of different pH, temperature, and media on radial growth of oyster mushrooms (*Pleurotus djamor*). *Bulletin of Environment, Pharmacology and Life Sciences*, **7**(11): 73–77.

Sitaula, H.P., Dhakal, R., D.C., G. and Kalauni, D. (2018). Effect of various substrates on growth and yield performance of oyster mushroom (*Pleurotus ostreatus*) in Chitwan, Nepal. *International Journal of Applied Sciences and Biotechnology*, **6**(3): 215–219.

Srivastava, H.C. and Bano, Z. (1970). Nutrition requirements of *Pleurotus flabellatus*. *Applied Microbiology*, **19**(1): 166–169.

Valverde, M.E., Hernández Pérez, T. and Paredes López, O. (2015). Edible mushrooms: Improving human health and promoting quality life. *International Journal of Microbiology*, **2015**, Article ID: 376387.

Kerketta, V., Shukla, C.S., Singh, H.K. and Kerketta, A. (2019). Effect of grain substrates on spawn development and their impact on yield and yield attributing characters of *Pleurotus* spp. *Journal of Pharmacognosy and Phytochemistry*, **8**(6): 722–725.

Yadav, A.K. (2014). Assessment of grain extract media on mycelial growth of *Pleurotus flabellatus* and *P. sapidus*. *Asian Journal of Environment & Ecology*, **17**(1): 10–16.