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The effect of substrates on the growth, yield, nutritional and phytochemical components of *Pleurotus ostreatus* supplemented with four medicinal plants

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The aim of this study was to investigate the effect of substrates on the growth, yield, nutritional, and medicinal value of *Pleurotus ostreatus* supplemented with four medicinal plants. A completely randomized block design was laid out with 4 treatments replicated 4 times with and without medicinal plants. T1 (sawdust), T2 (sawdust + corncobs), T3 (palm cones), and T4 (elephant stalks) were the treatments used. *Croton macrastarchus, Harungana madagascariensis, Tithonia diversifolia,* and *Rauwolfia vomitoria* were the medicinal plants used. Nutritional and phytochemical analysis was carried out. Sawdust + corn cobs indicated the highest effect on growth as it had the highest mean height (19.5 ± 3.3 cm), diameter (29.0 ± 4.3 cm) and mean weight of individual fruiting bodies (175.8 ± 84.3 cm). Biological efficiency was highest in palm cones (77.1%), second by sawdust + corn cobs (61.1%), saw dust (53.0%) and elephant stalk (6.3%). The protein content was highest in sawdust + corn cobs (12.4 g), lipid concentration highest in sawdust only (1.51 g), total carbohydrate highest in palm cones (82.98 g), and total ash highest in sawdust only (7.32 g) per 100 g. The supplementation of sawdust + corn cobs with *R. vomitoria* had the highest phytochemical components.

Key words: Medicinal properties, mushroom cultivation, growth and yield, nutritional content, *Pleurotus ostreatus*, medicinal plants substrates.

INTRODUCTION

Mushrooms are fleshy saprophytic fungi, and it can be found growing on damp rotten log of wood trunk of trees, decaying organic matter and in damp soil rich in organic substances. Edible mushrooms are highly nutritious and can be compared with eggs, milk and meat (Mata et al., 2005). *Pleurotus ostreatus* (*Basidiomycota*), is of the Pleurotaceae family. *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). Cultivation methods were developed in Germany during World War I and then successfully applied on a large scale. This was the result of the search for new food sources, due to the problem of hunger in Germany. In Poland, *P. ostreatus* is a common species (Wojewoda, 2003). *P. ostreatus* (white-rot fungus), also known as oyster mushroom, is

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commercially important in the world mushroom market.

Oyster mushroom is an edible mushroom having an excellent flavour and taste. *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). Oyster mushrooms are prized for their exclusive flavour and deliciousness. They are rich in proteins, contain less fat, less carbohydrates, salt and rich in fibres and have high vitamin B12 and folic acid which are uncommon in vegetables. High availability of lysine and tryptophan and other amino acids usually absent in cereals make them ideal for food for patients suffering from hypertension, diabetes and obesity (Carel et al., 2013). Mushrooms require carbon, nitrogen and inorganic compounds as their nutritional sources and the main nutrients are carbon sources such as cellulose, hemicellulose and lignin. The use of various wastes is recommended for the growth of *P. ostreatus* (Vendrusco et al., 2008)

The waste obtained from fruit processing industry is extremely diverse due to the use of wide variety of fruits and vegetables, the broad range of processes and the multiplicity of the product. Full utilization of horticultural produce is a requirement and a demand that needs to be met by countries wishing to implement low-waste technology in their agribusiness. Food processing industries generate high amount of waste and direct disposal of such residues poses a serious threat to the environment and represents an important loss of biomass (Vendrusco et al., 2008). Apple pomace is an important fruit industrial waste that remain unutilized and creating an environmental pollution. Therefore, it can be utilized along with the wheat straw as a substrate for *P. ostreatus* production. Several million tons of Apple pomace is generated because of its high carbohydrate content which is used for microbial processes for single cell protein, enzymes, ethanol, low alcoholic drinks and pigments (Bhushan et al., 2008). Therefore, this horticultural waste can be utilized and replaced with wheat straw for mushroom cultivation under mushroom house.

Extracts from wild species of *Pleurotus* have been reported to be used in treating some ailments (Osemwegie et al., 2007). Wild species are collected for consumption because they are a good source of carbohydrates, digestible proteins, fibres and vitamins (Barros et al., 2008). Structurally, polysaccharides and proteins comprise the main components of dry matter of *Pleurotus*, while lipid content is low. Chitin, glycogen, mannitol and trehalose are typical carbohydrate constituents. *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, fruiting and a low cost of culture, being slightly affected by 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 in a commercial point of view.

In general, the production of mushrooms may be divided into several stages: composting and filling, sterilization, inoculation, incubation, fruiting, and harvesting (Loss, 2009). Contrary to others, *Pleurotus* genus does not require a composting substrate (Fan et al., 2000), due to the presence of a powerful enzyme complex (with cellulases, hemicelulases, ligninases, peroxidases, laccases, proteases, among other enzymes) (Hyde et al., 2019). *Pleurotus* spp. only requires crushed materials to acquire the desired texture for a good mycelial colonization. The production of *Pleurotus* spp. has been tested using different substrates *e.g.*, cotton waste textile (Chang et al., 1981), rice straw (Mehta et al., 1990) by products of corn (Loss, 2009), bark of coffee (Dias et al., 2003), wheat straw (Ramos et al., 2011) and sugarcane (Cardoso et al., 2013). The adaptation of this genus to new wastes represents one of the main methods for bioconversion of agro-industrial waste into edible products with high nutritional value (Cohen et al., 2002). The recycle of different materials is one of the most important contributions of fungi in nature (Sanchez et al., 2002).

P. ostreatus is often attacked by various fungal, bacterial, viral pathogens and this leads to a great loss of yield. So, these pathogens must be controlled to save crop production (Dawoud et al., 2005). Hot water extract of *Curcuma aromatic* inhibited the mycelia growth of the causative agent of root rot of cotton and wilt of tomatoes (Raja and Kurcheve, 1999). The incorporation of medicinal plant products with *P. ostreatus* is for the phytochemical control of pathogenic microbes during mushroom cultivation. Some medicinal plants used during mushroom cultivation include Eucalyptus, Neem cake, Citrus lemon and Lemon grass. The fine powder of these medicinal plant's substances is mixed with mushroom substrates for yield improvement of various strains of oyster mushrooms (Nazir et al., 2010). The aim of this research was to investigate the effect of substrates on the growth, yield, nutritional and medicinal value of *P. ostreatus* when supplemented with four medicinal plants.

MATERIALS AND METHODS

Description of cultivation site

This research was conducted at the Mushroom Programme Training and Research Center (MUPTAREC) farmhouse at Ntarinkon Mankon-Bamenda. The farmhouse is situated in Mezam division in the Northwest Region of Cameroon. Bamenda also

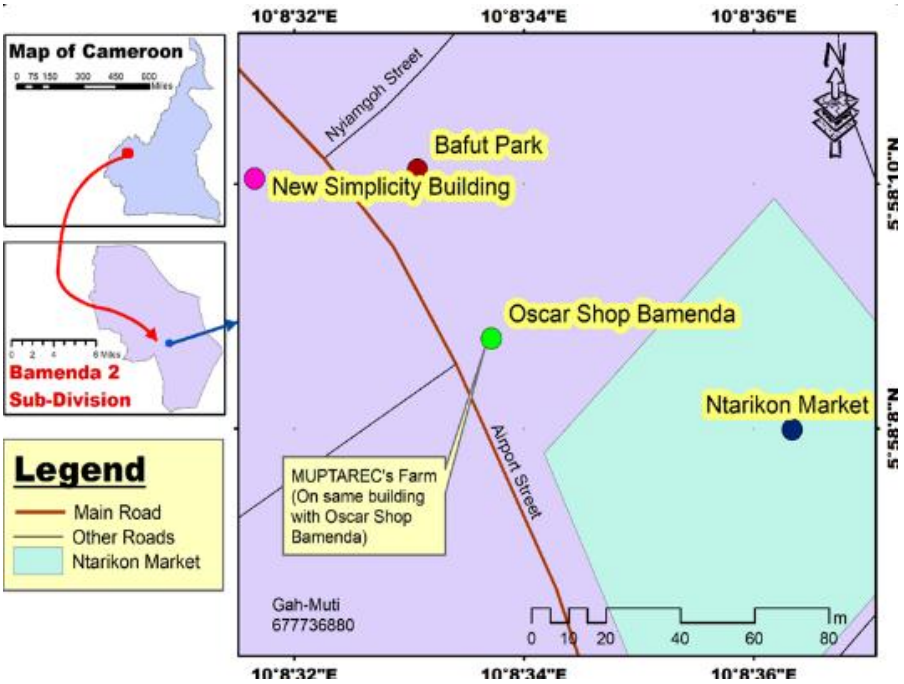

Figure 1. Map of Ntarinkon Mankon-Bamenda indicating MUPTAREC'S Farmhouse. Source: Authors

known as Abakwa town and Mankon town is a city in the Northwest Region and has a population of about 2 million inhabitants located at 366 km Northwest of the Cameroonian capital Yaounde. Bamenda is known for its cool climate. Mankon has coordinates 5°56[1]N 10E and 5.933N 10.167°E (Figure 1). The area has a tropical savanna climate, bordering on a tropical monsoon climate with long wet season and short dry season. The soil type in Mankon is sandy loam (Alakeh et al., 2020).

Sterilization procedure

All apparatus, equipment, metallic instruments, glass wares and culture media were sterilized locally in a drum. The culture room was cleaned by washing with detergents followed by 70% ethyl alcohol.

Mother spawn production

An exotic strain of *P. ostreatus* was obtained from Belgium and multiplied using Potato Dextrose Agar. 100 kg bag of sawdust, 25 kg bag of rice bran, 50 kg bag of rice husk, and 500 g of slake lime (Calcium Carbonate) were mixed using clean spades and 30 L of water added to obtain 65% moisture content. Mixing was done properly to obtain a homogenous mixture. This mixture was put in bottles and closed with bottle lids that have been perforated at the center of the lid using a nail and cotton fitted on the perforated area to permit the supply of small amount of oxygen to the mycelium after planting. These bottles were sterilized in a sterilization drum for 4 h. Cooling was allowed to take place for an hour followed by inoculation of the bottles in an inoculator for 90 min. Planting of substrate in bottles took place with seeds obtain from mother spawn that was already prepared three weeks before. The bottles were then placed in an already prepared spawn room for colonization for 3 weeks after which they were suitable for planting substrates.

Substrate preparation

Sawdust, sawdust mixed with corn cobs, palm cones and elephant grass were mixed with equal quantities (1:1) of rice bran and $CaCO_3$. Water was added to the mixture and mixed to homogeneity. The substrates used; sawdust, sawdust mixed with corn cobs, palm cones and elephant grass, were filled in plastic bags. Four (4) replications were made from each substrate, a kilogram in each bag. Tags were made on the bags for clear identification and differentiation. The substrate bags were tied and sterilized in a 25 ml metallic drum for 4 h and allowed in the drums for continuous gradual sterilization overnight. Bags were removed the next day and allowed to cool then spawning was done.

Substrates, composition and replications

The cultivation of *P. ostreatus* on different substrates was done using the procedure of Anagho (2008). This experiment was laid out in a completely randomized design with 4 treatments replicated 4 times. The treatments were supplemented with and without the 4 medicinal plants and each had 4 replications. Table 1 shows the various substrates (treatments) and composition used in the cultivation of *P. ostreatus* with the various replications.

Substrate preparation with medicinal plants

Substrates were mixed as stated earlier and equal amount of the different medicinal plants; 35 g each were added to the substrates

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

Treatment	Substrates and composition	Replications
Treatment 1	Saw dust+ rice bran	4
	Saw dust + rice bran +M1	4
	Saw dust + rice bran +M2	4
	Saw dust + rice bran +M3	4
	Saw dust +rice bran + M4	4
Treatment 2	Saw dust + corn cobs + rice bran	4
	Saw dust +corn cobs rice bran +M1	4
	Saw dust +corn cobs rice bran +M2	4
	Saw dust +corn cobs rice bran +M3	4
	Saw dust +corn cobs rice bran + M4	4
Treatment 3	Palm cones+ rice bran	4
	Palm cones + rice bran +M1	4
	Palm cones + rice bran +M2	4
	Palm cones + rice bran +M3	4
	Palm cones +rice bran + M4	4
Treatment 4	Straw + rice bran	4
	Straw + rice bran +M1	4
	Straw + rice bran +M2	4
	Straw + rice bran +M3	4
	Straw +rice bran + M4	4

M1= *Croton macrastarchus;* M2=*Harungana madagascariensis;* M3=*Tithonia diversifolia;* M4=*Rauwolfia vomitoria.*
Source: Authors

and mixed thoroughly for homogeneity. The four (4) medicinal plants used were *Croton macrastarchus*, *Harungana madasgariensis*, *Tithonia diversifolia* and *Rauwolfia vomitoria.* These medicinal plants were collected, chopped into pieces, dried in a bakery oven and ground into powder in a grinding mill. 20 replications were made from the substrates with different medicinal plants (4 replications per medicinal plant). 1 kg of compost was filled in each substrate bag. Tags were made on each substrate bag for clear identification. Bags were tied and sterilized in a 250 ml drum together with the control experiment for 4 h. Bags were removed the next day, and allowed to cool.

Spawning

The bags were removed from the drum and carefully placed in a spawning room. Cooling was allowed to take place for an hour before spawning. Washing and sterilization was done and spawning was carried out by use of a spoon. Hands and spoons were sterilized using medical alcohol. Planting spoons was used to prevent contamination and to mix spawn in the bags without damaging it. Bags were then untied and bottles of “sub mother” spawn weighing 760 g were used to plant substrates. 50 seeds were used to plant 100 substrates, that is planted at the ratio of 1 bottle of seed for two bags of substrate (1:2). The planted bags were properly tied to avoid the entering of microorganisms and to enhance proper colonization of the mycelium network. The substrates were then moved to the mushroom cultivation house with dark room with shelves where it was placed for colonization. After 21 days when the mycelium was fully colonized, the windows were open for ventilation to enhance and provide necessary conditions for fruiting. The temperature and moisture content of the room was maintained for healthy fruiting to take place. Watering of substrate to improve on the moisture content was done only after the first fruiting.

Morphological data collection

The trial was carried out according to the complete randomized block design with factorial arrangement using four replications for each treatment. The 100% saw dust substrate (treatment 1) was selected as the control. The pertinent data on growth and yield parameters such as time to spawn running, to pinhead formation, to first harvest; height of pileus, diameter of pileus, total yield, biological efficiency was collected during the experimental period according to Kinge et al. (2016).

Nutritional analysis

A laboratory analysis was done according to Association of Official Analytical Chemists (AOAC, 1984) in the National Polytechnic Bambui Nutritional Laboratory Bamenda to compare composition of nutrient in *P. ostreatus* cultivated on five substrates. The data was

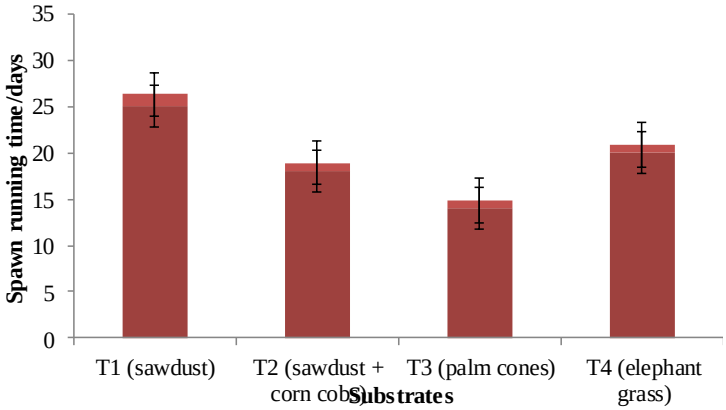

Figure 2. Days to spawn run on different substrates.
Source: Authors

recorded on crude protein, crude fat, crude fiber ash, organic matter, and dry matter according to Raghuramulu et al. (2003).

Phytochemical analysis

The phytochemical analysis was done using standard procedures (AOAC, 1984). The phytochemical test was analysed after extraction in 1:1 methanol and methylene chloride. The concentrated residues were used to detect the secondary fungi metabolites such as alkaloids, flavonoids, steroids, saponins, phenolics and tannins using standard methods with some modifications (MacNee, 2005).

Data analysis

The data for growth and yield parameters were analyzed by descriptive analysis using M.S Excel version 2010 where the results on growth and yield were compared using bar charts. Also, statistical evaluations on the growth and yield parameters were done by one way ANOVA in SPSS version 20 and the comparisons of the means realized by the Turkey HSD multiple comparison at $p<0.05$.

RESULTS

Spawn running time

Spawn running time tested on the 4 different substrates took 14 to 25 days. Palm cones took 14.4 ± 0.8 days; corn cobs+ saw dust 18.4 ± 0.9 days, elephant straw 20.4 ± 0.8 days and saw dust 24.2 ± 1.3 days. Figure 2 and Table 2 show spawn running time measured by counting number of days of the different substrates.

Time required for primordial initiation (pinhead formation)

Two replications from treatment 1 and one replication from treatment 2 were destroyed by green mould fungus. The time required for the formation of pinhead was least in treatment 3 (21.8 ± 2.1 days), treatment 2 (26.9 ± 2.2 days), treatment 4 (31.0 ± 2.8 days) and treatment 1(46.3 ± 1.7 days). Figure 3 and Table 2 show time required for pinhead formation measured by counting number of days of the different substrates.

Days from pinhead formation to harvesting

The number of days it took from fruiting to harvesting was equal in treatment 1, 2 and 3 (6.7 ± 0.7 days) and highest in treatment 4 (11.7 ± 0.7 days). Figure 4 and Table 2 show the time required for pinhead formation to harvesting measured by counting number of days of the different substrates.

Height of fruiting bodies

The mean height of individual fruiting bodies per treatment was measured in centimeters using a tailor's tape beginning from where the stalk gets attached to the substrate to the pileus. The highest mean height was recorded in treatment 2 (19.5 ± 3.3 cm), followed by treatment 1 (15.7 ± 2.6 cm), treatment 3 (14.3 ± 1.3 cm) and treatment 4 (10.1 ± 1.3 cm). Figure 5 shows the mean height of fruiting bodies of the different substrates.

Diameter of the pileus of individual fruiting bodies

The mean diameter of individual fruiting bodies measured (which is the distance round the pileus) using a tailor's tape in cm was recorded. The highest mean diameter was recorded in treatment 2 (29.0 ± 4.3 cm), treatment 1 (24.7 ± 3.5 cm), treatment 3 (23.5 ± 2.3 cm) and

Table 2. Comparison of growth and yield parameters based on treatment levels.

Parameter	Means ± SD of parameters based on treatment levels					Significant difference (ANOVA)		
	T 1 (control)	T2	T3	T 4	Total	df	F-value	*p* - value
Spawning running time (Days)	24.2±1.3	18.4±0.9	14.4±0.8	20.4±0.8	19.4±3.7	3	163.141	*0.000*
Primordial initiation (Days)	46.3±1.7	26.9±2.2	21.8±2.1	31.0±2.8	31.5±9.5	3	222.286	*0.000*
Pinhead formation to harvesting (days)	6.8±0.4	6.7±0.5	6.7±0.7	11.7±0.7	7.9±2.2	3	186.630	*0.000*
Height of fruiting bodies (cm)	15.7±2.6	19.5±3.3	14.3±1.3	10.1±1.3	14.9±4.0	3	29.182	*0.000*
Diameter of pileus fruiting bodies (cm)	24.7±3.5	29.0±4.3	23.5±2.3	11.3±1.2	22.1±7.3	3	61.282	*0.000*
Number of fruiting bodies in a cluster	5.1±0.9	4.7±1.4	4.5±1.2	2.2±0.6	4.1±1.5	3	14.971	*0.000*
Weight of individual fruiting bodies (g)	114.1±55.0	175.8±84.3	70.9±20.6	11.5±3.8	93.0±78.4	3	18.195	*0.000*
Biological yield (g)	2225.0±0.0	2645.0±0.0	1721.0±0.0	110.0±0.0	1675.3±1109.8	3	ND	ND
Biological efficiency (%)	53.0±5.8	61.1±17.7	77.1±7.1	6.3±3.1	49.4±28.4	3	91.339	*0.000*

Source: Authors

treatment 4 (11.3 ± 1.2 cm). Figure 6 and Table 2 show the mean diameter of the pileus of individual fruiting bodies measured in cm of the different substrates.

Number of individual fruiting bodies in a cluster

The mean number of individual fruiting bodies per treatment was recorded by counting. This number was almost equal in treatment 1, 2 and 3 (4.5 ± 1.2) and least in treatment 4 (2.2 ± 0.6). Figure 7 and Table 2 show the mean number of individual fruiting bodies of the different substrates.

Weight of individual fruiting bodies

The mean fresh weight of individual fruiting bodies was measured in grams and recorded. The highest mean weight of individual fruiting bodies was found in treatment 2 (175.8 ± 84.3 g), second in treatment 1 (114.1 ± 55.0 g), third in treatment 3 (70.9 ± 20.6 g) and least in treatment 4 (11.5 ± 3.8 g). Figure 8 and Table 2 show the mean weight of individual fruiting bodies of the different substrates.

Biological yield

This is the total fresh weight of fruiting bodies on the various treatments measured in grams using an electronic scale. Biological yield was highest in treatment 2 (2645 g), second in treatment 1 (2525 g), third in treatment 3 (1721 g) and least in treatment 4 (110 g). Figure 9 and Table 2 show the biological yield of the different substrates.

Biological efficiency

The biological efficiency as calculated from the total fresh weights of mushrooms divided by the dry weight of substrates x 100. The highest biological efficiency (77.5%) was obtained in treatment 3, treatment 2 (61.2%), treatment 1 (53.1%) and the lowest in treatment 4(6.3%). Figure 10 and Table 2 show the biological efficiencies of the different substrates.

Nutritional analysis

Table 3 shows the composition of various nutrients found in *P. ostreatus* on different substrates. Protein content was highest in sawdust + corn cobs (12.4 g), lipid concentration highest in sawdust only (1.51 g), total carbohydrate highest in palm cones (82.98 g), fibers highest in sawdust + corn cobs (19.78 g) and total ash highest in sawdust only (7.32 g) per 100 g.

Phytochemical analysis

Table 4 shows the various phytochemical compounds and their concentrations found in *P. ostreatus* on different treatments. According to our finding, steroids were highly concentrated in most of the treatments (those with and without medicinal

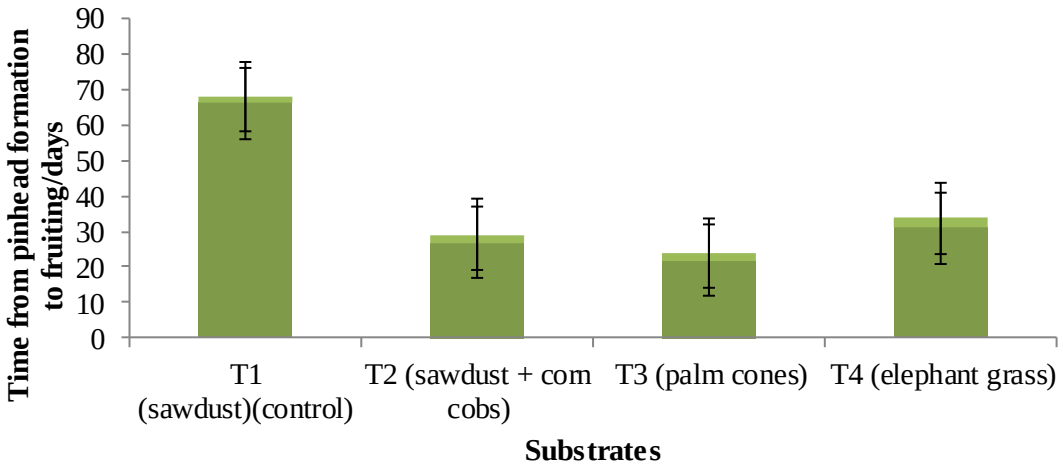

Figure 3. Days of pinhead formation on substrates.
Source: Authors

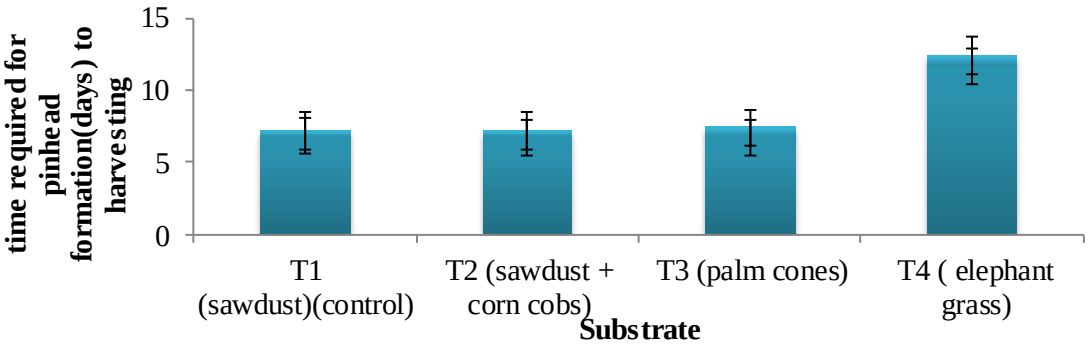

Figure 4. Time from pinhead formation to harvesting on substrates.
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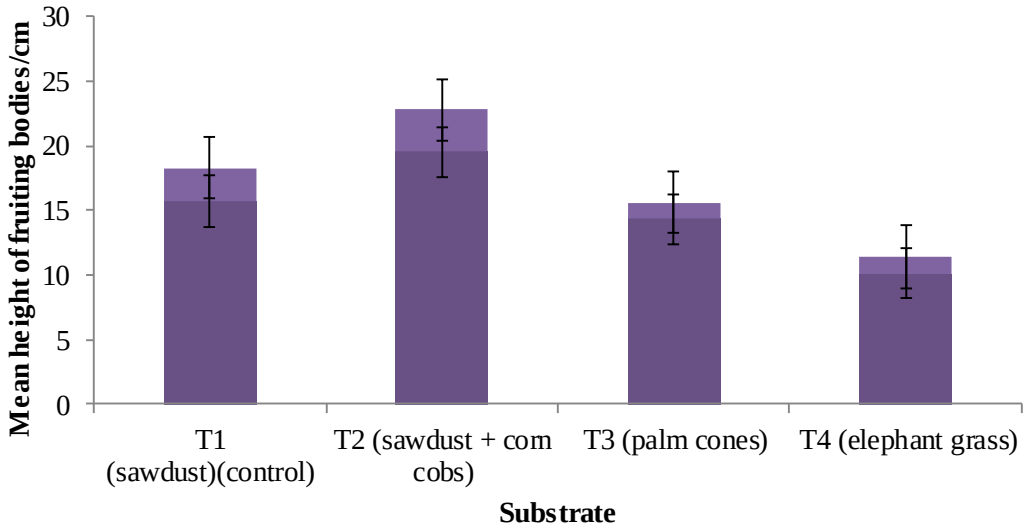

Figure 5. Mean height of individual fruiting bodies on substrates.
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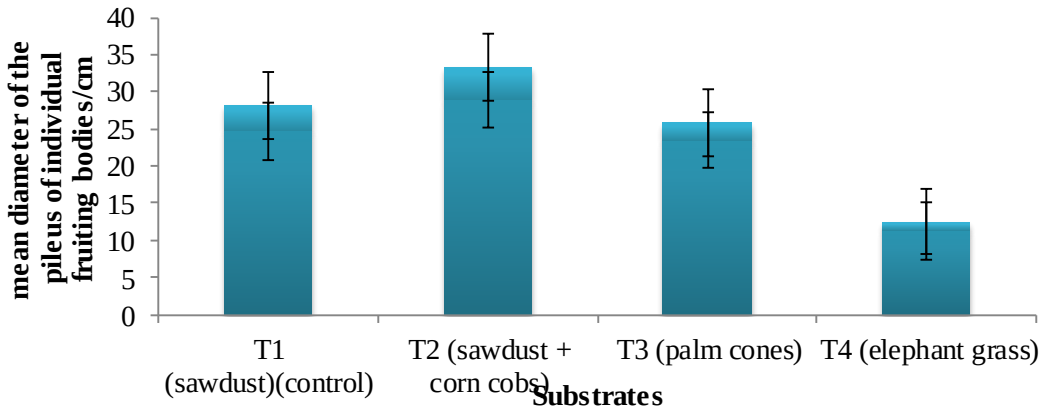

Figure 6. Mean diameter of the pileus of fruiting bodies on substrate.
Source: Authors

plants). Supplementation of SCB (Sawdust + corncobs) with *R. vomitoria* presented the highest concentrations of steroids, flavonoids and saponins (+++) and presence of alkaloids (+). SCB (sawdust + corncobs without supplementation) presented the lowest concentration of bioactive components with steroids highly concentrated (+++), saponins just present (+) and other components absent. Alkaloids were present in some of the treatments supplemented with medicinal plants (DBH, DBT, DBR, SCR, PBC, PBH and PBR).

DISCUSSION

Effects of different substrates on the growth and yield of *P. ostreatus*

Mycelia growth was faster in palm cones (T3) (14.4 ± 0.8 days) and slowest in sawdust (24.2 ± 1.3 days). Formation of pinhead appeared earlier in palm cones (21.8 ± 2.1 days) after the period of incubation and last in sawdust (46.3 ± 1.7 days). Pin head formation is affected by temperature and humidity; below 17°C pinhead formation is delayed. Composition of growth medium also affects pinhead formation time (Pathmashini et al., 2008); shortest time of primordial initiation in corncobs and palm cones has been reported by Ajonina and Tata (2012). Pinhead formation was prevented in some replications as a result of fungal infection; two replications of the treatment 1 and 1 replication of treatment 2 (sawdust and corn cobs + sawdust). This prevented the expected mycelium growth. Earnshaw et al. (2012) and Oseni et al. (2012) have reported similar infections and their detrimental effects on mycelium.

Days from pinhead formation to harvesting was significantly similar in the treatments 1, 2, 3 (corn cobs and palm cones) when compared with the control (sawdust) (6.8 ± 0.4 days) and higher in treatment 4 (elephant grass) (11.7 ± 0.7 days). The time from the pinhead formation to the first harvest for *P. ostreatus* was around 6±1 days, agreeing with those of Iqbal et al. (2005) who conducted similar research and who reported 46±3 days for this stage of development. Height of fruiting bodies differed on the different substrates. T2 (corn cobs + sawdust) presented the highest height (19.5 ± 3.3 cm) while the least height was recorded in T4 (elephant grass) when compared with sawdust (control). This result is in line with the findings of Rambey et al. (2018) who reported that sawdust can be mixed with corncobs to enhance productivity. Diameter of pileus of the fruiting bodies was higher in corncobs + sawdust (29.0 ± 4.3 cm) and lowest in elephant grass (11.3 ± 1.2 cm) when compared with sawdust (control) in *P. ostreatus*. Pileus diameter on corn cobs + saw dust was statistically similar. The outcome is in line with the findings of Ajonina and Tatah (2012) who reported highest diameter of pileus in corncobs and palm cones during their experiment. Mean number of effective fruiting bodies in the various treatments were significantly similar in treatment 1, 2 and 3 (4.7 ± 1.4) (corncobs + sawdust and palm cones) and lower in treatment 4 (elephant grass) when compared with sawdust. This outcome is similar to the findings of Ajonina and Tatah (2012) who reported highest number of fruiting bodies in sawdust and corn cobs. These results might be due to the presence of glucose, fructose and trehalose in the substrate as reported by Kitamoto et al. (1995). The highest weight of individual fruiting bodies of *P. ostreatus* was recorded in corncobs + sawdust (175.8 ± 84.3 g) and lowest in elephant grass (11.5 ± 3.8 g) when compared with sawdust. This result is similar to the findings of Rambey et al. (2018) who confirmed best yield of *P. ostreatus* when cultivated on corncobs mixed with sawdust.

Biological yield varied significantly because of different substrate compositions. The highest biological yield (2645 g) was obtained in corncobs + sawdust and the lowest biological yield (110 g) was obtained in elephant grass. Biological yield in corncobs+ sawdust was

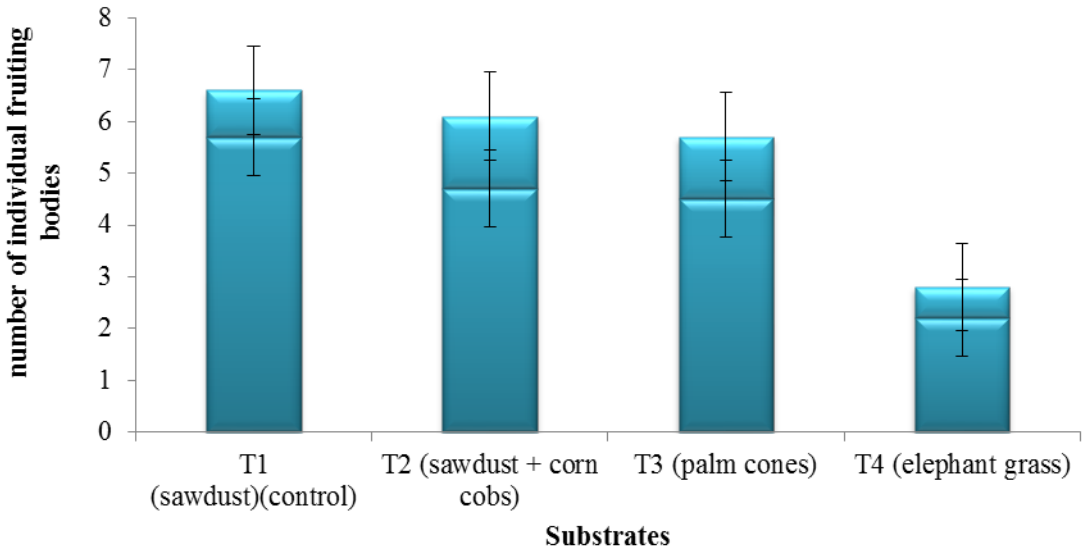

Figure 7. Mean number of fruiting bodies on substrate
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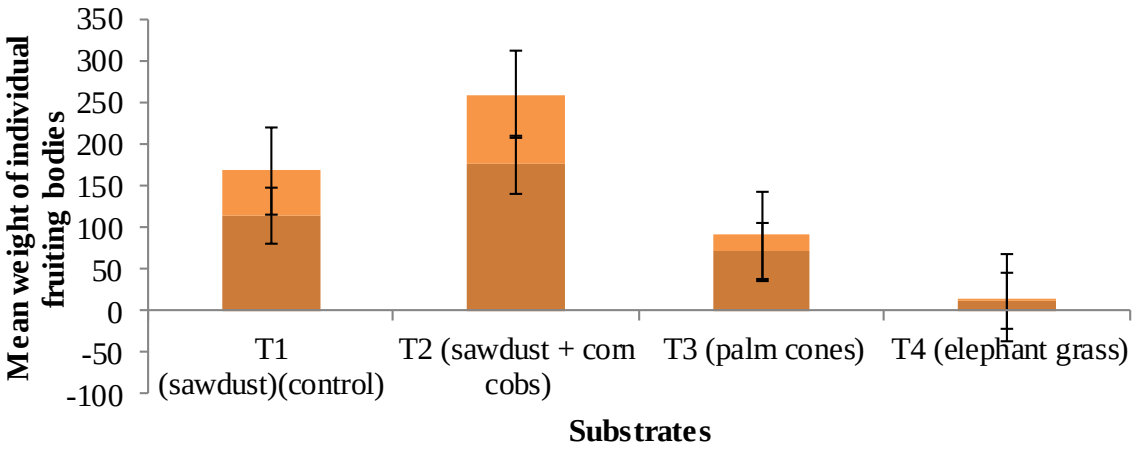

Figure 8. Mean weight of individual fruiting bodies on substrate.
Source: Authors

Significantly similar with biological yield on sawdust (control). Biological yield value of 2645 g obtained on the sawdust + corn cobs substrate was higher than the biological yield value of 213.5 g reported by Hawrez (2018) on wheat straw. Biological efficiency is the most important parameter in mushroom growing. Highest biological efficiency (77.1 ± 7.1%) was recorded in palm cones, (61.1 ± 17.7%) in corncobs + sawdust, (53.0 ± 5.8%) in sawdust (control), and lowest in elephant grass (6.3 ± 3.1%). The highest biological efficiency value of (77.1%) obtained on the palm corn substrate was higher than the biological efficiency (66.41 %) reported by Hawrez (2018) on wheat straw substrate.

Nutritional analysis

Nutritional composition of mushroom is affected by many factors among which the composition of the substrate is of major importance. This can be supported by the results in the findings where *Pleurotus florida* and *P. ostreatus* grown on sawdust gave a significant nutritional value than that cultivated on corn cobs (Shah et al., 2004). During our trial we discovered that protein content was highest in sawdust + corn cobs substrate (12.48 g/100 g) and least in palm cones (8.22 g/100 g). It is well known that the protein content of mushrooms varies with the type of substrate as a result of the differences in nutrient

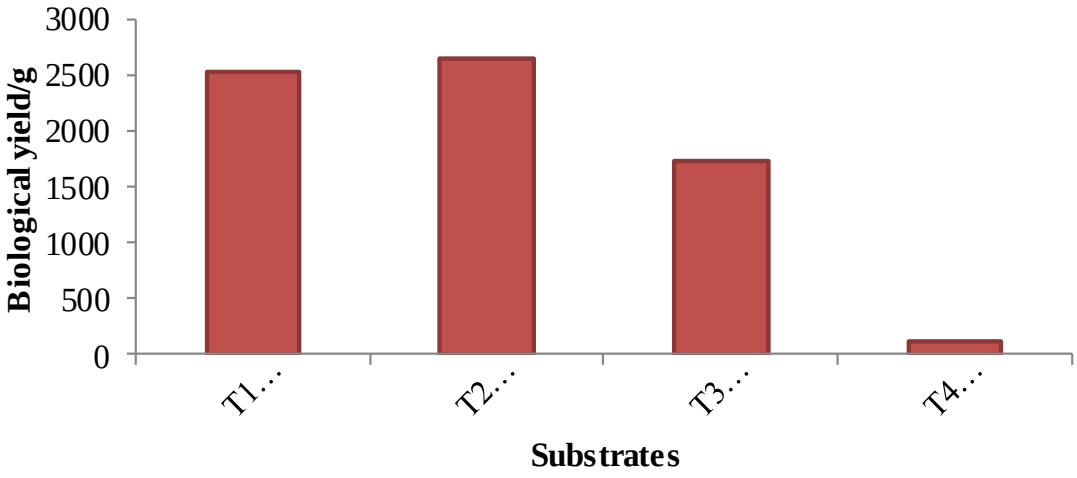

Figure 9. Biological yield of fruiting bodies on substrate.
Source: Authors

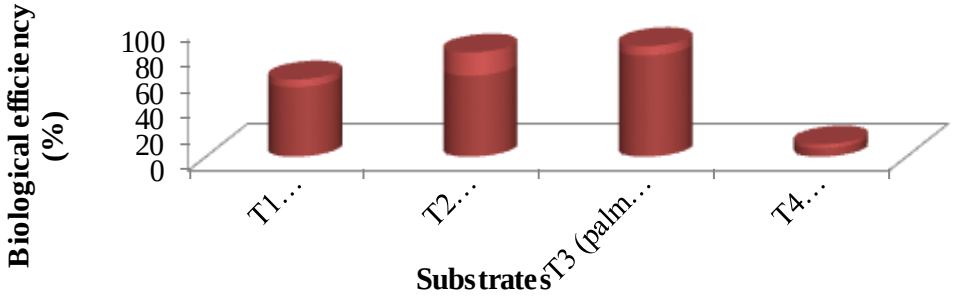

Figure 10. Biological efficiency of *P. ostreatus* on different types of substrates.
Source: Authors

supply (Gupta et al., 2013). This result is similar to that of Jin et al. (2018) who reported a protein value closer to our finding but contradicts the findings of Kinge et al. (2016) and Hawrez (2018) who reported of protein value higher than that of our findings.

Lipid content ranged from 1.43 to 1.51 g/100 g. Lipid content of oyster mushroom was affected by different substrates. Lipid content was maximum in sawdust (1.51 g/100 g) which was the control and minimum in palm cones (1.43 g/100 g). This result is similar to the findings of Barros et al. (2015) who reported lipid content range from 1.16-1.67 g/100 g for *P. ostreatus* but contradicts the report of Kinge et al. (2016) who reported a higher value of lipid content to that of our findings. Total carbohydrate ranged from 78.89 to 82.98 g/100 g. Total carbohydrates content varies depending on the type of substrates used. Total carbohydrate content was highest in palm cones (82.98 g) and minimum in sawdust (78.89g). The results of our finding were higher than the reported 61.9% for Croatian wild variety of *P. osreatus* studied by Beluhan and Ranogajec (2011), but less than the reported values of 73.2 to 78.1% in *P. ostreatus* studied by Fernandes et al. (2015). Crude fibre content ranged from 17.07 to 19.78 g/100 g. Crude fibre content varies depending on the type of substrate used. Fibre content was maximum in sawdust + corncobs (19.78 g) and minimum in the control which is saw dust only (17.07 g). This result is similar to the findings of Kinge et al. (2016) who reported a crude fibre content value maximum in corncobs (16.69 g) and minimum in sawdust (5.08 g) but contradicted by the findings of Teke et al. (2021) who reported a crude fibre value of 30.69 g obtained from the analysis of eight most preferred wild mushroom species from the Kilium-Ijim mountain forest in Cameroon. Total ash content varied depending on the type of substrate material used. Ash content ranged from 6.85 to 7.32 g/100 g. Total ash content was recorded as maximum in sawdust (7.32 g) which is the control and minimum in sawdust + corncobs (6.85 g). This result is similar to that of Hawrez (2018) who reported the ash

Table 3. Nutrient composition of various mushroom samples.

Sample (g/100 g)	T1	T2	T3
Protein	12.09±0.20	12.48±0.50	8.22±0.27
Lipids	1.51±0.17	1.45±0.11	1.43±0.21
Total carbohydrates	78.89±1.25	79.88±0.00	82.98±1.78
Fibers	17.07±0.51	19.78±0.50	18.75±0.61
Ash	7.32±.21	6.85±0.32	7.10±0.25

Source: Authors

content value in the range of 6.38 to 7.48 g/100 g.

Phytochemical analysis

Lindequist et al. (2005) stated that the nutritional and chemical compositions of mushroom are responsible for their medicinal values. The phytochemical analysis of *P. ostreatus* when supplemented with 4 medicinal plants revealed the presence of the following bioactive components; steroids, flavonoids, alkaloid, phenolic, tannins and saponins present in different concentrations in the different substrates and replications. Flavonoids, phenolic, tannins and saponins were present in *P. ostreatus* while alkaloid was absent (Kinge et al., 2016). During our experiment, we observed an increase in the concentration of bioactive components in *P. ostreatus*. The concentration and type of bioactive component varied depending on the substrate material used and the type of medicinal plant supplement. This is similar to the findings reported by Lindequist et al. (2005) who stated that the nutritional and chemical values of mushrooms are responsible for their medicinal values and equally, nutritional value of mushroom is affected by the type of substrate material used (Kinge et al., 2016).

The increase in the phytochemical component and thus the medicinal component of *P. ostreatus* is as a result of the supplementation with medicinal plants as medicinal plants contain phytochemical components which give them their medicinal properties. This result is in line with the findings of some researchers who reported an increase in the yield of oyster mushroom as a result of phytochemical components. This has been supported by an increase in the concentration of substances present in Neem cake which contain a mixture of tetranor triterpenoids (Govindachari et al., 1998), flavonoid, nimbosterol, liminoids, quercentinm, tannic acid, and substances found in citrus lemon, that is, volatile oil (Zeringue and Bhatnagar, 1994), limonene, alpha-pinene, beta-pinene, citral, coumarins, and bioflavonoids (Sammbamurty and Subrahmanyan, 2000). According to our findings, the concentrations of flavonoids, phenolic, tannins and saponins was highest in sawdust+ corn cobs + *R. vomitoria*. Alkaloids which are reported to be absent in oyster mushroom (Kinge et al., 2016) were present in some of the replications with medicinal plants in our trial. Alkaloids were present in replications: PBH, DBT, DBR, SCR, PBC, PBH, and PBR. Therefore, the supplementation of substrate with medicinal plants does not only improve on the growth and yield of the oyster mushroom according to Nazir et al. (2010) but also improves on the medicinal value.

Conclusion

Cultivation of oyster mushroom (*P. ostreatus*) on corn cobs substrate mixture with sawdust has a significant positive effect on the growth and yield of *P. ostreatus*. The nutritional content of oyster mushroom depends on the type of substrate material used. Protein and fibres content was highest in sawdust + corn cobs substrate making it the best substrate under investigation. Phytochemical components were present in oyster mushroom supplemented with medicinal plants more than in the control. Best result was presented by supplementation of sawdust + corn cobs with *R. vomitoria*. *R. vomitoria* is an anti-cancer plant. Oyster mushroom substrate can be supplemented with this plant during cultivation and the mushroom giving to cancer patients as a treatment. Therefore, mushroom growers are encouraged to supplement their oyster mushroom substrates with medicinal plants to improve on its medicinal value.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Table 4. Phytochemical screening of *P. ostreatus* supplemented with and without medicinal plants.

Sample	Phytochemical constituents					
	Steroid	**Flavonoid**	**Alkaloid**	**Phenolic**	**Tannins**	**Saponins**
DB	+++	+	-	-	-	+
DBC	+++	-	-	-	-	+
DBH	+++	-	+	-	+	-
DBT	+++	-	+	-	+	-
DBR	+++	-	+	-	-	-
SCB	+++	-	-	-	-	+
SCC	+++	+	-	-	-	+
SCH	+++	++	-	+	+	++
SCT	+++	+	-	++	+	++
SCR	+++	+++	+	-	-	+++
PB	+++	+	+	-	+	+
PBC	+++	+	+	-	-	-
PBH	+++	+	+	-	-	+
PBT	+++	-	-	-	+	+
PBR	+++	+	+	-	-	-

(+) Present, (-) absent, (++) moderate concentration, (+++) abundant concentration. DB= Sawdust + rice bran; DBC= Sawdust + rice bran+ *Croton macrastarchus;* DBH= Sawdust + rice bran+ *Harungana madagascariences;* DBT =Sawdust + rice bran+ *Tithonia diversifolia;* DBR= Sawdust + rice bran+ *Rauwolfia vomitoria;* SCB=Sawdust + corncobs + rice bran; SCC= Sawdust + corncobs + rice bran + *Croton macrastarchus;* SCH= Sawdust + corncobs + rice bran + *Harungan madagascariences;* SCT= Sawdust + corncobs + rice bran + *Tithonia diversifolia;* SCR= Sawdust + corncobs + rice bran + *Rauwolfia vomitoria;* PB= Palm cones + rice bran; PBC= Palm cones + rice bran + *Croton macratarchus;* PBH= Palm cones + rice bran + *Harungana madagascariences;* PBT= Palm cones + rice bran + *Tithonia diversifolia;* PBR= Palm cones + rice bran + *Rauwolfia vomitoria.*
Source: Authors

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