

## Research Article

# Growth and Yield Performance of Oyster Mushroom (*P. ostreatus* (Jacq.: Fr.) Kummer) Using Waste Leaves and Sawdust

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Mushroom is a fungus growing on decomposing substrates. It is the substrate type that affects the yield and quality of oyster mushroom. It can be cultivated by landless people to alleviate poverty. The objective of this study was to evaluate the growth and yield performance of oyster mushroom in waste leaves and sawdust. Spawn were purchased from YB Plant Micropropagation Plc; Mekelle, Tigray, Ethiopia. *Euclea racemosa* waste leaves, *Cordia africana* waste leaves, and sawdust were prepared and inoculated with the spawn. Cotton husks were used as a control. 60 grams of spawn was used for 1000 g of each substrate and supplemented with 3% wheat bran and 1% gypsum. The data were analyzed using SPSS version 20. A one-way ANOVA model was used to indicate significant mean differences at 95% confidence interval between flushes. Treatment means were compared using Turkey's *t* test. In the first flush, primordial initiation was fastest and took 6.33 days in *Cordia africana* waste leaves and provide higher (166 ± 48.49, 131.6 ± 32.71, 49.66 ± 15.53 gram) mean yield and BE (16.6 ± 4.84, 13.16 ± 3.27, 4.96 ± 5.5%) in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> flushes, respectively. However, in the 2<sup>nd</sup> and 3<sup>rd</sup> flushes, a lower (24.66 ± 4.61, 14.66 ± 0.57 gram) mean yield was recorded in *Euclea racemosa* waste leaves. Higher (10.63 ± 1.00, 7.83 ± 3.92, 6.56 ± 2.26 cm) mean pileus diameter and pileus thickness (8.3 ± 1.47, 7.76 ± 1.32, 4.10 ± 0.85 mm) were noted in sawdust in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> flushes, respectively. This study confirmed that the waste leaves of *Cordia africana* and *Euclea racemosa* could be used as an alternative substrate for the cultivation of oyster mushroom.

## 1. Introduction

Mushroom, which is a flesh saprophytic macrofungi, has been valued throughout the world as either food or medicine. Edible mushrooms can be saprophytes, symbionts, and parasites of different plants and all need organic matter to grow [1]. Mushroom production is an economically viable biotechnology process for recycling of various lignocellulosic biomass and agro-industrial wastes into food [2]. Mushroom cultivation is relatively a new applied technology, and the mushroom industry is still small compared to other crop types [3]. Also, it has been reported as an alternative way to alleviate poverty, especially in developing countries, due to its low cost of production and high profitability [4]. Edible mushrooms are able to colonize and degrade a large variety of lignocellulosic

substrates and convert various wastes which are produced primarily through the activities of the agricultural, forest, and food-processing industries [5, 6]. Mushrooms have long been used for medicinal and food purposes for over a thousand years [7] and possess a variety of nutrients such as high protein, carbohydrate, crude fiber, fat, minerals, mycocellulose, vitamins, riboflavin, as well as niacin. Beyond their food sources, mushrooms are also used as medicinal and have some biotechnology-based applications [8, 9].

Oyster mushroom is an edible mushroom having excellent flavor and taste. *Pleurotus* species are popular and widely cultivated throughout the world owing to their simple and low-cost production technology [10]. Improving the performance of mushroom in terms of high production and fast growth rate is essential in mushroom cultivation. Oyster

mushrooms are easier to cultivate compared to other types of mushroom species [11], and their production has increased dramatically in recent years [12]. It can be cultivated at moderate temperatures, ranging from 20 to 30°C, and at a humidity of 55–70% because of its ability to adapt a variety of factors [13].

Many mushroom growers from different corners of the world cultivate mushroom using cotton seed husk, sawdust, sugarcane bagasse, corncob and rice straw. However, it is not easily accessible. In the capital city of Ethiopia (Addis Ababa), most mushroom growers use cotton husk, but this was not easily available in the remote areas of the country. However, in different parts of the world, many substrates have been applied for mushroom cultivation such as wheat straw, grass, barley straw, and bean straw. Most of our farmers use these substrates for animal feed and mushroom growers could not access them easily with fair price, and there is a need for cheap and easily available substrates.

In Ethiopia, mushroom cultivation is a young project that has not been expanded to the community. This could be due to lack of awareness of the people towards the consumption of mushroom and lack of cost-effective substrates. Therefore, the current study focuses in assessing the growth and yield performance of oyster mushroom using locally available substrates such as waste leaves and sawdust for oyster mushroom cultivation.

## 2. Materials and Methods

**2.1. Description of the Study Area.** The study was conducted in the Aksum University Microbiology Laboratory. Axum is located at an altitude of 2133 m in the north part of Ethiopia at 14°11'N and 38°73'E. Axum's average annual temperature is 18.3°C, and its average annual precipitation is 652 mm. The average annual relative humidity is 57.7%.

**2.2. Spawn Preparation.** For spawn preparation, 10 kg of untreated sorghum was used and washed three times, i.e., fresh water was added upon every discarding until all the dirty materials were removed. Then, the sorghum was soaked overnight, and excess water was drained off. The soaked sorghum seeds were thoroughly mixed with 5% wheat bran and 2% gypsum. Optimum moisture was adjusted to 51–54%. Then, the mixture was distributed equally into 250 ml sterile plastic bags, at the rate of 250 g of sorghum seed per plastic bag. This was autoclaved, at 121°C for 30 minutes. After cooling, culturing of the spawn was done under laminar air-flow. Each of the filled sterile plastic bag was inoculated with oyster mushroom culture (*Pleurotus ostreatus* (Jacq.: Fr.) Kummer; obtained from YB Plant Micropropagation Plc; Mekelle, Ethiopia). The inoculated plastic bags were incubated for 15 days at 25°C. When the mixture was totally invaded by mycelium, the spawn was ready to be used for the inoculation of the solid substrate [14].

**2.3. Substrate Preparation and Inoculation.** Waste leaves of *Euclea racemosa* and *Cordia africana* were collected from around Axum town, and sawdust was collected from

different wood works. Cotton husks were also purchased from Addis Ababa. The collected substrates were transported to the Aksum University Microbiology Laboratory and soaked overnight. One kilogram of each substrate was weighed, prepared in triplicate, and autoclaved at 121°C for 15 minutes. The excess water was drained off, and the moisture content of the room was held 60–70%. This was cooled and 3% wheat bran and 1% gypsum were added to the cooled substrate. For 1 kg of substrate, 60 grams of spawn was added. Finally, it was placed in the dark room until mycelium was covered the substrate. After mycelium running, it was taken into a room, which can transmit light partially.

**2.4. Cropping and Harvesting of Mushroom.** Matured mushrooms were picked by clean hands without harming the substrates. Mycelium running (days), primordial initiation, number of total primordia, maturity (days), number of flushes, number of effective fruiting bodies, pileus thickness (mm), pileus diameter (cm), and stipe length (cm) were recorded, and the total weight of all fruiting bodies harvested for all the flushes were measured as the total yield of the mushroom, while the biological efficiency (B.E.) was calculated as given in [15] using the following formula:

$$B.E = \frac{\text{weight of fresh mushroom harvested (g)}}{\text{weight of dried substrate (g)}} \times 100\% \quad (1)$$

The pileus diameter and the stipe length were measured with graduated transparent ruler.

**2.5. Data Collection Methods.** *Pleurotus ostreatus* were grown in triplicates and data were recorded periodically for three flush, namely, 1<sup>st</sup> flush, 2<sup>nd</sup> flush, and 3<sup>rd</sup> flush. Data on total yield, BE, mycelium running, primordial initiation, stalk length, pileus diameter, pileus thickness, number of total primordia, maturity, and number of effective fruiting bodies were determined.

**2.6. Data Analysis.** The data were analyzed by using SPSS version 20. An analysis of variance (ANOVA) was used to indicate significant mean differences at the 95% confidence interval. Mean treatments were compared using Turkey's *t* test.

## 3. Results and Discussion

**3.1. Results.** Mushroom is an attractive crop to cultivate in developing countries for many reasons. Oyster mushroom (*Pleurotus* spp.) cultivation has increased tremendously throughout the world. In the current study, spawn were purchased from Mekelle town, and three substrates, namely, waste leaves of *Cordia africana* and *Euclea racemosa*, as well as sawdust, were tested for the cultivation of *P. ostreatus* (Table 1). Mycelia extension, maturity, pileus diameter, pileus thickness, stalk length, yield, and BE were tested and collected for three consecutive flushes. *P. ostreatus* were

TABLE 1: Substrates used in the different treatment groups for the cultivation of oyster mushroom.

Treatment group	Type of substrate	Amount of dry substrate (kg)	Number of replication of each trial
T1	<i>Cordia africana</i> waste leaves	1	Three
T2	<i>Euclea racemosa</i> waste leaves	1	Three
T3	Sawdust	1	Three
T4	Cotton husk (control)	1	Three

harvested when the in-rolled margins of the mushroom began to flatten (Supplementary Figure 3).

Mycelium running across treatments varied, but statistically there was no significant difference (Supplementary Figure 13).

Results from Table 2 confirmed that T3 took much time for primordial initiation ( $9.00 \pm 1.00$ ,  $16.33 \pm 13.51$ , and  $14.00 \pm 2.63$ ), respectively, in all flushes, and no significant difference was observed in all the treatments except in the second flush (Supplementary Figure 10).

Furthermore, there was no significant difference in mushroom primordial initiation between T2 and T4 ( $P > 0.05$ ) at flush one and flush three, but there was a significant difference between T1 and T4 at flush 1. Statistically, there was no significant difference in maturity among each treatment (Supplementary Figure 14).

A higher number of total primordia were recorded in T1 and T4 in all the flushes, but less number of total primordia were counted in T2 and T3 (Supplementary Figure 11).

The result from Table 3 also revealed that T4 produce higher mean mushroom number of effective fruiting bodies ( $15.66 \pm 3.21$  and  $13.33 \pm 5.50$ ), respectively, at flush one and three (Supplementary Figure 12). There was a significant difference between T4 and other treatments at flush one and three ( $P < 0.05$ ). But the mean mushroom number of effective fruiting bodies in both T4 and T3 were more or less the same at flush two ( $P > 0.05$ ). T1 gave the second highest mean mushroom number of effective fruiting bodies ( $8.00 \pm 2.00$ ,  $10.33 \pm 4.72$ , and  $11.33 \pm 6.11$ ), respectively, in all flushes. T2 produced the least mean mushroom number of effective fruiting bodies at the second and third flushes (Table 3).

The result from Table 4 revealed that the T4 group produce higher mean stalk lengths ( $4.63 \pm 1.18$ ,  $4.20 \pm 0.34$ , and  $3.60 \pm 0.52$  cm), respectively, throughout the flushes. Furthermore, there was significant difference between T2 and other treatments at flush one and two ( $P < 0.05$ ). But the mean stalk length in all treatments was more or less the same at flush three ( $P > 0.05$ ). Besides, T1 gave the second highest ( $4.33 \pm 0.55$ ) mean stalk length only in the first flush. However, there was no significance difference as compared to T3 ( $P > 0.05$ ), and T2 produced the least mean stalk length (Supplementary Figure 5).

T3 gave higher mushroom pileus diameters ( $10.63 \pm 1.00$ ,  $7.83 \pm 3.92$ , and  $6.56 \pm 2.26$ ), respectively, throughout the flushes, and there was a significant difference with T1 and T2 at the first flush. Furthermore, the difference between T3 and T4 was not significantly different ( $P > 0.05$ ) throughout all flushes, and T3 produced a higher mushroom

pileus diameter compared to other treatments, especially at the first flush (Supplementary Figure 6).

Treatment three (T3) produced higher pileus thickness in the first and second flush (Supplementary Figure 7) compared to T1 and T2, and no significant association was observed between T3 and T4 ( $P > 0.05$ ) at flushes 1 and 3 (Table 5).

T1 and T3 produce higher mean yield in all flushes. However, this was lower compared to the control T4 which is  $480.33 \pm 48.29$ ,  $285.00 \pm 56.34$ , and  $128.00 \pm 9.84$  gm, respectively, throughout the flushes and there was a significant difference between T4 and other treatments in each flush ( $P < 0.05$ ). But the mean mushroom yield for T1 and T3 was more or less the same in all flushes, with a lower ( $65.66 \pm 18$  gm) mean yield obtained in T2 (Supplementary Figure 8).

The mean BE in each flush for T1 and T3 was ( $16.6 \pm 4.84$ ,  $13.16 \pm 3.2$ ,  $4.96 \pm 1.55$ ) and ( $15.06 \pm 4.20$ ,  $13.66 \pm 6.47$ ,  $5.63 \pm 2.88$ ), respectively. Less mean BE was obtained from T2 compared to all treatments, with T4 giving the highest BE value (Supplementary Figure 9).

**3.2. Discussion.** *P. ostreatus* is an easily cultivable mushroom that colonizes different substrates. The results showed that the changes in stalk length, pileus diameter, yield, BE, maturity, number of effective fruiting bodies, and number of total primordial of oyster mushroom grown in different substrates vary depending on the substrates (Supplementary Figure 11).

Although statistically there was no significant difference in mycelium running between treatments, the lowest days to complete mycelium running were recorded on sawdust (Table 6). However, higher rates (25 days) were reported for *P. ostreatus* cultivated on different waste leaves to complete mycelium running [16]. Other studies reported that mycelium running of *Pleurotus ostreatus* cultivated on 50% cotton waste + 50% maize leaves took 45 days which was higher than the current study [17]. Similarly, the authors in [18] reported 21 days for mycelium running of *P. ostreatus* cultivated on banana leaves. However, higher numbers (78 days) were reported for mycelium running in banana leaves supplemented with 15% wheat bran. In the current study, sawdust was best for the cultivation of *P. ostreatus* in terms of mycelium running rate. The shorter number of days taken for mycelium running of sawdust and cotton husk might be due to the higher nutrient composition and the presence of a suitable C:N ratio which could be responsible for the higher mycelial growth (Supplementary Figure 1).

TABLE 2: Effect of substrates on primordial initiation (days) and maturity per flush of *P. ostreatus*.

Substrates	Primordial initiation (days) (mean $\pm$ SD)			Maturity (days) (mean $\pm$ SD)		
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush
T1	6.33 $\pm$ 0.57 <sup>b</sup>	5.66 $\pm$ 0.57 <sup>b</sup>	6.00 $\pm$ 1.00 <sup>a</sup>	4.00 $\pm$ 0.00 <sup>a</sup>	4.33 $\pm$ 0.57 <sup>a</sup>	4.00 $\pm$ 0.00 <sup>a</sup>
T2	8.33 $\pm$ 2.30 <sup>ab</sup>	17.00 $\pm$ 1.73 <sup>a</sup>	10.00 $\pm$ 2.00 <sup>a</sup>	3.66 $\pm$ 0.57 <sup>a</sup>	3.33 $\pm$ 0.57 <sup>a</sup>	3.00 $\pm$ 0.00 <sup>a</sup>
T3	9.00 $\pm$ 1.00 <sup>a</sup>	16.33 $\pm$ 3.51 <sup>a</sup>	14.00 $\pm$ 2.64 <sup>a</sup>	4.33 $\pm$ 0.57 <sup>a</sup>	4.33 $\pm$ 0.57 <sup>a</sup>	4.00 $\pm$ 0.00 <sup>a</sup>
T4	8.33 $\pm$ 0.57 <sup>ab</sup>	6.33 $\pm$ 1.52 <sup>b</sup>	10.66 $\pm$ 7.37 <sup>a</sup>	4.33 $\pm$ 0.57 <sup>a</sup>	3.66 $\pm$ 0.57 <sup>a</sup>	4.00 $\pm$ 0.00 <sup>a</sup>

Means followed by the same superscript letter along the column are not statistically different ( $P > 0.05$ ).

TABLE 3: Effect of substrates on number of primordia and effective fruiting bodies per flush.

Substrates	Number of total primordia (mean $\pm$ SD)			Number of effective fruiting bodies (mean $\pm$ SD)		
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush
T1	40.66 $\pm$ 6.80 <sup>a</sup>	18.00 $\pm$ 1.00 <sup>ab</sup>	21.33 $\pm$ 7.57 <sup>a</sup>	8.00 $\pm$ 2.00 <sup>c</sup>	10.33 $\pm$ 4.72 <sup>a</sup>	11.33 $\pm$ 6.11 <sup>ab</sup>
T2	14.66 $\pm$ 1.52 <sup>c</sup>	4.00 $\pm$ 1.00 <sup>c</sup>	3.66 $\pm$ 0.57 <sup>b</sup>	4.66 $\pm$ 1.15 <sup>bc</sup>	2.66 $\pm$ 1.52 <sup>c</sup>	2.66 $\pm$ 0.57 <sup>c</sup>
T3	8.00 $\pm$ 6.55 <sup>c</sup>	9.66 $\pm$ 8.96 <sup>bc</sup>	5.66 $\pm$ 2.51 <sup>b</sup>	3.00 $\pm$ 1.00 <sup>b</sup>	6.33 $\pm$ 4.50 <sup>ab</sup>	4.33 $\pm$ 1.52 <sup>bc</sup>
T4	25.00 $\pm$ 3.00 <sup>b</sup>	18.66 $\pm$ 0.57 <sup>a</sup>	19.00 $\pm$ 1.00 <sup>a</sup>	15.66 $\pm$ 3.21 <sup>a</sup>	8.00 $\pm$ 1.00 <sup>ab</sup>	13.33 $\pm$ 5.50 <sup>a</sup>

Means followed by the same superscript letters within a column are not statistically different ( $P > 0.05$ ).

TABLE 4: Effect of substrates on stalk length (cm) per flush of *P. ostreatus*.

Substrates	Mean $\pm$ SD of stalk length in each flush		
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush
T1	4.33 $\pm$ 0.55 <sup>ab</sup>	3.23 $\pm$ 0.76 <sup>ab</sup>	2.40 $\pm$ 1.4 <sup>a</sup>
T2	2.86 $\pm$ 0.61 <sup>c</sup>	2.30 $\pm$ 0.26 <sup>b</sup>	3.00 $\pm$ 0.00 <sup>a</sup>
T3	3.22 $\pm$ 0.57 <sup>ab</sup>	3.33 $\pm$ 0.57 <sup>a</sup>	2.36 $\pm$ 0.35 <sup>a</sup>
T4	4.63 $\pm$ 1.18 <sup>a</sup>	4.20 $\pm$ 0.34 <sup>a</sup>	3.60 $\pm$ 0.52 <sup>a</sup>

Means followed by the same superscript letter along the column are not statistically different ( $P > 0.05$ ).

TABLE 5: Effect of substrates on pileus diameter and thickness per flush of *P. ostreatus*.

Substrates	Pileus diameter (cm) (mean $\pm$ SD) (cm)			Pileus thickness (mm) (mean $\pm$ SD) (mm)		
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush
T1	5.26 $\pm$ 1.48 <sup>b</sup>	5.63 $\pm$ 0.7 <sup>a</sup>	4.83 $\pm$ 0.85 <sup>a</sup>	4.33 $\pm$ 0.35 <sup>b</sup>	4.00 $\pm$ 1.30 <sup>b</sup>	3.10 $\pm$ 0.10 <sup>a</sup>
T2	5.70 $\pm$ 0.69 <sup>b</sup>	4.06 $\pm$ 0.05 <sup>a</sup>	4.03 $\pm$ 0.05 <sup>a</sup>	2.76 $\pm$ 0.20 <sup>b</sup>	4.46 $\pm$ 0.40 <sup>b</sup>	4.33 $\pm$ 0.57 <sup>a</sup>
T3	10.63 $\pm$ 1.00 <sup>a</sup>	7.83 $\pm$ 3.92 <sup>a</sup>	6.56 $\pm$ 2.26 <sup>a</sup>	8.30 $\pm$ 1.47 <sup>a</sup>	7.76 $\pm$ 1.32 <sup>a</sup>	4.10 $\pm$ 0.85 <sup>a</sup>
T4	8.70 $\pm$ 1.47 <sup>a</sup>	7.20 $\pm$ 1.31 <sup>a</sup>	6.50 $\pm$ 2.17 <sup>a</sup>	6.10 $\pm$ 2.00 <sup>a</sup>	4.53 $\pm$ 1.85 <sup>b</sup>	5.33 $\pm$ 4.04 <sup>a</sup>

Means followed by the same superscript letter along the column are not statistically different ( $P > 0.05$ ).

TABLE 6: Effect of substrates on mycelium extension (days) per flush of *P. ostreatus*.

Substrates	Mean $\pm$ SD of mycelium extension in each flush		
	Flush 1	Flush 2	Flush 3
T1	19.00 $\pm$ 0.00 <sup>a</sup>	18.00 $\pm$ 0.00 <sup>a</sup>	19.00 $\pm$ 0.00 <sup>a</sup>
T2	19.00 $\pm$ 0.00 <sup>a</sup>	18.00 $\pm$ 0.00 <sup>ba</sup>	18.00 $\pm$ 0.00 <sup>a</sup>
T3	17.00 $\pm$ 0.00 <sup>a</sup>	17.00 $\pm$ 0.00 <sup>a</sup>	17.00 $\pm$ 0.00 <sup>a</sup>
T4	15.00 $\pm$ 0.00 <sup>a</sup>	15.00 $\pm$ 0.00 <sup>a</sup>	15.00 $\pm$ 0.00 <sup>a</sup>

Means followed by the same superscript letter along the column are not statistically different ( $P > 0.05$ ).

Maturity of *P. ostreatus* was determined and the variation in cropping period among different substrates could come from variations in the time elapsed for formation of pinheads, maturation of fruiting bodies, period between

flushes, and number of flushes and yield, which in turn are affected by the nature of the substrates [19]. However, statistically, there were no significant differences in the maturity of the different substrates (Table 2). The result of the current study is similar to the work of [20], who reported that the maturity of *P. ostreatus* cultivated on sawdust and cotton waste took (4.09  $\pm$  0.16) days. Higher maturity periods were also reported for *P. ostreatus* cultivated on rice straw supplemented with *Trianthem portulacastrum* weed and took 34 days for harvesting [21]. Similarly, a higher number of days for maturity of *P. ostreatus* were reported, i.e., 27 days for cotton seed and 40.67 days for wheat straw [22]. The variation in maturity may emanate from the variation in the growing environment and physiological requirements for mushroom cultivation like temperature and humidity.

TABLE 7: Effect of substrates on yield and biological efficiency per flush of *P. ostreatus*.

Substrates	Yield (mean $\pm$ SD) (gm)			Biological efficiency (mean $\pm$ SD) (%)		
	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush	1 <sup>st</sup> flush	2 <sup>nd</sup> flush	3 <sup>rd</sup> flush
T1	166 $\pm$ 48.49 <sup>b</sup>	131.6 $\pm$ 32.71 <sup>b</sup>	49.66 $\pm$ 15.53 <sup>b</sup>	16.6 $\pm$ 4.84 <sup>b</sup>	13.16 $\pm$ 3.27 <sup>b</sup>	4.96 $\pm$ 1.55 <sup>b</sup>
T2	65.66 $\pm$ 18.00 <sup>c</sup>	24.66 $\pm$ 4.61 <sup>c</sup>	14.66 $\pm$ 0.57 <sup>c</sup>	6.56 $\pm$ 1.800 <sup>c</sup>	2.46 $\pm$ 0.46 <sup>c</sup>	1.46 $\pm$ 0.05 <sup>c</sup>
T3	150.66 $\pm$ 42.00 <sup>b</sup>	136.66 $\pm$ 64.76 <sup>b</sup>	56.33 $\pm$ 28.88 <sup>b</sup>	15.06 $\pm$ 4.20 <sup>b</sup>	13.66 $\pm$ 6.47 <sup>b</sup>	5.63 $\pm$ 2.88 <sup>b</sup>
T4	480.33 $\pm$ 48.29 <sup>a</sup>	285.00 $\pm$ 56.34 <sup>a</sup>	128.00 $\pm$ 9.84 <sup>a</sup>	48.03 $\pm$ 4.82 <sup>a</sup>	28.50 $\pm$ 5.63 <sup>a</sup>	12.80 $\pm$ 0.98 <sup>a</sup>

Means followed by the same superscript letter along the column are not statistically different ( $P > 0.05$ ).

Primordial initiation was observed following the invasion of substrates by mycelia growth. In the current study, the time required for the formation of pinheads for sawdust and *Euclea racemosa* waste leaves after spawning took 6–9 days (Table 3). Higher (33 days) of primordial initiation rates of *Pleurotus ostreatus* mushroom cultivated on cotton husk were reported in [23]. Similarly, the study conducted on sawdust supplemented with rice bran found a higher (31.60  $\pm$  1.14) number of days for primordial initiation [24]. Other studies were also reported by [20] for primordial initiation which cultivated on sawdust and cotton waste and took 6.44  $\pm$  0.57 and 6.77  $\pm$  0.57 days, respectively. The variation in primordial initiation could be due to the difference in nutrient composition of the substrates.

The highest number of total primordia was recorded from *Cordia africana* waste leaves, but low numbers of primordia were obtained from *Euclea racemosa* waste leaves and sawdust (Table 3). Substrates containing glucose, fructose, and trehalose provide the highest number of primordia while those containing glycerol, xylose, sucrose, and fructose produced abnormal fruiting bodies [25]. Compared to the current study, a higher number of total primordia were described in the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> flushes of *P. ostreatus* cultivated on sawdust, and it was reported as 29.39, 51.34, and 32.5 days, respectively [26].

From the different types of substrates, a stalk length of *P. ostreatus* was measured and cotton husk (the control) gave a higher stalk length in all flushes, and this was followed by waste leaves of *Cordia africana* and sawdust (Table 4). Our result is similar with that of [27], who reported a stalk length of (3.59 cm) from Kadom sawdust and the lowest (2.20 cm) from coconut sawdust. Mushrooms with higher stalk length will have poor quality [28]. Besides, fruit bodies with larger pileus and shorter stipes are better than those with smaller pileus and longer stipes [29]. However, the stipes contain more insoluble dietary fibers that can be used for the preparation of biologically active polysaccharide complexes utilizable as food supplements than the pilei. The current study revealed that sawdust provides the better quality of mushroom with larger diameter and shorter stalk length (Supplementary Figure 2).

In the first flush, higher pileus diameter and pileus thickness were observed (Table 5). The pileus diameter differed on the different substrates. In the first flush, less (5.6 cm) pileus diameter of *Pleurotus ostreatus* mushroom cultivated on sawdust was reported in [30]. The performance of oyster mushroom grown in different substrates with respect to stipe length and pileus diameter also depends on

the structure, compactness, and physical properties of the substrate (Supplementary Figure 4). The substrates with higher moisture retaining capacity perform better than those with lower moisture retaining capacity [31]. In the current study, waste leaves of *Cordia africana* and *Euclea racemosa* produced less pileus diameter and pileus thickness, indicating that these substrates might retain less moisture content than cotton husk and sawdust. On the other hand, the existence of higher pileus diameter of *P. ostreatus* in sawdust and cotton husk might be due to the presence of adequate nutrients with high moisture retention capacity in the substrates. Moreover, the variation in the pileus thickness could be due to the difference in the rate of absorption of nutrients in the substrates.

The cultivated mushrooms were harvested in three flushes, and in the first flush, higher yields were obtained on cotton husk (Supplementary Figure 3) and *Cordia africana* waste leaves (Table 7). Followed this, the authors in [32] reported less (46 gm) yield of *P. ostreatus* cultivated on sawdust. Moreover, the authors in [33] found less (123  $\pm$  1.77 gm) yield of *P. ostreatus* cultivated on coconut sawdust. On the other hand, oyster mushroom cultivated on mixed sawdust of Simbal, Mango, Kail, and Kikar yield less (95 gm, 75 gm, and 63 gm) in three flushes compared to the current study [34]. The highest yield on cotton husk and sawdust might be due to the availability of different nutrients on the substrate.

In all flushes of the current study, maximum biological efficiency was obtained on waste leaves of *Cordia africana*, and this was followed by sawdust. The lowest biological efficiency was obtained from *Euclea racemosa* waste leaves (Table 7). Similar result (14.9%) of biological efficiency was reported from sawdust [31]. On the other hand, 50.2% of BE were reported for *P. ostreatus* cultivated on sawdust [35]. Moreover, 15.86% BE were obtained in *P. ostreatus* cultivated on *Grevillea robusta* leaves [3]. *Pleurotus florida* were also cultivated on sawdust for consecutive three flushes and found a higher (85%, 65%, and 48%) biological efficiency, and the biological efficiency of a species is directly related to strain, substrate nutrition, and growth conditions [18].

Number of effective fruiting bodies of the different substrates varies across the three flushes. In the first and second flushes, a higher numbers of effective fruiting body were obtained from the control substrate, and this was followed by *Cordia africana* waste leaves. The lowest numbers of fruiting body were recorded on sawdust. In the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> flushes of *P. ostreatus* cultivation, less (8.00  $\pm$  1.53, 10.67  $\pm$  1.20, and 9.00  $\pm$  0.58) numbers of

effective fruiting body were reported from dry plantain leaves [36]. The number of fruiting bodies produced per flush decreased from flush to flush [37]. The difference might be due to the variation in the digestibility of the substrates by the enzyme.

#### 4. Conclusion

The fastest mycelium runners were found in sawdust and cotton husk. Besides, higher BE and yield of *P. ostreatus* were observed in *Cordia africana* waste leaves and cotton husk (control). *Euclea racemosa* waste leaves show the fastest maturity of pinheads, but sawdust produced a better quality of mushroom, i.e., a higher pileus diameter with a shorter stalk length.

#### Data Availability

Datasets supporting the conclusion are available from the corresponding author upon request.

#### Disclosure

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#### Conflicts of Interest

The authors declare that there are no conflicts of interest.

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#### Supplementary Materials

Figure 1: mycelium running of *P. ostreatus* in different substrates. Figure 2: growth of *P. ostreatus* on sawdust. Figure 3: matured *P. ostreatus* on Cotton husk. Figure 4: growth of *P. ostreatus* on *Cordia africana* and *Euclea racemosa* waste leaves. Figure 5: effect of substrate on stalk length of *P. ostreatus* mushroom. Figure 6: effect of substrate on pileus diameter of *P. ostreatus* mushroom over flushes. Figure 7: effect of substrate on pileus thickness of *P. ostreatus* mushroom over flushes. Figure 8: effect of substrate on yield of *P. ostreatus* mushroom over flushes. Figure 9: effect of substrate on BE of *P. ostreatus* mushroom over flushes. Figure 10: effect of substrate on primordial initiation of *P. ostreatus* mushroom over flushes. Figure 11: effect of substrate on total primordial of *P. ostreatus* mushroom over flushes. Figure 12: effect of substrate on number of effective fruiting bodies of *P. ostreatus* mushroom over flushes. Figure 13: effect of substrate on mycelium running. Figure 14: effect of substrate on maturity of *P. ostreatus* mushroom over flushes. (*Supplementary Materials*)

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