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EVALUATION OF GROWTH AND PRODUCTIVITY OF MUSHROOM (*Pleurotus ostreatus*) UNDER DIFFERENT ORGANIC SUBSTRATE COMBINATIONS

***Teke A.N.A., Muyang R.F. and Ngongbi J.N.**

Department of Biology, Higher Teacher Training College,
The University of Bamenda, Bamenda, P.O. Box 39, Bambili, Cameroon

*Corresponding author's email: teke1976a@gmail.com

ABSTRACT

Mushrooms are fleshy saprophytic fungi noted for their health, nutritional and economic benefits. Recent studies have revealed the ability of Pleurotus mushroom to grow on agricultural wastes within a short period of time. In Cameroon, the technological knowledge on the use of organic waste materials as substrates for mushroom production still remains under exploited. The aim of this study was to investigate the effect of different organic substrate combinations on the growth and yield of Pleurotus ostreatus. A completely randomized design was laid out with four treatments and four replications, giving a total of 16 treatments. The treatments consisted of T1 (sawdust/rice bran/corn flour), T2 (sawdust/corncobs/rice bran/corn flour), T3 (palm cones/rice bran/corn flour) and T4 (elephant grass straw/rice bran/corn flour). Results obtained revealed that growth and yield of P. ostreatus depends largely on the type of substrate combination used. Significant differences ($p < 0.05$) were recorded in growth parameters among treatments. The highest mean height (19.5 cm), diameter of pileus (29 cm) and mean weight of individual fruiting bodies (75.6 g) were recorded in T2. The highest biological yield was also due to T2 which was significantly ($p < 0.05$) different from T3 and T4 but not T1. The combination of sawdust + corn cobs + rice bran + corn flour showed a suitable substrate for the cultivation of P. ostreatus and therefore can be recommended to local communities in the Northwest region of Cameroon where large volumes of agricultural wastes such as sawdust and corncobs are still highly under-utilized.

Key words: cultivation, mushrooms, organic waste, substrate combinations

INTRODUCTION

Mushrooms are fleshy saprophytic fungi that can be found growing on damp rotten logs of wood, tree trunks, decaying organic matter and in damp soil rich in organic materials. *Pleurotus ostreatus* (oyster mushroom) belongs to the family Pleurotaceae. *Pleurotus* originated from China; however, nowadays it is distributed all over the world, except for the North-West Pacific because of the arctic climate (Wojewoda, 2003). The mushrooms of the genus *Pleurotus* occupy the third position in the production of edible mushrooms, behind the species of the genus *Agaricus* and *Lentinula* (Cardoso *et al*., 2013). *Pleurotus* spp. are found in tropical and subtropical rainforests around the world, and can be artificially cultivated (Bonatti, 2004) due to their ability to colonize and degrade a wide variety of substrates containing cellulose, hemicellulose and lignin, using them in their own development (Pokhrel *et al*., 2013). Furthermore, these species have a quick mycelium growth and fruiting, less prone to diseases, and requiring minimal monitoring of the cultivation environment due to an easy adaptation and maintenance (Pokhrel *et al*., 2013). Therefore, due to nutritional and functional characteristics, *Pleurotus* spp. is considered increasingly popular from a commercial point of view.

P. ostreatus has received increased attention for applications in bio-bleaching and the catalysis of difficult chemical conversions in the paper industry, textile dye decolorization, and detoxification of environmental pollutants (Park *et al.,* 2014). They are rich in proteins, contain less fat, less carbohydrates and salt and rich in fibres and have high vitamin B12 and folic acid which are uncommon in vegetables (Park *et al.,* 2014). High availability of lysine and tryptophan and other amino acids usually absent in cereals makes them an ideal food for hypertension, diabetes and obesity patients (Carel *et al.,* 2013). Mushrooms require carbon, nitrogen and inorganic compounds as nutrients and their major sources of carbon are cellulose, hemi-cellulose and lignin. The use of various wastes is recommended for *P. ostreatus* growth (Vendruscolo *et al.*, 2008). The production of *Pleurotus* spp. has been tested with different substrates *e.g*., cotton waste (Chang *et al.,* 1981), rice straw (Mehta *et al.*, 1990), by products of corn chain (Loss, 2009), bark of coffee (Dias *et al*., 2003), wheat straw and sugarcane bagasse (Cardoso *et al*., 2013). The substrates used for cultivation depend largely on their local availability (Cohen *et al*., 2002). The recycling of different materials is one of the most important contributions of fungi in nature (Sanchez *et al*., 2002).

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Current population growth coupled with inadequate supply of food, diminishing quality of health, high rate of unemployment and increasing environmental degradation are some of the key underlying problems affecting the future well-being of humankind (Chang and Miles, 2004). In Cameroon, most attention on agricultural research is centered around cash crops for exports such as cocoa, coffee, bananas, tea, oil palm and a number of studies on vegetable crops while less attention has been given to mushroom research (Achancho, 2013). Women and children scramble for mushroom after the first drop of rain, due to the fact that its availability still remains seasonal in most areas where mushroom farming and domestication is rare (Oguntoye *et al.,* 2022). These mushrooms can be sustainably grown for food, health and also to generate income from common agricultural waste in the environment such as coffee pulp, sawdust, corncobs, rice straw, cassava peels, cocoa pots (CEDEP, 2012). Areas such as the North West Region of Cameroon produce a lot of corncobs, sawdust, rice straw which are waste products. These waste products can be used by local farmers in mushroom production thereby reducing pollution.

The level of self-employment in the Cameroonian economy is quite low as the government still employs more than the private sector. Therefore, this study will help inform the government and the local population on the importance of creating strategies and methods to enhance self-employment. Mushroom cultivation serves as the most efficient and economically viable biotechnology for the conversion of long cellulose waste materials into high quality protein food (Bonginkhosi *et al*., 2012). This does not depend on weather conditions such as rainfall and can thus be grown all year round in cropping houses (Bonginkhosi *et al*.,2012). If the local communities are informed on the methods of cultivating mushroom on a small scale, this will assist as an income generator and therefore improve standards of living. Mushroom is an attractive crop cultivated in developing countries for many reasons such as nutrition, health and economic reasons. They are grown on agricultural wastes, enabling the acquisition of substrate materials at low prices or even for free and to conserve the environment by recycling wastes thereby creating new job opportunities especially in rural areas and increasing revenue generation (Talaro and Talaro, 2002). Therefore, this study was aimed at evaluating the combination of different organic substrates in improving yield of *Pleurotus ostreatus* to meet up consumer's demand in local markets in Bambili, NW Region of Cameroon.

MATERIALS AND METHODS

Study Site

This research was conducted at "Hotspot", Bamenda I Subdivision, situated in Mezam Division in the North West Region of Cameroon (Figure 1) from the month of June to October 2022. Bamenda is a city in the North West Region with a population of about 2 million inhabitants. The area has a tropical savanna climate, bordering on a tropical monsoon climate with long wet season and short dry season.

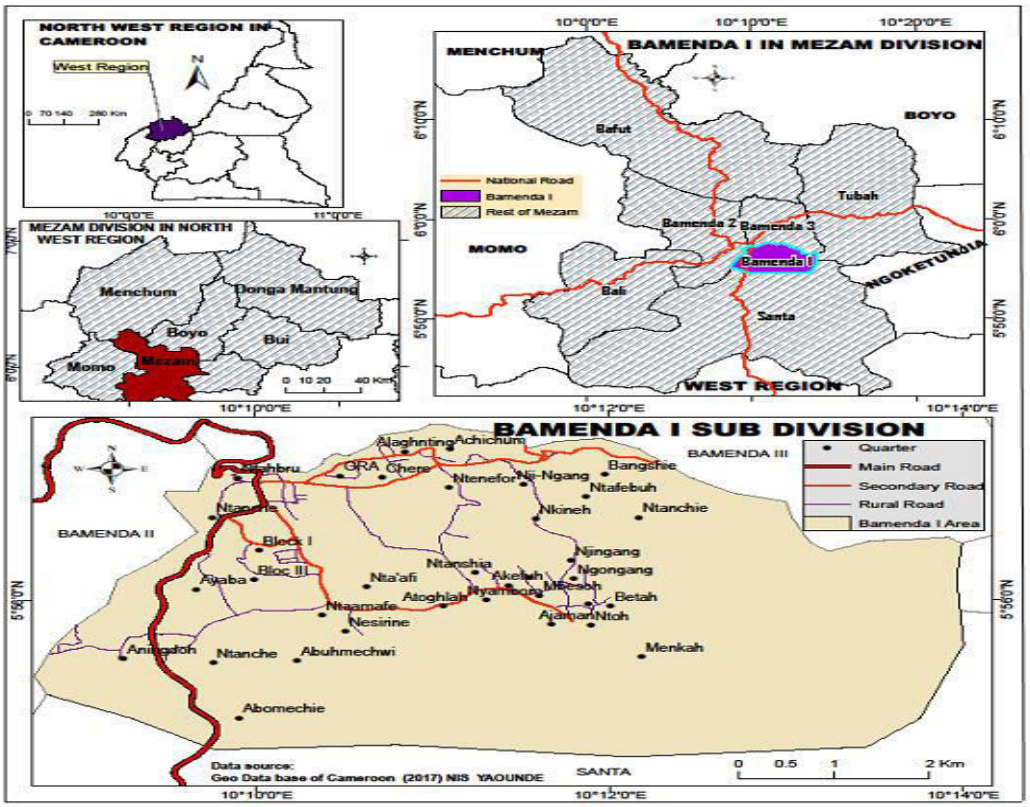

Figure 1: Map of Bamenda I Subdivision indicating Nkineh (site of cultivation)

Collection and Preparation of Samples
The spawn for this study was bought from Bimaih and Bernard (B&B) Mushroom Farm situated at Hotspot, Bamenda in the Northwest Region of Cameroon. The substrates used for the study included sawdust, corncobs, palm cones, elephant grass straw (*Pennisetum purperum),* rice bran and corn flour. Sawdust from was collected from a saw mill. Corncobs and corn flour were bought from local vendors. Rice bran was collected at a rice milling factory, Palm cones from Bamali-Ndop and elephant grass straw was collected from a farmland. The palm cones and elephant grass straw were chopped into small pieces of 3-5 cm and sun-dried for 6 h for threw days. Calcium hydroxide and plastic bags were bought from the local market. Cultivation method of *P. ostreatus* on different substrates was done using the protocol of Anagho (2008). The experiment was laid out in a completely randomized design with each treatment replicated four times making a total of 16 treatments. Table 1 shows the various substrates (treatments) and composition used in the cultivation of *P. ostreatus*.

Treatment 1
In the first treatment, 4.5 kg of fine sawdust and 800 g of rice bran were mixed with tap water. 200g of corn flour was weighed using a weighing balance and added to the sawdust and rice bran mixture.

Treatment 2
In the second treatment, 2.25 kg of sawdust and 2.25 kg of crushed corncobs were measured using a scaled balance. The corncobs were soaked with tap water in a 5L bucket overnight to enable the substrate absorb enough moisture after which it was drained the next morning. 200 g of corn flour and 800 g of rice bran were weighed using a weighing balance and added to the mixture.

Treatment 3
In the third treatment, 4.5 kg of palm cones were measured using a scaled balance. The palm cones were soaked with 5 L of water over night to enable it absorb enough moisture after which it was drained the next morning. Then, 200 g of corn flour and 800 g of rice bran were measured and added to the palm cones.

Treatment 4
In this treatment, 4.5 kg of elephant grass straw was measured using a weighing balance and soaked in tap water overnight to enable it absorb enough moisture after which it was drained the next morning. Then, 200 g of corn flour and 800 g of rice bran were measured and added to the elephant grass straw.

To each of the above treatments, 25 g of hydrated calcium hydroxide was added to the mixture to neutralize the pH. The ingredients in each treatment were homogenized using a clean spade. The substrates were allowed to drain for 3-4 h to remove excess water. Each treatment was replicated four times, put in plastic bags and tied.

Substrate Sterilization and Spawning
Substrates in the tied polythene bags for the different treatments were sterilized separately in boiling water for 4 h in a large drum. The bags were allowed to cool and inoculated with the spawn. Eight spawn bottles or seeds were used to plant 16 substrate bags at a ratio of, 1 bottle of spawn (seed) for two bags of substrate (1:2). With the aid of sterilized razor blades, tiny perforations were made on the substrate bags to enable fruiting bodies to develop. The substrate bags were then labeled and incubated in a dark room with shelves for colonization at a relative humidity of about 70-85% and temperature of about 25-32 °C (Figure 2). After 21 days when the mycelium was fully colonized, the windows were open for ventilation to enhance primordial pinhead formation and full fruiting of the mushroom. Watering of substrates started after the first fruiting to ensure available moisture for continuous fructification.

Harvesting
Maturation period varied in the different treatments. Harvesting started six weeks after spawning when the fruiting bodies were matured. Harvesting was done after every three days. The process of harvesting involved the removal with the hand of the matured fruiting bodies from their substrate without any destruction on the substrate bag. The matured mushroom was held on their stipe below the pileus and close to the substrate level and was gradually pulled out. All matured fruiting bodies of a particular substrate bag were harvested at the same time (Figure 6).

Measurement of Growth Parameters
Four bags, one from each treatment were sampled at random using the paper balloting system. These bags were monitored daily for data collection. The means for each parameter were then calculated and recorded. Growth parameters such as spawn running, pinhead formation and harvest times were recorded in days.

Measurement of Yield Parameters
Yield parameters measured were number of fruiting bodies by counting before harvesting, height and diameter of fruiting bodies using a measuring tape, and fresh weight using a weighing balance. The biological yield was obtained by taking and calculating the total fresh weight of the fruiting bodies per bag. Biological efficiency was calculated using the following equation (Kinge *et al*., 2016):

$$\frac{\text{Fresh weight of harvested mushroom (g)}}{\text{dry weight of substrate (g)}} \times 100\%.$$

Table 1: Substrates and compositions used in the cultivation of *P. ostreatus* and replications

Treatments	Substrates and Composition
Treatment 1	Sawdust + rice bran + corn flour + calcium hydroxide
Treatment 2	Sawdust + corncobs + rice bran + corn flour + calcium hydroxide
Treatment 3	Palm cones + rice bran + corn flour + calcium hydroxide
Treatment 4	Elephant grass straw + rice bran + corn flour + calcium hydroxide

Figure 2: Different substrate treatments incubated for colonization

Data Analysis

The data were analyzed using Microsoft Excel 2019 version. Means were analyzed by one-way analysis of variance and means separated using Tukey's Honestly Significant Difference (HSD) test to compare if there were significant differences at $p < 0.05$ between the substrates tested.

RESULTS

Effects of Different Substrates on the Growth and Yield of *Pleurotus ostreatus*

Spawn running time

Spawn running time tested on the four different substrates varied from 15 to 26 days with significant differences at $p < 0.05$ between treatments (Figure 3). Treatment T3 (palm cones/rice bran/corn flour) took the shortest time to spawn (15.6±0.7 days) not significantly different from T2 (sawdust/corn cobs/rice bran/corn flour) 18.4±0.9 days but was significantly different from T4 (elephant grass straw/rice bran/corn flour), 20.4±0.8 days and T1 (sawdust/rice bran/corn flour), spawned only after 24.2±1.3 days.

Duration for pinhead formation

Pinhead formation was observed after 21.0±1.2 days in T1 (sawdust/rice bran/corn flour) which was similar to T2 (sawdust/corn cobs/rice bran/corn flour) (28.1±1.6 days), but significantly different from T3 (palm cones/rice bran/corn flour) that took 35.0±2.8 days and T4 (elephant grass straw/rice bran/corn flour) that took 46.3±1.7 days (Figure 4).

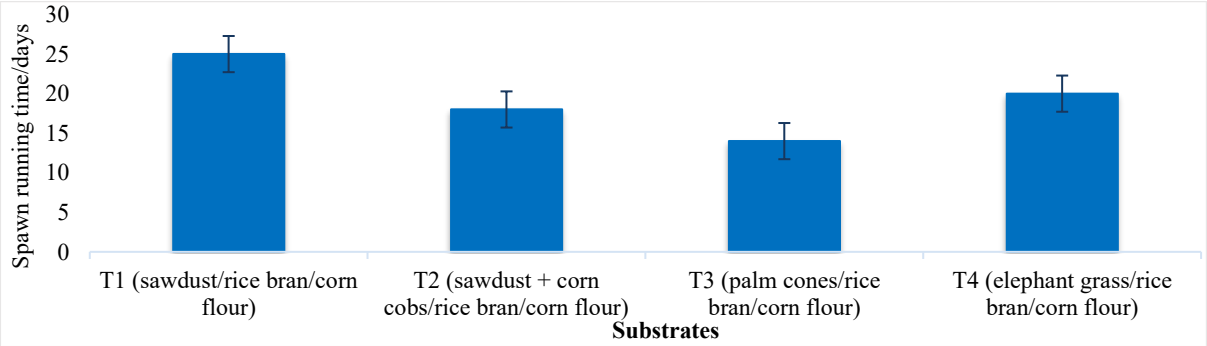

Figure 3: Spawning duration of different substrate combinations

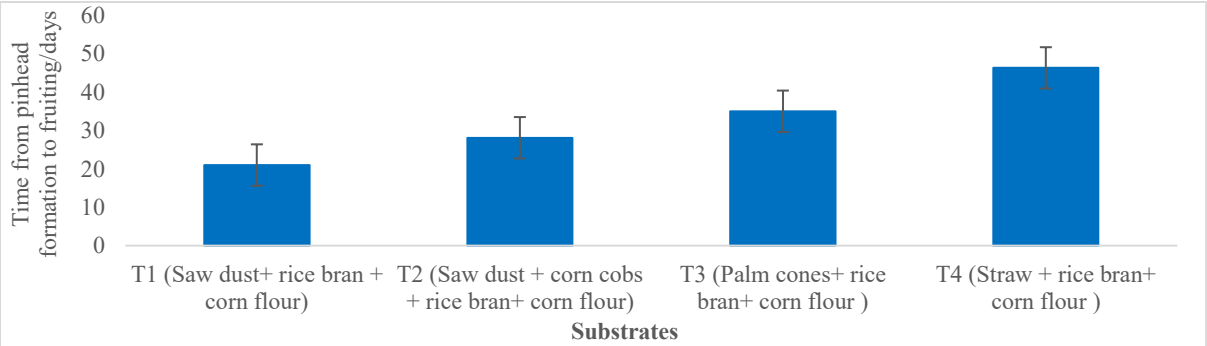

Figure 4: Duration for pinhead formation on substrate combinations

Duration from pinhead formation to harvesting
The duration from pinhead formation to harvesting showed no significant differences among T1 (sawdust/rice bran/corn flour), T2 (sawdust/corn cobs/rice bran/corn flour) and T3 (palm cones/rice bran/corn flour). Duration from pinhead formation in T4 (elephant grass straw/rice bran/corn flour) which was 11.7±0.7 days was significantly different from the other treatments (Figure 5).

Height of fruiting bodies
The length of fruiting bodies showed no significant differences at $p < 0.05$ with respect to the different treatments (Figure 6). Mean height recorded ranged from 19.5±3.3 cm in T2 (sawdust/corn cobs/rice bran/corn flour) to 10.1±1.3 cm in T4 (elephant grass straw/rice bran/corn flour).

Diameter of the pileus of individual fruiting bodies
Significant differences were recorded in pileus diameter between T2 (sawdust/ corn cobs/rice bran/corn flour) and T4 (elephant grass straw/rice bran/corn flour). The largest mean diameter was recorded in T2 (29.0±4.3 cm) (Figure 7).

Number of individual fruiting bodies in a cluster
The mean number of individual fruiting bodies revealed no significant difference among treatments T1, T2 and T3. However, T4 significantly differed from the other treatments (Figure 8).

Weight of individual fruiting bodies
Mean fresh weights of the different treatments revealed T2 (sawdust/ corn cobs/rice bran/corn flour) with the highest fruiting bodies weight of 75.6±34.3 g which was significantly different from the other treatments. The least weight was recorded in treatment 4 (11.2±3.8 g) (Figure 9).

Biological yield
Biological yield showed a significant difference at between all the treatments at $p < 0.05$. Treatment 2 recorded the highest yield of 1950 g, followed by treatment 1 (1240 g), and treatment 3 (750 g). Treatment 4 recorded the least yield of 102 g (Figure 10).

Biological efficiency
Biological efficiency was highest in treatment 3 (77.1±7.1%) which showed a significant difference from the other treatments. However, no significant difference was recorded in terms of biological efficiency between treatment 1 (53.0±5.8%) and treatment 2 (61.1±17.7%). Biological efficiency was lowest in treatment 4 (6.3±3.1%) (Figure 11).

DISCUSSION

Growth and yield parameters in the cultivation of *P. ostreatus* vary depending on the type of substrates used. Spawn running time took 15 to 24 days in all the treatments tested. This observation was similar to

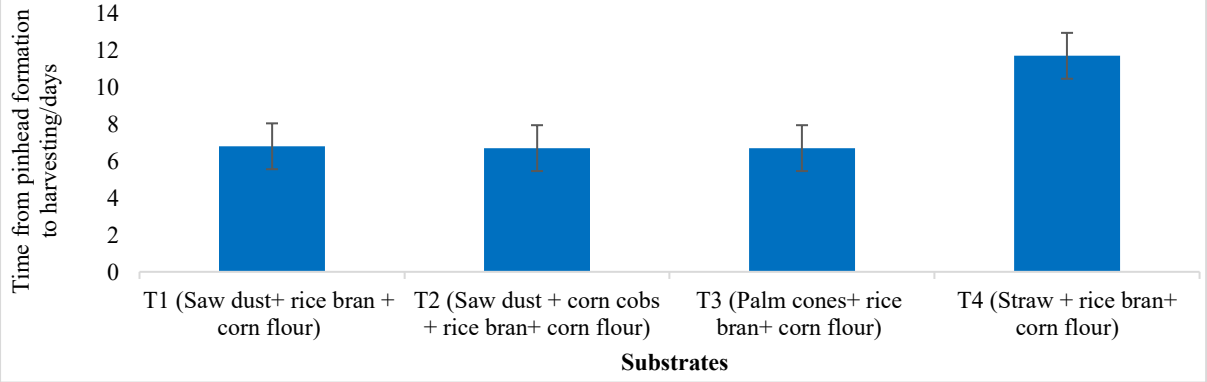

Figure 5: Duration from pinhead formation to harvesting on substrates

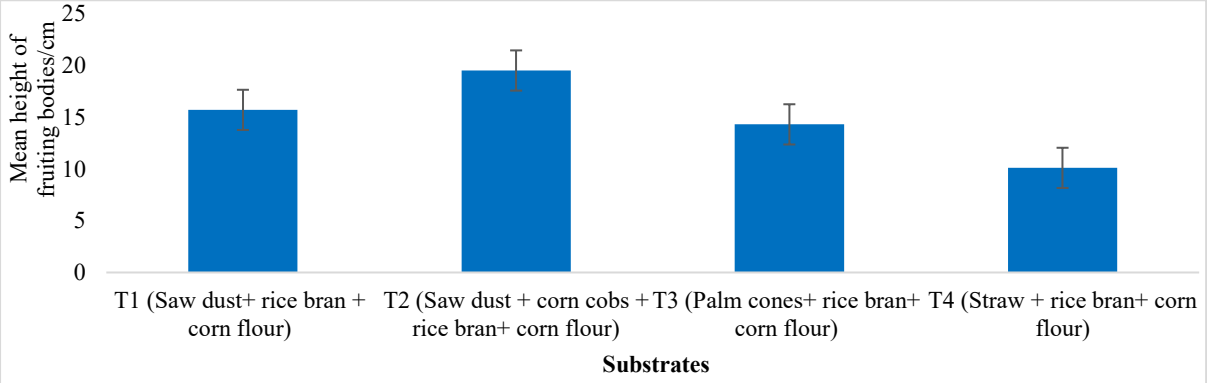

Figure 6: Mean height of individual fruiting bodies on substrates

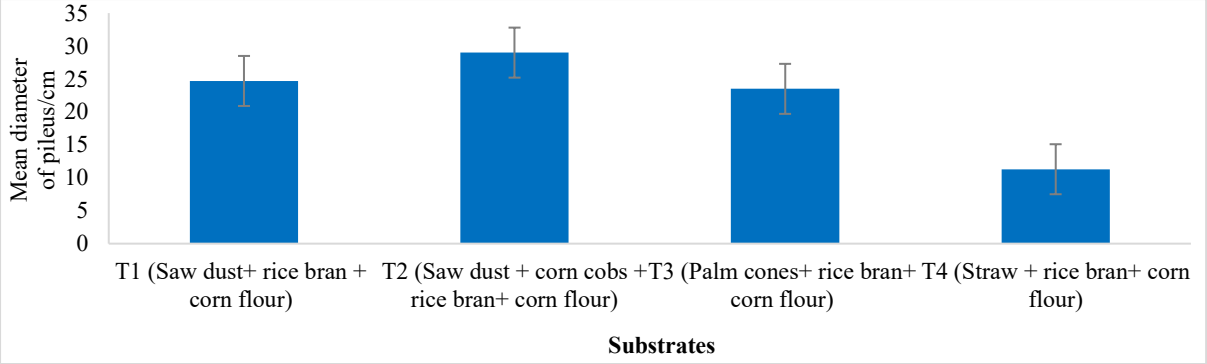

Figure 7: Mean diameter of the pileus of fruiting bodies on substrate

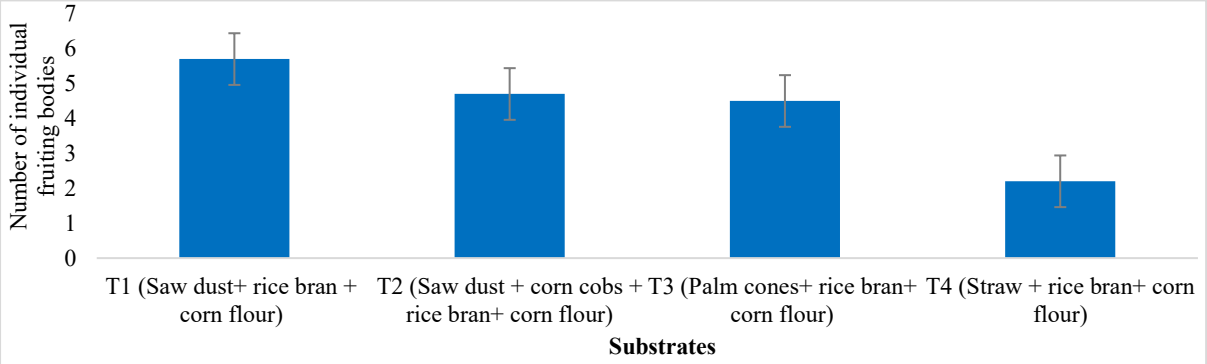

Figure 8: Mean number of individual fruiting bodies per treatment

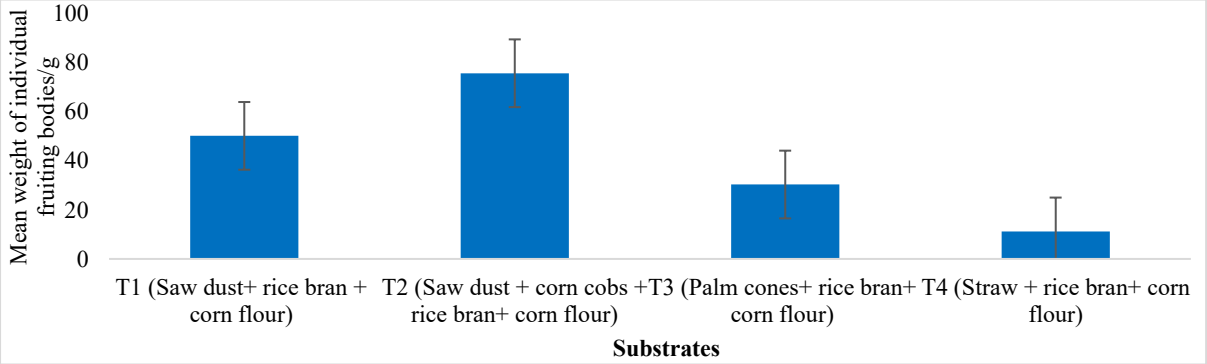

Figure 9: Mean weight of individual fruiting bodies on substrate

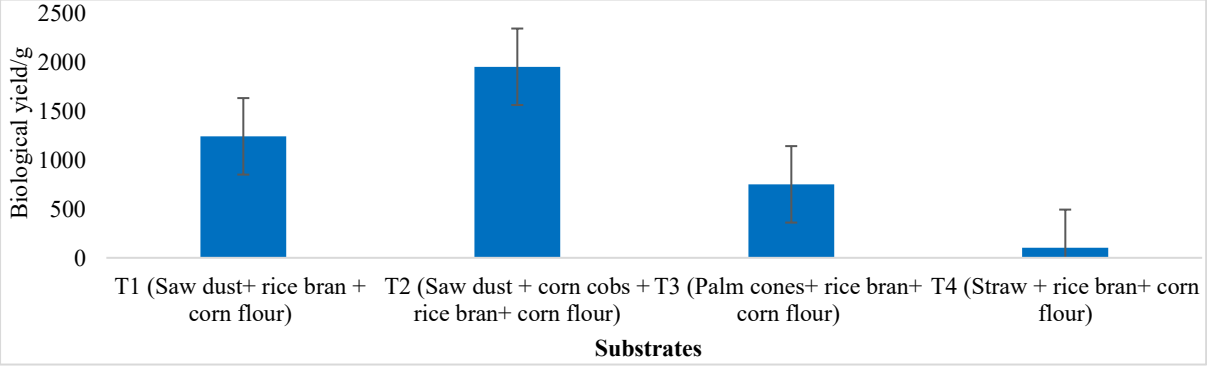

Figure 10: Biological yield of fruiting bodies on substrate

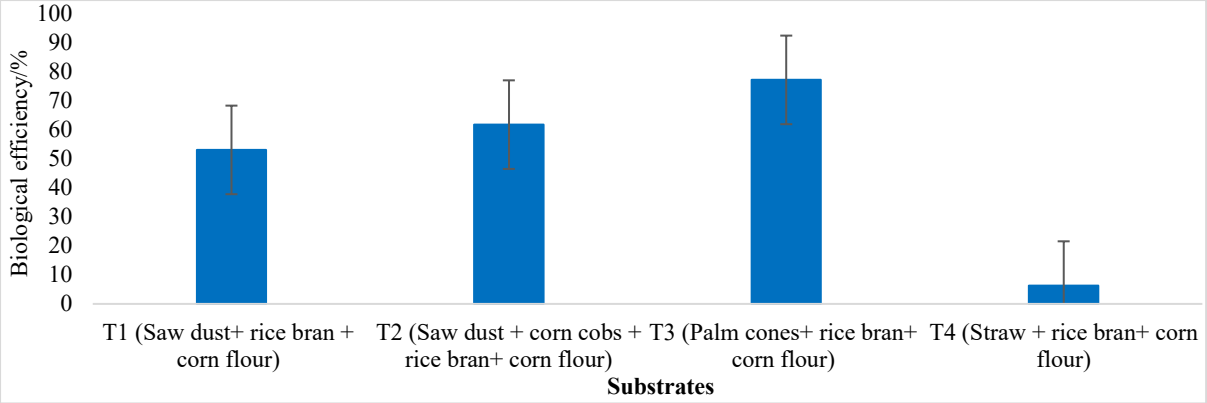

Figure 11: Biological efficiency of *P. ostreatus* on different types of substrates

those of Iqbal *et al*. (2005) and Pathmashini *et al*. (2008) who reported two to three weeks of spawn running time. T3 (Palm cones/rice bran/corn flour) took the shortest time to spawn. This outcome was similar to Nsoh *et al*. (2022) who reported shortest time of mycelia growth in *P. ostreatus* when cultivated using palm fruit bunch. This might be due to the fact that palm cones contain essential components that facilitates mycelial growth in *P. ostreatus.*

Duration from incubation to the formation of pinhead varied in all the different treatments. This is in line with the findings of Pathmashini *et al*. (2008) who reported that composition of growth medium also affects pinhead formation time. Pinhead formation appeared earliest in T3(Palm cones/rice bran/corn flour). Nsoh *et al*. (2022) and Ajonina and Tata (2012) also reported shortest time of primordial initiation in corncobs and palm cones during their experiment. The time from the pinhead formation to the first harvest for *Pleurotus ostreatus* was approximately 6±1 days. This finding is in contrast with that of Iqbal *et al*. (2005) who conducted a similar study and reported 46±3 days for pin head formation while using sugarcane bagasse and banana leaves as substrates.

Treatment 2 (sawdust/corn cobs/rice bran/corn flour) substrate combination recorded the highest stipe length, pileus diameter, number of fruiting bodies and mean weight of fruiting bodies. These results confirm the findings of Rambey *et al*. (2018) and Ajonina and Tatah (2012) who all reported that a combination of sawdust and corncobs can enhance growth and productivity in *P. ostreatus* mushrooms. Kitamoto *et al.* (1995) reported that substrates with high sugar contents such as glucose, fructose and tetrahalose often results in high yield.

The highest biological yield (1950 g) was obtained in corncobs/sawdust/rice bran/corn flour substrate combination. This value was lower than that (2645 g) reported by Nsoh *et al*. (2022) but higher than the biological yield value of 213.5 g reported by Hawrez (2019) on wheat straw. The highest biological efficiency value of 77.1% was obtained in T3 (palm cones/rice bran/corn flour) substrate combination. This was, however, lower than that of Dehariya and Vyas (2013) who obtained a biological efficiency of 93.3% with soybean straw as substrate. Somashekhar *et al.* (2020) reported a biological efficiency of 70.4% on a similar study using ragi straw as substrate.

CONCLUSION

Variations in growth and yield parameters obtained from the study revealed that yield in *P. ostreatus* is greatly dependent on the combination of growing substrates. Corncobs mixed with sawdust are basic substrates for the cultivation of *P. ostreatus* and show significant positive effect on the growth and yield of *P. ostreatus*. A combination of corncobs/sawdust /rice bran /corn flour resulted in highest biological yield than all the other substrate combinations tested in the study and can be recommended to mushroom farmers in the Northwest Region of Cameroon where large volumes of these materials remain underutilized and are often discarded as agricultural waste material. Further research is recommended on the chemical contents of these substrate materials.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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