
Growth, yield performance, and economic assessments of *Pleurotus ostreatus* var. *floridag* grown in Philippines agricultural waste enriched with dried banana leaves

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Abstract Dried banana leaves, rice straw, and sawdust are locally available and abundant in the farming community in the Philippines. These substrates enriched with rice bran are suitable for tropical mushroom cultivation. Based on various combinations of these substrates, cultivation of *Pleurotus ostreatus* var. *florida* finds that the optimum mycelial increment and the favorable number of days of full mycelial colonization were recorded in 40% rice straw + 30% banana leaves + 30% sawdust + 10% rice bran and 10% rice straw + 60% banana leaves + 30% sawdust + 10% rice bran combinations respectively. Moreover, the combination of 70% banana leaves + 30% sawdust + 10% rice bran obtained the maximum number of flushes, the highest return on investment, and achieved the highest economic yield of 751 g. However, biological efficiency is not significantly (0.05) different among substrate formulations. Enrichment of dried banana leaves to the traditional formulation of rice straw and sawdust substrate in oyster mushroom production is highly suggested since mycelial colonization and fruiting can be achieved in a short period of days.

Keywords: *Pleurotus ostreatus* var. *florida*, Substrates, Mycelial performance, Biological efficiency

Introduction

Mushroom cultivation and production are valued in Asia and Central-Eastern Europe because of its taste and its high nutritional value as important source of protein, carbohydrates, vitamins, calcium, and iron (Siwulski, 2019). According to Emiru *et al.* (2016), mushrooms were seen as food for nobility and the best option to alleviate food shortage and poverty.

The growing interests in the cultivation of mushrooms resulted to the dynamic development in its production and import recording to over 10 million metric tons of mushrooms produced (FAOSTAT, 2018). For instance, China

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alone recorded an almost eight million tons of mushroom produced on 2016 (Sardar *et al.*, 2017). According to Rosmiza *et al.* (2016), oyster mushroom is the most common mushroom with the highest performance yield and production in Malaysia with over 90.89% total of mushroom cultivated. In the Philippines, mushroom has no steady supply in the market since small-scale mushroom growers only produce about 200 metric tons of oyster mushroom per year which dominate the mushroom production (Zurbano *et al.*, 2017).

Mushroom farmers encountered various challenges particularly in utilizing substrates. According to Núñez and Mendoza (2002), the physiochemical properties of substrates might differ in favour of the spread of mycelia from the applied spawn until it fully ramified. Consequently, agricultural wastes used as substrates are locally available and abundant and it contains lignocellulosic materials that are generally low in protein, which is insufficient for commercially cultivating mushrooms (Mane *et al.*, 2007). These agro-industrial wastes can be enhanced with sufficient amounts of nitrogen, phosphate, potassium, and vitamins for better growth and yield of mushrooms (Mangat *et al.*, 2008). Furthermore, in the report of Oei *et al.* (2005), the nutrient composition of substrates is one of the limiting factors to the mycelial colonization of cultivated mushrooms particularly fruiting of *P. florida*.

The growing percentage of agro-industrial waste discarded every year contributes to environmental degradation (Panesar *et al.*, 2015). These wastes, however, contain lignocellulosic materials and cellulose, which can be an ideal substrate for the cultivation and production of Oyster mushroom (*P. florida*). Particularly, the banana leaves are ideal substrates for the production of *P. florida* since it contains cellulose that is good for the mushroom cultivation and production (Silva *et al.*, 2018).

Banana is the number one fruit commodity in the Philippines (Espino and Espino, 2015) producing nearly 9.1 million metric tons of bananas on 443,270 hectares of land (PSA, 2015). Consequently, during its production, a million metric tons of banana waste products are generated (Padam *et al.*, 2014). The million metric tons of banana waste if remains untapped and contributes to environmental pollution both in soil and in water (Alzate *et al.*, 2021). On the other hand, Philippines rice farmer only generated 32 % of the 22 million tons' rice straw after harvest (Mendoza, 2015); while an approximately two million tons annually of sawdust were produced in the country for the production of lumber (Mwango and Kambole 2019). Hence, using these agricultural wastes as substrate for mushroom production is a good strategy to harness its full biotic potential while promoting healthy farming environment.

At present, cultivating *P. florida* is now a profitable business. In fact, there are many small-scale mushroom growers ventured in this livelihood because it

requires minimal production costs and uses agricultural waste as substrate in cultivation. Thus, this study was conducted to determine the mycelial performance, biological efficiency, total days of full colonization and economic viability of *P. florida* inoculated in rice straw-based formulation enriched with dried banana leaves.

Materials and methods

Preparation of fruiting bags

Rice straw and banana leaves (*M. acuminata* × *balbisiana* leaves) were soaked for 24 hours and were drenched after. Subsequently, the banana leaves and rice straw were chopped at approximately one inch. Then the test combinations were mixed (Bellere, 2019). The substrate formulation followed the following mixtures as shown in Table 1.

Table 1. Formulation of substrates for *P. florida*

Treatments	Rice Straw	Dried Banana Leaves	Sawdust	Rice Bran
Treatment 1 (T1)	70%	0 %	30%	10%
Treatment 2 (T2)	60%	10%	30%	10%
Treatment 3 (T3)	50%	20%	30%	10%
Treatment 4 (T4)	40%	30%	30%	10%
Treatment 5 (T5)	30%	40%	30%	10%
Treatment 6 (T6)	20%	50%	30%	10%
Treatment 7 (T7)	10%	60%	30%	10%
Treatment 8 (T8)	0	70%	30%	10%

*note: all substrate will be added with 10 % rice bran as additive

Once the combinations were mixed homogeneously, the substrate formulation was air dried until 65% of moisture was attained, then 500 grams of the mixture was placed in a 6x12x0.2 polypropylene sheet and was placed with ¾ PVC as neck. Then a cotton plug was placed and secured with used paper. Subsequently, the fruiting bags were undergo steam sterilization for one hour at 121°C / 15 psi. Then allowed to cool and was inoculated with *P. florida* spawn. After that, it was stored at a dark room at room temperature ranging from 20°C to 30 °C and allowed to fully ramified. Once fully ramified the fruiting bags was placed in the growing house at Tara Sipocot, Camarines Sur. It was opened after five days of acclimatization. The fruiting bags were sprayed with tap water three times a day and harvesting was done if the edge of the *P. florida* caps begin to flatten or turn upwards. Moreover, the mycelial performance of *P. florida* in rice straw-based formulation enriched with dried banana leaves were determined by conducting daily mycelial run to determine the mycelial increment and also adopting the parameters of Silva *et al.* (2018) for the mycelial density of the treatments.

Statistical analysis

This study used eight treatments and were done in nine replicates. Turkey's Honest Significant Difference (HSD) was utilized to determine the significance of the data. For the biological efficiency, the formula below was used (Babar *et al.*, 2016).

$$\text{Biological Efficiency (BE)} = \frac{\text{Weight of fruiting Bodies}}{\text{Weight of Substrates}} \times 100$$

Additionally, the Return on Investment (ROI) was determined (Bandera & Bendas, 2020) by the formula:

$$\text{ROI} = \frac{\text{Cost of Production}}{\text{Net Income}} \times 100$$

Furthermore, the cost of production were calculated by adding all the cost of raw materials such as fruiting bags, PVC, spray bottle and cotton used throughout the oyster mushroom cultivation while the net income was determined by the total profit earned in selling the *P. floridain* the market were in one kilogram was sold as high as 200 pesos.

Results

Mycelial density

The mycelial density of *P. florida* shown in Table 2 were noted to be very thickened in 50% RS+ 20% BL + 30% SD + 10% RB, 40% RS+ 30% BL + 30% SD + 10% RB, 10% RS + 60% BL + 30% SD + 10% RB and 70% BL + 30% SD + 10% RB. Meanwhile, 30% RS + 40% BL+ 30% SD + 10% RB noted with very thin mycelial density.

Table 2. Mycelial density of *P. floridain* rice straw based formulation enriched with dried banana leaves

Treatments	Substrates	Mycelial Density
T1	70% RS + 30% SD + 10% RB	++
T2	60% RS + 10% BL + 30% SD + 10% RB	+++
T3	50% RS + 20% BL + 30% SD + 10% RB	++++
T4	40% RS + 30% BL + 30% SD + 10% RB	++++
T5	30% RS + 40% BL + 30% SD + 10% RB	+
T6	20% RS + 50% BL + 30% SD + 10% RB	+++
T7	10% RS + 60% BL + 30% SD + 10% RB	++++
T8	70% BL + 30% SD + 10% RB	++++

¹/The parameters of the mycelial density were adopted from Silva *et al.* (2018).

²/(+) very thin, (++) thin, (+++) thick, (++++) very thick.

³/BL = Banana leaves, RS = Rice straw, SD = Sawdust, RB = Rice bran

Daily hyphal increment

The shorter period of day of colonization is ideal in mushroom production. It dictate the number of days need for incubation which can provide data for fruiting production. The widest and fastest mycelial growth increment *P. florida* was revealed in 40% RS + 30% BL + 30% SD + 10% RB while the formulation 50% RS + 20% BL + 30% SD +10% RB obtained the lowest mycelial growth as shown in table 3 and figure 1.

Table 3. Growth increment *P. florida* in evaluated substrate combination

Treatment	Substrates	Daily Mean of Mycelial Increment					Range of Mycelial from Day 1 to Day 13	Rank
		1	3	6	9	13		
1	70% RS + 30% SD + 10% RB	3.08	16.44	44.50	73.78	117.25	114.17	5
2	60% RS + 10% BL + 30% SD +10% RB	6.81	20.86	48.69	78.39	118.53	111.72	7
3	50% RS + 20% BL + 30% SD +10% RB	19.25	28.33	51.22	79.03	118.92	99.67	8
4	40% RS + 30% BL + 30% SD + 10% RB	7.93	27.94	56.33	87.47	126.52	118.59	1
5	30% RS + 40% BL + 30% SD + 10% RB	6.19	20.19	48.72	81.14	123.34	117.15	3.5
6	20% RS + 50% BL + 30% SD + 10% RB	13.94	29.75	58.08	89.08	131.81	117.87	2
7	10% RS + 60% BL + 30% SD + 10% RB	17.19	31.78	61.75	91.11	130.11	112.92	6
8	70% BL + 30% SD +10% RB	14.22	29.90	56.94	88.69	131.37	117.15	3.5

¹/Range measure the difference between the largest and the smallest value of mycelial density of *P. florida* in different substrate formulation to determine the variability of mycelia distribution.

²/Millimeters (mm) is the unit used to determine the range of daily mycelial density of *P. florida*

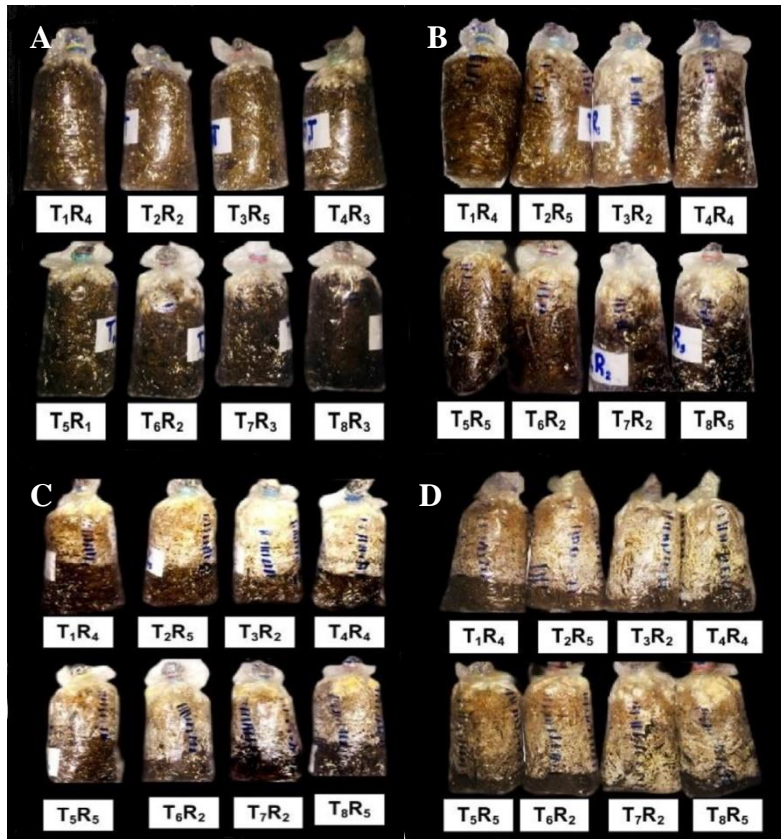


Figure 1. Growth increment of *P. florida* in evaluated substrate formulation on day 1 (A), day 5 (B), (C) day 10 and day 13 (D)

Return on investment (ROI)

Return on investment is profitability metric which used to evaluate the profitability of the business. To evaluate the return on investment of the different substrate formulation the total costs of production, harvests, and net income were considered.

The highest return on investment (ROI) was recorded on 70% BL + 30% SD + 10% RB and achieved the highest economic yield of 751 g followed by 30% RS + 40% BL + 30% SD + 10% RB, 10% RS + 60% BL + 30% SD + 10% RB, 60% RS +10% BL + 30% SD +10% RB, 50% RS +20% BL + 30% SD +10% RB and 20% RS + 50% BL + 30% SD + 10% RB. However, the lowest return on investment was obtained in 40% RS+ 30% BL + 30% SD + 10% RB in nine production bags each treatment, which also attains the lowest economic yield of 492 g.

Table 4. Return on investment of Oyster Mushroom (*P. florida*)

Treatments	Cost of Production	Economic yield(grams)	Net Income (Php)	Return on Investment	Rank
T1	90.375	492g	98.4	108.88%	7
T2	90.375	588g	117.6	130.12%	4
T3	90.375	582g	116.4	128.8%	5
T4	90.375	451g	90.2	99.81%	8
T5	90.375	739g	147.8	163.54%	2
T6	90.375	512g	102.4	113.31%	6
T7	90.375	717g	143.4	158.67%	3
T8	90.375	751g	150.2	166.2%	1

¹/T1= Treatment 1, T2= Treatment 2, T3= Treatment 3, T4= Treatment 4, T5= Treatment 5, T6= Treatment 6, T7= Treatment 7, T8= Treatment 8.

Total days of full mycelial colonization of P. florida

The total days of mycelial growth of *P. florida* in rice straw-based formulation enriched with dried banana leaves elucidated that the formulation 50% RS+ 20% BL + 30% SD + 10% RB, 70% RS + 30% SD + 10% RB and, 60% RS +10% BL + 30% SD + 10% RB were significantly ($p=0.05$) noted with shorter period of days of incubation compared to the rest of the treatment.

Table 5. Days after spawning of *P. florida* in different substrate formation

Treatments	Substrates	Incubation Period (Days)
T1	70% RS + 30% SD + 10% RB	15.92a
T2	60% RS +10% BL + 30% SD + 10% RB	15.81a
T3	50% RS+ 20% BL + 30% SD + 10% RB	16.00a
T4	40% RS+ 30% BL + 30% SD + 10% RB	14.42b
T5	30% RS + 40% BL+ 30% SD + 10% RB	14.42b
T6	20% RS +50% BL + 30% SD + 10% RB	14.00b
T7	10% RS +60% BL + 30% SD + 10% RB	13.47b
T8	70% BL + 30% SD + 10% RB	13.78b

¹/Data presented are means of eight replicates.

²/Means with the same letter are not significantly different at 5% level of significance using Tukeys's Honest Significance Difference (HSD) test.

³/RS = Rice straw, SD = Sawdust, RB = Rice bran

Difference on the Biological Efficiency of P. florida in Rice Straw-Based Formulation Enriched with Dried Banana Leaves

In this study, the biological efficiency was determined as the ratio of fresh weight mushroom and weight of substrates. The highest biological efficiency was recorded in 70% BL + 30% SD + 10% RB while the lowest biological efficiency of *P. florida* obtained in treatment with 40% RS+ 30% BL + 30% SD + 10% RB. However, no significance (0.05) difference were recorded for the biological efficiency among substrate formulation.

Table 6. Biological efficiency of *P. florida*

Treatments	Substrates	Biological Efficiency (%)
T1	70% RS + 30% SD + 10% RB	10.92a
T 2	60% RS +10% BL + 30% SD + 10% RB	13.07a
T 3	50% RS+ 20% BL + 30% SD + 10% RB	12.93a
T4	40% RS+ 30% BL + 30% SD + 10% RB	10.62a
T5	30% RS + 40% BL+ 30% SD + 10% RB	16.42a
T6	20% RS +50% BL + 30% SD + 10% RB	11.38a
T7	10% RS +60% BL + 30% SD + 10% RB	15.93a
T8	70% BL + 30% SD + 10% RB	16.69a

¹/Data presented are means of nine replicates.

²/Means with the same letter are not significantly different at 5% level of significance using Tuukeys's Honest Significance Difference (HSD) test.

³/RS = Rice straw, SD = Sawdust, RB = Rice bran

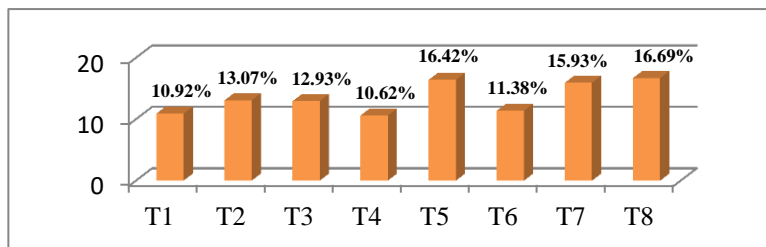


Figure 2. Biological efficiency of *P. florida* in different substrate combination

The growth of *P. Florida* is considered to be dependent upon the performance of the substrates and directly influence the time frame to attain its maximum yield as shown in figure 2 and figure 3.

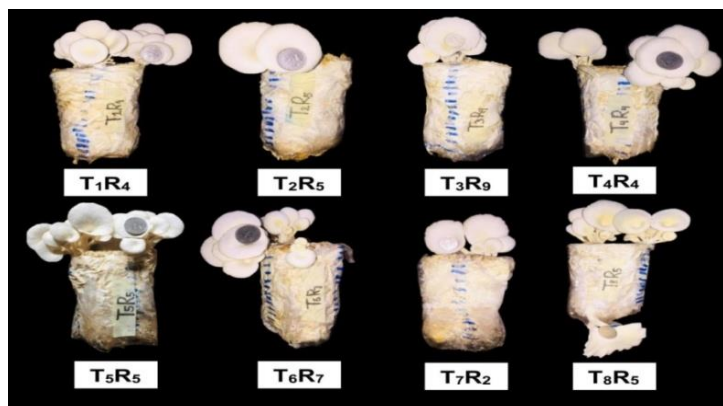


Figure 3. Fruiting bodies of *P. florida* in different substrate formulation

Number of flushes of P. florida in different substrate formulation

The flushes of *P. florida* were counted in each fruiting bag. Substrates consisting 70% BL + 30% SD + 10% RB obtained the maximum number of flushes with a mean of 2.44 that is significantly (0.05) higher to 1.33 flush mean of the traditional combination of 70% RS + 30% SD + 10% RB.

Table 7. Number of Flushes of *P. florida* in different substrate formulation

Treatments	Substrates	Number of Flushes
T1	70% RS + 30% SD + 10% RB	1.33b
T2	60% RS + 10% BL + 30% SD + 10% RB	1.78ab
T3	50% RS + 20% BL + 30% SD + 10% RB	1.67ab
T4	40% RS + 30% BL + 30% SD + 10% RB	1.56ab
T5	30% RS + 40% BL + 30% SD + 10% RB	2.22ab
T6	20% RS + 50% BL + 30% SD + 10% RB	2.00ab
T7	10% RS + 60% BL + 30% SD + 10% RB	1.89ab
T8	70% BL + 30% SD + 10% RB	2.44a

¹/Mean with the same letter are not significantly different (0.05)

Discussion

One of the principal factors in the cultivation of mushroom is the growth and performance of mycelia in the substrate. As a result of this study, the mycelia density of *P. florida* were noted to be very thick in 50% RS + 20% BL + 30% SD + 10% RB, 40% RS + 30% BL + 30% SD + 10% RB, 10% RS + 60% BL + 30% SD + 10% RB and 70% BL + 30% SD + 10% RB while very thin mycelia was observed in treatment with 30% RS + 40% BL + 30% SD + 10% RB. These findings correspond with the observation of Chang and Miles (2004), that the difference in the duration of the incubation period in a given substrate could be due to the fungal strain, growth conditions, and substrate type. Likewise, Bhatti *et al.* (1987), explained that the variation could also be attributed to the difference in chemical composition as well as in carbon and nitrogen ratio of the substrates. In terms of daily mycelial increment of *P. florida*, it showed that 40% RS + 30% BL + 30% SD + 10% RB recorded the highest mycelial increment while 50% RS + 20% BL + 30% SD + 10% RB obtained the lowest mycelial growth of *P. florida*. This result revealed that the ability of banana leaves and rice straw to attain maximum mycelial colonization can be attributed to carbon and nitrogen content of banana leaves and rice straw since according to Mondal *et al.* (2010), banana leaves and rice straw contain high amount of carbon and nitrogen. However, the lower mycelial performance of mushroom might be due to the various kinds of

polyphenolic substances, low content of cellulose and moisture holding capacity of substrates (Neupane *et al.*, 2018).

Furthermore, the main purpose of mushroom cultivation is yield. The present study reveals that 70% BL + 30% SD + 10% RB recorded the highest value of return on investment. Similarly to the observation of Muswati *et al.* (2021), substrate with higher mycelium growth rate and water holding capacity of the substrate as well as the suitability of C: N ratio might be the responsible of maximum yield of oyster mushroom. Correspondingly with the result of the present study which is evidently shown in substrate formulation with 30% RS + 40% BL + 30% SD + 10% RB, 60% RS + 10% BL + 30% SD + 10% RB, 50% RS + 20% BL + 30% SD + 10% RB, 20% RS + 50% BL + 30% SD + 10% RB. However, this is not in congruent for the treatment that consisting of 70% RS + 30% SD + 10% RB and 40% RS + 30% BL + 30% SD + 10% RB which obtained the lowest return on investment.

Comparing all the mean of treatments in different substrate formulation, 10% RS + 60% BL + 30% SD + 10% RB (13.47 days) recorded the shortest period of colonization while treatment with 50% RS + 20% BL + 30% SD + 10% RB obtained the longest incubation period. This significant variation of total days of mycelial growth and colonization of *P. florida* can be associated to the substrate formulations and substrates used in cultivating the mushrooms since according to Rzymiski *et al.* (2016), the mycelial performance of mushroom depends on the chemical composition and properties of the growing substrate. Colonization period in the present study is not congruent with the result obtained by Mondal *et al.* (2010), since this study is shorter in terms of mycelial colonization of *P. florida* among substrates, hence is more ideal. Additionally, based on Tukey's Honest Significant (HSD) test the data revealed that formulation containing 30%, 40%, 50%, 60% and 70% banana leaves is significantly (0.05) requires shorter period of days in terms of mycelial full colonization of fruiting bags compared to substrate with 10% and 20% banana leaves as well as the substrate without banana leaves. This finding signifies that banana leaves in the formulation of rice straw based sawdust is ideal as additive in 30%, 40%, 50%, 60% and 70% composition. This finding suggests that substrate type is one of the major factors affecting the growth, fruiting and quality of *P. florida*. Several researchers, Chukwurah *et al.* (2013), Dubey *et al.* (2019) and Onyeka *et al.* (2018), also observed this significant differences among different substrates formulation.

Biological efficiency (BE) is another important parameter to be considered in mushroom cultivation. It is used to evaluate the efficiency of substrate conversion into fruiting bodies (Girmay *et al.*, 2016). In this study, 70% BL + 30% SD + 10% RB obtained the highest biological efficiency among

treatments. Meanwhile, treatment with 40% RS + 30% BL + 30% SD + 10% RB recorded the lowest biological efficiency of *P. florida* production. These findings show that the variation of BE among each treatment probably affect the fructification of *P. florida* since according to Hoa *et al.* (2015), differences in the physical and chemical compositions of substrates including the cellulose/lignin ratio, mineral contents, electrolyte conductivity (EC) and carbon nitrogen ratio of substrates can be a factor in the differences of biological efficiency of *P. florida*. Moreover, Reddy and Yang (2015), also explained that this can be attributed to the higher percentage of banana leaves among the substrate since banana leaves contain 26% cellulose, 17% hemicelluloses and 25% lignin which can be a key factor in the fast conversion of substrate into mushroom fruiting bodies.

The nutritional content of the substrates can affect the number of flushes since according to Liebig's Law of Minimum, the rate of growth of a plant, the size to which it grows, and its overall health depend on the amount of the scarcest of its essential nutrients that is available to it. In this study, 70% BL + 30% SD + 10% RB recorded the maximum number of flushes followed by 30% RS + 40% BL + 30% SD + 10% RB, 20% RS + 50% BL + 30% SD + 10% RB, 10% RS + 60% BL + 30% SD + 10% RB, 60% RS + 10% BL + 30% SD + 10% RB, 50% RS + 20% BL + 30% SD + 10% RB and 40% RS + 30% BL + 30% SD + 10% RB while 70% RS + 30% SD + 10% RB which has no banana leaves on the substrate obtained the lowest number of flushes among different substrate formulation. These results closely correspond to the observation of Oliveira *et al.* (2007), that the number of flushes of *P. florida* produced can be associated to the nutritional content of banana leaves since it contains higher amount of cellulose, lignin and sugar that can support the fructification of mushroom.

From the present study, it is confirmed that the performance and productivity of oyster mushroom were highly influenced by the substrate from which it was grown. Substrate formulation consisting of 10% RS + 60% BL + 30% SD + 10% RB were identified as the most suitable substrates for *P. florida* cultivation among the evaluated substrate since it can able to provide widest mycelial increment, maximum mycelial density and fastest colonization as well as short period of initial fruiting. Additionally, 70% BL + 30% SD + 10% RB is also efficient and ideal substrates considering that it can generate highest return on investment among the treatments. Therefore, mushroom growers and farmers can use 10% RS + 60% BL + 30% SD + 10% RB and 70% BL + 30% SD + 10% RB as substrate formulation in cultivating oyster mushroom. Moreover, a follow up studies on the other agricultural waste as

well as different varieties of banana leaves are being considered and subject for further evaluation.

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