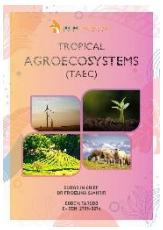
ZIBELINE INTERNATIONAL™ PUBLISHING

ISSN: 2735-0274 (Online)
CODEN: TARGDB

Tropical Agroecosystems (TAEC)

DOI: http://doi.org/10.26480/taec.01.2022.07.11

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*RESEARCH ARTICLE*

# EFFECT OF DIFFERENT SUBSTRATES ON GROWTH AND YIELD PERFORMANCE OF OYSTER (*Pleurotes oestratus*) MUSHROOM AT GOKULESHWOR, BAITADI

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**ARTICLE DETAILS**

***Article History:***

Received 07 January 2022
Accepted 11 February 2022
Available online 22 February 2022

**ABSTRACT**

To determine the growth and yield of oyster mushroom, types of substrates is of crucial importance. Five different types of substrates namely rice straw, wheat straw, banana leaves, rice straw and wheat straw (1:1) and Banana leaves and rice straw (1:1) were used for evaluating the yield and related attributes of *Pleurotus ostreatus*. Standard cultivation practice was followed with steam sterilization and spawned substrate packed in a plastic bag. The data of two flushes were recorded. The parameters observed were days to primordial formation, yield of each harvest and length of longest stipe (Cm). Our results reveal that wheat straw was the quickest to full spawn run (23.25 days) followed by rice straw (24.25 days) and rice straw mixed with wheat straw (24.75 days) and the slowest in banana leaves (50.75 days). However, the duration from full mycelial run to primordia formation was quickest in rice straw and slowest in the mixture of rice straw and banana leaves. From the yield perspective, rice straw (1.5 kg) excelled followed by the mixture of rice straw and wheat straw (1.34 kg) and lowest in banana leaves (0.5kg). The longest stipe was observed on rice straw (17.9 cm) and mixture of rice straw and banana leaves (17.9 cm) with the least on banana leaves (12.32 cm). Results revealed that there was significant reduction of yield and stipe length from first to second harvest. The average duration to complete one cycle in rice straw was 32 days, wheat straw 31.5 days, rice and wheat mixture 33.5 days, banana leaves 54.75 days and the mixture of rice straw and banana leaves with 46 days. From over all results, rice straw was found to be the most suitable substrate for oyster mushroom under Gokuleshwor condition. If other substrates are to be used, they should be mixed with rice straw.

**KEYWORDS**

Agricultural waste, yield, stipe length, cropping duration, *Pleurotus ostreatus*

## 1. INTRODUCTION

Mushroom are the spore bearing fruiting bodies of saprophytic, parasitic and mycorrhizal fungi belonging to order Ascomycetes or Basidomycetes which can be found in the soil rich in organic matter and humus, moist wood, animal waste after heavy rain or when there is the sudden temperature change, and they disappear leaving no sign but vegetative mycelium soon after the hours or day (Zied et al., 2011). They are highly nutritious specially rich in proteins and minerals like potassium, phosphorous, copper and iron, vitamin B, C and D (Panjikkaran and Mathew, 2013). They contain all essential nine amino acids, 20-35% protein whereas they are comparatively low in lipid content and also in calcium (Kalač, 2009). Mushrooms are high value crop with distinct texture, flavour, great nutritional value and high productivity per unit area this is an excellent food source for malnutrition alleviation in the third world countries (Eswaran and Ramabadran, 2000). They contain bioactive constituents thus receiving great attention from the food and pharmaceutical researchers (Chang et al., 2007). The β-glucans like Lentinan, terpenes, steroids, polysaccharides, and phenolic compounds are the biomolecules found in mushroom that have various biological activities (Bao et al., 2020). They are considered as food of great medicinal importance because of the presence of variety of compounds with anti-fungal activity, antigen toxicity, antitumorigenic, antioxidation, antiproliferative, antihypertensive, anti-nociceptive, immune stimulation (Bao et al., 2013; Feeney et al., 2014; Shaffique et al., 2021; McBride et al., 2005; Mortimer et al., 2012; Vaz et al., 2011).

Agriculture is considered as one of the low profitable and risky enterprise which takes very long time to give adequate profit (Reardon et al., 1999). Low production due to subsistence farming and untimely availability of fertilizers and other inputs has resulted in widespread malnutrition and protein gap in our country which has necessitate the search for alternative source of subsistence farming and protein because the pulse protein and animal protein has not kept pace with our requirement due to high population growth which can easily be maintained by producing mushroom in rural and semi urban areas using various agro byproducts (Wasser, 2011).There are roughly 110 mushroom species edible, 45 toxic and 13 of medicinal value in Nepal (Poudel and Bajracharya, 2011). *Pleurotus* species also known as oyster mushroom are edible fungi that are cultivated all around the world (Mbassi et al., 2018). The Latin word '*Pleurotus*' means beside the ear and '*ostreatus*' means oyster shaped (Cohen et al., 2002). In Nepal it is often called "Kanya chyau". Due to its excellent flavor and taste, its popularity as edible mushroom has been exceeding. Oyster mushrooms were more recently domesticated and are now rank second in world production and first in Nepal (Neupane et al., 2018). Oyster mushroom is highly nutritious which has been reported with protein (25-50%), fat (2-5%), sugars (17-47%), mycocellulose (7-38%) and minerals (Na, K, P, Ca) of about 8-12% (Asneti, 2013). They are also rich in vitamins such as niacin, riboflavin, vitamin D, C, B1, B5, B6 (Patil, 2012).

Apart from food value, its medicinal value for diabetics and in cancer therapy has also been emphasized (Sivrikaya et al., 2002). It is an ideal

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| | **Website:** www.taec.com.my | **DOI:** 10.26480/taec.01.2022.07.11 |

Cite The Article: Janaki Mahat, Debaki Dhami, Deepa Bhatt, Kamana Bhandari (2022). Effect of Different Substrates on Growth and yield Performance of Oyster (*Pleurotes oestratus*) Mushroom at Gokuleshwor, Baitadi. *Tropical Agroecosystems, 3(1): 07-11.*

food for patients suffering from hypertension and heart diseases (hypercholesterolemia) as it contains high K: N ratio (Gunde-Cimerman, 1999). These species normally grow within a temperature range of 15-25$^0$C (Hasan et al., 2010). The best growing season is during March/April to Sep/Oct in the upper region and in the lower regions from Sep/Oct to March/April (Dubey et al., 2019). In cultivation, it can grow well in substrates rich in lignocellulosic materials such as rice straw, wheat straw, sugarcane baggage, maize cobs, sawdust and aquatic weeds with varying yield and performance (LUCKY, 2015). The substrate which contains adequate amount of nitrogen and carbohydrate is ideal for mushroom growth (Zied et al., 2011).

In oyster mushroom, mycelium colonization and development of fruiting bodies are determined by total carbonic acid, total nitrogen and C:N ratio and yield of mycelium decreases under lower and higher C:N ratio (Chen et al., 2021). In rice straw, composition of carbohydrate is mobilized by *Pleuroteus* fungi through degradation of cellulose and hemicellulose (Fazaeli and Masoodi, 2006). Rice straw has the chemical composition of (in percentage dry masses basis) 0.96% N,73.01 NDF,41.59% ADF, 31.42% hemicellulose, 33.35% cellulose and 4.84% acid detergent lignin in different growth stages, lignocellulosic C:N ratio of 72% (Paudel and Dhakal, 2020). Wheat straw contain 37.75–38.18 g,15.67–16.07 g, 28.25–28.98 g per 100-gram raw material of cellulose, lignin and hemicellulose respectively (Sharifi-Rad et al., 2020). Prominent level of lignocellulose is found in leaves and pseudo stem of banana. Hemicellulose and lignin contents is close to other biomass fuels but had the lowest cellulose content, of 26.7% in semi-dried and wet leaves (Fernandes et al., 2015).

Since rice straw is only being used as a substrate for the production of mushroom although there are wide ranges of crop residue to grow mushroom but there are some challenges to select the right substrate to grow it. Therefore, the objective of this study is to determine the growth and performance of oyster mushroom (*Pleurotus spp*) on different substrates/ agricultural wastes (rice straw, wheat straw, rice and wheat straw, banana leaves, banana leaves and rice straw) under Gokuleshwor condition.

## 2. Materials and Methods

### 2.1 Site of study

The research was carried out at Gokuleshwor Agriculture and Animal Science College, Gokuleshwor, Baitadi dated 2077/11/14 to 2078/01/21: situating 29$^0$403" N latitude and 80$^0$3240" E longitude with elevation of 700 m above sea level. Research was conducted during the summer month temperature maintained to 24-28$^0$C and RH about 80-90%.

### 2.2 Treatment's detail

The research was conducted in Completely Randomized Design (CRD) with 5 treatments each replicated for 4 times. Thus, making total 20 bags each of 4 kg. The treatments used were T1 (Paddy straw). T2 (Wheat Straw), T3 (Rice: Wheat (1:1) straw), T4 (Banana Leaves), T5 (Banana leaves+ rice straw (1:1)).

### 2.3 Substrate's collection

Different substrates were collected from different sources like rice straw and wheat straw were collected from nearby farm whereas banana leaves were collected from farm as well as from college periphery of Gokuleshwor Agriculture and Animal Science College, Gokuleshwor, Baitadi.

Preparation of substrates: The substrates thus obtained were chopped into finer length of 3-5cm: soaked in water for next 12hrs in a separate drum and washed thoroughly for 3-4 times in next morning until the clear water was obtained. The moisture level from the substrates was reduced to an extent that the water didn't ooze out when squeezed by hand.

Steam sterilization was carried out for sterilization. 4"-6" water was filled in the drum and bricks were kept inside so that the top of the brick was just above the water level. The processed substrates were kept in the drum above the perforated tin plate. The opening of the drum was closed with jute sack and plastic sheet and left for 1-2 hours. The substrates were then cooled to the temperature of 25◦C and was used for filling.

Bag filling and inoculation/ spawning: Cultivation of *Pleurotus* was carried out in a transparent plastic bag of dimension 16" × 24". 4 layers of substrates of total 4kg were filled in bag and spawning was done in each layer aseptically. Total 100 g spawn and 50 g gram powder was used for each 4kg substrates. After inoculating, mouth of the bag was secured tightly with nylon thread. Small holes were made on the lateral side and top of bag for aeration

Spawn running: The inoculated substrates were then kept in a dark room above a chair individually at a temperature of about 25-30◦C and 65-75% RH. The observation was done on regular basis until the whitish mycelia growth (colonization) had spread to both upper and lower sides of the bags from the inoculated zone. Plastic was removed on 23$^{rd}$ day when maximum colonization was done. Ventilation was allowed in the room to allow the entry of fresh air and irrigation was done twice or thrice depending on weather condition.

Harvesting: The fruiting bodies were harvested when most of them get matured completely which was usually 4-5 days after pinhead formation. The substrate was incubated under the same conditions after each harvest for further flush

Data recording and analysis: The required data were collected at different times. Data collection started from the time of spawning to 2$^{nd}$ harvest. The observed parameters were Days to primordia formation (Days**),** yield of each harvest, total yield (Kg) and length of the longest stipe (Cm).

The obtained data were compiled by using Ms-excel program. Data were then analyzed using R Stat. Mean were separated using Duncan's Multiple Range Test (DMRT) at ($p \leq 0.05$). Graphs and tables were also constructed by using Ms- excel.

## 3. Result and Discussion

Five different treatments were compared and each shows variation in days to colonization, days to primordia formation, stipe length and yield.

### 3.1 Effect of different substrates on the days to primordia formation from the day of spawning

**Table 1:** Effect of different substrates on days to primordia formation of Pleurotus mushroom under Gokuleshwor condition

| Treatment name | Days to primordia formation |
|---|---|
| Rice straw | 24.25 |
| Wheat straw | 23.25 |
| Rice + wheat straw | 24.75 |
| Banana leaves | 50.75 |
| Rice straw + Banana leaves | 38 |

Treatments means are separated by Duncan's Multiple Range Test (DMRT) and the columns represented by same letter(s) are not significantly different among each other at 5%.

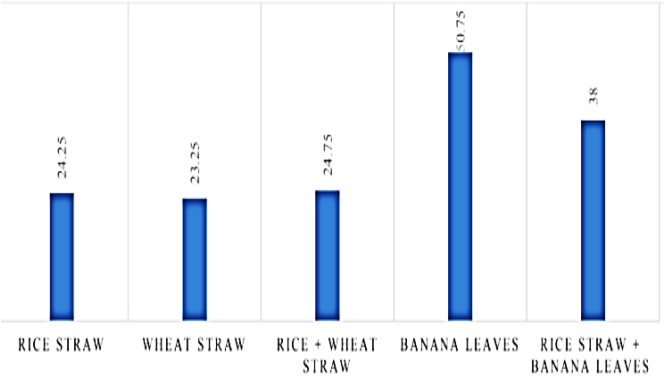


**Figure 1:** Days to primordia formation

From, table no.1, it was observed that wheat straw took shortest time for primordia formation i.e 23.25 days followed by rice straw and rice + wheat straw which took 24.25 days and 24.75 days respectively. The longest time was taken by banana leaves which took 50.75 days. The result was similar to and (Hossain, 2017; Saifullah et al., 2016). According to wheat straw took shortest time for primordia formation which was only 20 days (Saifullah et al., 2016). According too rice straw took 24days for pin head formation (Hossain, 2017). This might be due to greater amount of cellulose, hemicellulose and lignin in wheat straw followed by rice straw and lowest in banana leaves (Sarnklong et al., 2010; Tufail et al., 2018; Fernandes et al., 2013).

### 3.2 Effect of different substrates on the days to primordia formation from the day of full colonization

**Table 2:** Effect of different substrates on the days to primordia formation from the day of full colonization of Pleurotus spp under Gokuleshwor

| Treatments | Days to primordia formation |
|---|---|
| Rice straw | 1-2 |
| Wheat straw | 2-3 |
| Rice + Wheat straw | 4 |
| Banana leaves | 9 |
| Banana leaves + Rice straw | 12 |

Treatments means are separated by Duncan's Multiple Range Test (DMRT) and the the columns represented by same letter(s) are not significantly different among each other at 5%.

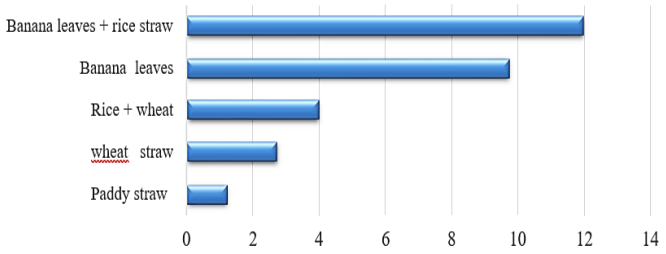


**Figure 2:** Days to primordia formation from full mycelial

After full colonization, the shortest time for primordia formation was taken by rice straw followed by wheat straw. Longest time for the primordia formation was taken by banana leaves. According to a study, it took 17-20 days to complete spawn running in *P. ostreatus* on different substrates and after 3-10 days i.e at 23-27days pinhead formation was noted (Ahmed, 1998). This is according to our result. The primordia formation in rice straw is quicker than wheat straw might be due to the quicker hydrolysis lignocellulose to sugar.

### 3.3 Effect of different substrates on total yield

**Table 3:** Effects of different substrates on total yield of *Pleurotus* mushroom under Gokuleshwor condition

| Treatments | Total yield (kg) |
|---|---|
| Rice straw | 1.581 |
| Wheat straw | 1.1825 |
| Rice + Wheat straw | 1.34 |
| Banana leaves | 0.5085 |
| Banana leaves + Rice straw | 1.02875 |

Treatments means are separated by Duncan's Multiple Range Test (DMRT) and the columns represented by same letter(s) are not significantly different among each other at 5%.

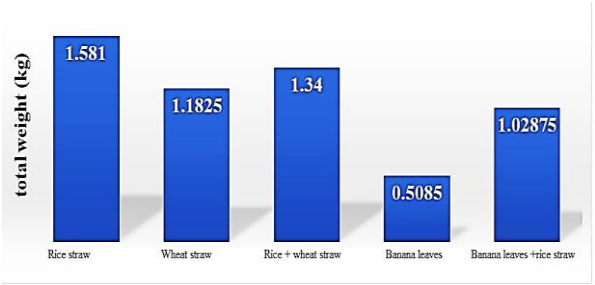


**Figure 3:** Total yield in different substrates

The highest yield of 1.581 kg was obtained in rice straw followed by rice and wheat straw which is 1.34kg. 100% wheat straw produced the yield of 1.1825kg. Banana leaves supplemented with rice straw yielded 1.02875 kg. Sole banana leaves produced the yield of 0.5085 kg which was lowest among the used treatments. In *Pleurotus ostreatus* the better yield performance was obtained in paddy straw (100%) (Sitaula et al., 2018). This might be the due to easier extraction of sugar from the substrates than on wheat and banana leaves. Banana leaves has the greatest yield among rice straw, wheat straw, rice and wheat straw, saw dust and banana leaves which is contrasting to our result (Neupane et al., 2018). And the reason might be the use of semi dried leaves in our experiment which contain low lignocellulose to hydrolyze into sugar.

### 3.4 Effect of different substrates on yield and stipe length on 1st harvest

**Table 4:** Effects of different substrates on yield and stipe length on 1st harvest of *Pleurotus* mushroom under Gokuleshwor condition

| Treatments | Yield on 1st Harvest (kg) | Length of stipe on 1st Harvest (cm) |
|---|---|---|
| Rice straw | 1.1725$^{a}$ | 17.9$^{a}$ |
| Wheat Straw | 0.805$^{b}$ | 16.75$^{a}$ |
| Rice+ wheat (1:1) | 1.03$^{a}$ | 17.25$^{a}$ |
| Banana leaves | 0.268$^{c}$ | 12.32$^{b}$ |
| Banana leaves+ rice straw (1:1) | 0.69$^{b}$ | 17.9$^{a}$ |
| Significance at 5% | *** | * |
| CV% | 16.244 | 12.59 |
| Mean | 0.79 | 16.07 |

Treatments means are separated by Duncan's Multiple Range Test (DMRT) and the the columns represented by same letter(s) are not significantly different among each other at 5%.

Analysis of variance show highly significant($p \leq 0.001$) result in yield of mushroom and significant ($p \leq 0.05$) result in length of stipe of mushroom between the different substrates used as a treatment. Highest yield was obtained in rice straw followed by rice + wheat straw and wheat straw. The lowest yield was obtained in banana leaves. Yield of rice straw and rice + wheat straw were statistically similar but yield of banana was statistically different from those of other treatments.

According to Zang, higher mushroom yield was found in paddy straw 10% more than wheat straw under the similar environmental condition (Zang, 1988). This is similar to our result. The reason for higher yield in rice straw might be that the nutrient required for the mushroom particularly for the spawn run and pin head development were supplied by rice straw substrates which decomposed quicker than other substrates. Banana yields more than rice straw and wheat straw (Neupane et al., 2018). This is contrasting to our result and the reason might be the use of semi dried leaves in our experiment rather than properly dried leaves which contain low amount of lignocellulose required for growth and development of mushroom.

In case of stipe length highest stipe length was obtained in rice straw followed by rice and wheat straw and the lowest stipe length was obtained in banana leaves. Highest yield and stipe length was observed in rice straw than other substrates (Dubey et al., 2019). The stipe length of all the substrates except banana leaves were statistically similar. The reason might be that the main factor that affects the stipe length is external factors like fresh air, temperature, humidity etc which were almost similar in case of our experiment and the only reason for the difference in stipe length of banana leaves is its low lignocellulose content.

### 3.5 Effect of different substrates on length of the stipe on first and second harvest

**Table 5:** Effect of different substrates on length of the stipe on first and second harvest of *Pleurotus spp* under Gokuleshwor condition

| Treatments | Stipe length on 1st harvest (cm) | Stipe length on 2nd harvest (cm) |
|---|---|---|
| Rice straw | 17.9 | 15 |
| Wheat straw | 16.75 | 13 |
| Rice + Wheat straw | 17.25 | 11.37 |
| Banana leaves | 12.3 | 12.5 |
| Banana leaves + Rice straw | 17.9 | 12.62 |

Treatments means are separated by Duncan's Multiple Range Test (DMRT) and the columns represented by same letter(s) are not significantly different among each other at 5%.

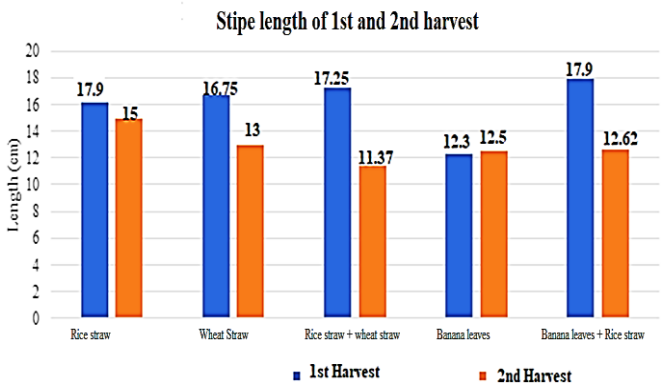


**Figure 4:** Length of stipe in 1st and 2nd harvest

The length of the stipe on all the treatments had reduced in 2nd harvest compared to 1st harvest except in case of banana leaves where the stipe length has slightly been increased. Our result is in accordance with Tesfaye et al., 2020 which states that the length of the stipe on 2nd harvest is lower than 1st harvest but stipe length in case of banana is contrary and the reason might be the greater use of hydrolyzed sugar in 2nd harvest than on 1st harvest since banana absorb more water than any other substrates. This water helps in quick degradation of substrates thus liberating sugar.

### 3.6 Effect of different substrates on the yield on 1st and 2nd harvest

**Table 6:** Effect of different substrates on the yield on 1st and 2nd harvest of Pleurotus spp under Gokuleshwor condition

| Treatments | Yield on 1st harvest (kg) | Yield on 2nd harvest (kg) |
|---|---|---|
| Rice straw | 1.1 | 0.4 |
| Wheat straw | 0.8 | 0.37 |
| Rice + wheat straw | 1.03 | 0.31 |
| Banana leaves | 0.27 | 0.24 |
| Banana leaves + Rice straw | 0.69 | 0.34 |

Treatments means are separated by Duncan's Multiple Range Test (DMRT) and the columns represented by same letter(s) are not significantly different among each other at 5%.

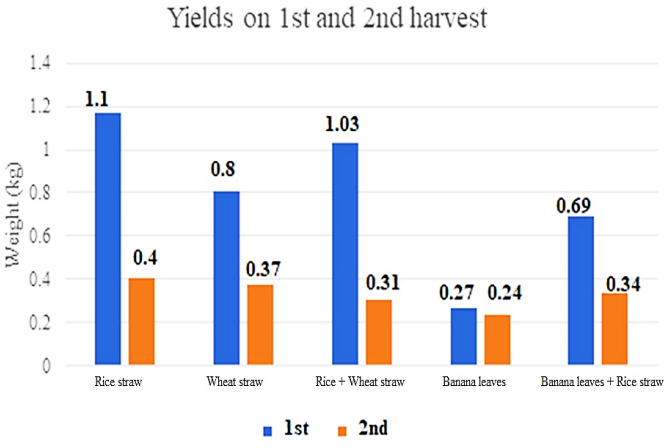


**Figure 5:** Yields on 1st and 2nd harvest

From the table no 6, yield in the 2nd harvest is lower compared to 1st harvest in all treatments. Our result is in accordance to which states that the yield is higher in first flush than on second and third flush (Shah et al., 2004). The reason for the decreased yield in the subsequent flush might be due to decreased nutrient content from the substrates.

### 3.7 Effect of different substrates on the duration to complete one life cycle

**Table 7:** Effect of different substrates on the duration to complete one life cycle of Pleurotus spp under Gokuleshwor condition

| Treatments | Days |
|---|---|
| Rice straw | 32 |
| Wheat straw | 31.5 |
| Rice + Wheat straw | 33.5 |
| Banana leaves | 54.75 |
| Banana leaves + Rice straw | 46 |

Treatments means are separated by Duncan's Multiple Range Test (DMRT) and the columns represented by same letter(s) are not significantly different among each other at 5%.

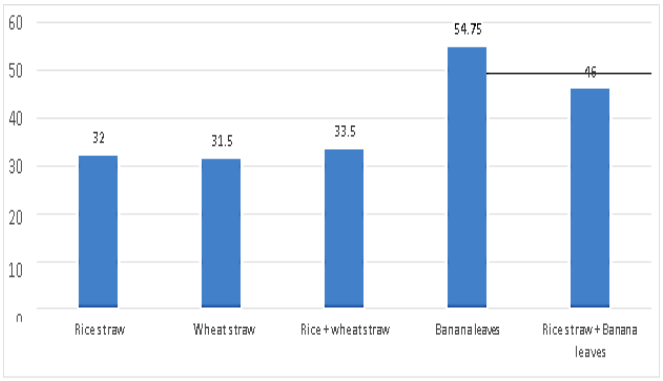


**Figure 6:** Duration to complete one cycle

From table no 7, from the day of spawning to one complete harvest the shortest time was taken by wheat straw followed by rice straw and rice and wheat straw. The banana leaves took longest time for the completion of one cycle. Rice straw took 26.25 days, wheat straw took 23.5, days, rice + wheat straw took 24.5 days which is similar to our result and banana leaves took23 days for the completion of one harvest which is contrasting to our result (Neupane et al., 2018). Banana leaves took longest time for the harvest i.e 10.5 days after primordia formation (Mondal et al., 1970). According to Ahmed,1988P. ostreatus 17-20 days for the completion of spawn running on different substrates and 23- 27daysfor pinhead formation. Wheat straw took 55 days for one complete harvest (Muswati et al., 2021). This is contrasting to our finding.

## 4. Conclusion

From the result obtained through research conducted in Gokuleshwor condition, wheat straw was quickest to primordia formation even quicker than rice straw. The highest stipe length and yield was obtained in rice straw followed by wheat straw and lowest in banana leaves. Using banana leaves alone or wheat straw alone is not found to be profitable.

## Suggestion

After the completion of the research, we came to know that there are various parameters that affect the mushroom production both in qualitative and quantitative way. Among the five substrates used, rice straw is found to be most profitable under Gokuleshwor condition. The mixture of rice and wheat straw can be used as a substitute for rice straw. If properly dried leaves of banana are used, it also gives the good result. As the research was conducted in only one season and only on the substrates of cereals crops result will be varied in upcoming seasons and ither substrates. Different research needs to be conducted to know the cause-and-effect relationship of different parameters and substrates for the production of *Pleurotus* mushroom.

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