



Effect of local substrates on the growth and yield of *Pleurotus ostreatus* K. in the North West Region, Cameroon

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Kinge TR, Djidjou TAM, Nji TM, Ache NA, Mih AM 2016 – Effect of local substrates on the growth and yield of *Pleurotus ostreatus* K. in the North West Region, Cameroon. Current Research in Environmental & Applied Mycology 6(1), 11–19, Doi 10.5943/cream/6/1/2

Abstract

Food security to the ever growing world population is a major challenge of the 21st century. Scientists all over the world are seriously exploring ways and means to bring more food on the table. Cultivation of highly nutritional and medicinal mushrooms on local substrates is one of such effort. In an attempt to bring in our own contribution, locally available substrates for the cultivation of *Pleurotus ostreatus* was investigated at the Mbeng-Adio mushroom cultivation center Banjah Bamenda, Cameroon from March to September 2015. *Pleurotus ostreatus* was cultivated on different supplemented substrates such as Corn cobs on corn flour T1, Corn cobs on rice bran T2, Iroko on corn flour T3, Iroko on rice bran T4, Eucalyptus on corn flour T5 and Eucalyptus on rice bran T6; supplemented with 1% CaCO₃. The experiment was laid in a completely randomized design. The effects of various substrates on comparative growth and yield performance of oyster mushroom were then analyzed. The highest degree of colonization after inoculation (95%) and lowest time from primordia initiation to harvest (3.25days) were obtained in T6. The highest biological yield (0.47 kg/packet), economic yield (0.43 kg/packet) and 75% contamination were obtained with T1. Highest fresh weight (0.47 kg/packet), highest dry weight(0.09 kg/packet), highest average number of primordia/packet(226.3), highest average number of fruiting body/packet(43.25), the highest average weight of individual fruiting body(0.02 kg), highest mean thickness of stipe (12.08) and the highest average number of effective fruiting body/packet(26.25) were obtained in T5. Among many aspects, T5 was found as the best substrate with biological yield (0.47 kg/packet) and economic yield (0.43 kg/packet) followed by T1, T6, T4, and T3, with T2 rejected due to 100% contamination for the production of the oyster mushroom.

Key words – Basidiomycetes – domestication – macrofungi – Oyster mushroom

Introduction

Mushrooms are reproductive structures of some fungi mostly the Basidiomycetes and some Ascomycetes roughly equivalent to the flowers on an apple tree, which contain the “seeds” of the future tree (Nicholas & Kerry 2006). They have been universally recognized now as food and are increasingly

being grown on commercial scale in many parts of the world. China which is the largest producer of various mushrooms in the world has had a 24.3% increase in production over a ten year period between 2000 to 2010 (Li 2012). Cultivation is a wonderful activity as the fungi does not depend on weather conditions such as rainfall and can thus be grown all year round in cropping houses (Bonginkhosi et al. 2012). Mushrooms are appreciated for their good taste and nutritional value. The income from edible mushrooms is an important source of revenue for farmers, especially in developing countries. The increased consumer demand over the years has resulted in production of mushrooms in large proportions through cultivation which is a highly efficient method for recycling the agricultural residues so as to produce nutritious food (Atri & Lata 2013).

Commercial production of fresh edible mushrooms is a rapidly growing industrial activity that can be carried out in a large or small scale. It is an efficient and relatively short biological process of food protein recovery from negative value lignocellulosic materials, utilizing the degrading capabilities of mushrooms (Ediriweera et al. 2015). The Oyster mushroom *Pleurotus ostreatus* is characterized by its rapid growth on agro-wastes such as dried sugar cane leaves, saw dust, maize stover, banana leaves, palm cones, coffee husks and wheat bran which are substrates for mushroom production (Beetz & Kustida 2004, Lourdes et al. 2008, Ajonina & Tatah 2012). Sawdust types differ but softwood sawdust like mango and cashew are known to be more suitable than hardwood sawdust (Pathmashini et al. 2008). These substrate materials can be supplemented with corn flour, rice bran, molasses, soya bean or kernel cake in accordance with the particular substrate material used. Even though there has been considerable research on the taxonomy and phylogeny of mushrooms, there has been far less research on their domestication (Thawthong et al. 2014).

In Cameroon, agriculture and forestry are two prominent sectors which bring in revenue to the government (Degrande et al. 2007). Unfortunately most of the resulting waste products like sawdust from timber exploitation, rice husk or maize stover, are not often recycled appropriately through their use in the cultivation of mushroom. This may be due to lack of knowledge on the use of these substrate materials in mushroom cultivation. Cameroon's food supply in most rural areas is diminishing caused by increasing environmental deterioration (Kinge et al. 2014). The consequences are malnutrition, starvation, diseases and low life expectancy of these populations. Mushroom cultivation therefore comes not only to provide an opportunity to improve the local farmers livelihoods and reduce dependence on natural farm resources, but also to provide them with mushrooms having valued medicinal properties like immunomodulatory and antitumor activities (Wang et al. 1996). The need for commercial production of this edible mushroom in the country cannot be over emphasized. Unfortunately, very few individuals are involved in mushroom cultivation. Most of the research works in Cameroon have been on the taxonomy and documentation of the diversity of macrofungi and very few on modes of cultivation (Douanla-Meli 2007, Egbe et al. 2013, Kinge et al. 2014). This study therefore aimed at investigating the effect of local substrates in the cultivation of *Pleurotus ostreatus* in the North West Region of Cameroon. This may subsequently help in increasing living standards, environmental cleaning, providing protein and self employment in the country.

Materials & Methods

The Study Area

The research was conducted at Mbeng-Adio mushroom research center Banjah (Fig. 1), in Bamenda, North West Region of Cameroon from March to September 2015. The research center is located at an altitude of 1647m above sea level and has the following latitude and longitude coordinates; N 05°57.431' and E 010°13.093' respectively. An average temperature of 21 -22°C was found during the period of cultivation.

Sterilization Procedure and Preparation of Spawns

In the laboratory, all of the apparatus, equipment, metallic instruments, glassware and culture media were sterilized in drums at 121°C for about 2 hour at 1.5 kg/cm pressure. The culture room was

cleaned by gently washing with detergent followed by 70% ethyl alcohol regularly. All the instruments and equipment used were sterilized with alcohol before use.

The mother culture substrate was prepared by sawdust and corn flour in the ratio 2:1 with 0.1% calcium carbonate (Amin et al. 2007). It was mixed thoroughly with hands and moisture maintained by adding sufficient water for 65% moisture content, controlled using the hand squeezing test. Then 1000g of this mixture was packed tightly in 15×5cm mayonnaise glass bottles. Each of the bottles was covered tightly with middle- holed covers. The holes on the covers were plugged with cotton and this served as a sterile opening through which the fungus breaths. The bottles were sterilized for 2 hour at 121°C with 1.5kg/ cm² pressure in drum sand after they were kept for cooling. Mushroom seeds were placed aseptically on them other spawn bottles in a sterilized glove box. The bottles after inoculation were kept at 20-22°C for spawn run in a dark cupboard. The whole bottle containing substrate became white due to fungal mycelia proliferation within 20-25 days, and thus ready for spawning the substrate.

Two sawdust species (Eucalyptus and iroko), were obtained from carpenter shops at Nkwen Mile 4 and each divided into two equal portions. One portion of each was supplemented with rice husk, and rice bran in the ratio 4:2:1 and 0.25kg of quick lime. The other portion supplemented with rice husk, corn flour and quick lime in the same ratio. The material was mixed thoroughly on a clean cemented floor using a spade. The moisture increased by adding water until it reached around 65% moisture content. Then polypropylene bags (25×18 cm) were filled, four with 2.0 kg prepared substrate of each of the four prepared portions of substrate materials and packed tightly. The packets were sterilized in drums at 121°C for 2 hours and kept 12 hours for cooling. Three table spoonfuls of mother culture materials from mother spawn containing mycelia were placed aseptically after opening in each packet. The two differently supplemented portions of each sawdust being treatments each were replicated 4 times, giving a total of 16 packets. The packets were then marked treatment wise with paper notices after tying with different coloured threads and kept on the shelves in an incubation room at 25±1 °C under 80% to 85% relative humidity and allowed to complete the whitish mycelia growth.

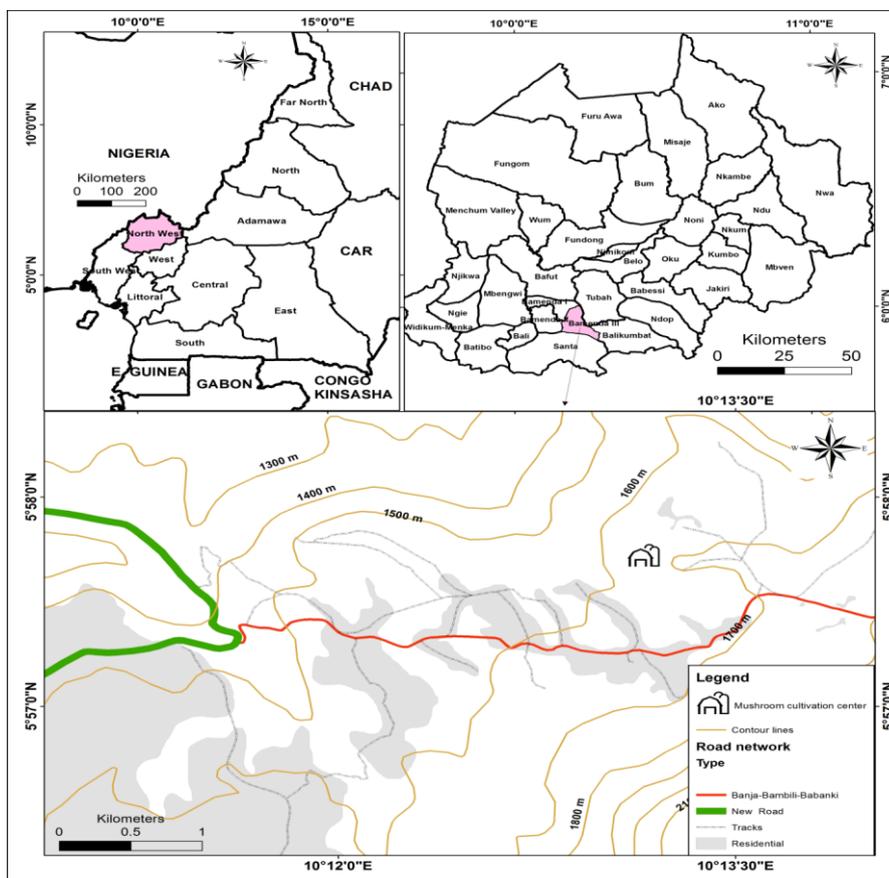


Fig. 1– Locational map of Banjah (Kinge et al. 2015)

Preparation of maize stover substrates

The maize stover was obtained from farms in Bandjoun (West Region) and sun dried. It was divided into two portions. These two portions were supplemented each separately. One with rice bran in the ratio 4:2 and 0.25kg of quick lime, the other portion with corn flour and quick lime in the same amounts as above. The material was mixed thoroughly on a clean cemented floor using a spade. The moisture increased by adding water until it reached around 65% moisture content. Then polypropylene bags (25×18 cm) were filled 4 each with 2.0Kg of one portion of prepared substrate and packed tightly. The packets were sterilized in drums at 121°C for 2 hours and kept 12 hours for cooling. Three table spoonful of mother spawn were inoculated aseptically. The two differently supplemented portions were carried out in four replicates. All bags were incubated at 25±1°C under 80-85% relative humidity until completely colonized. The experiment was laid out in a completely randomized design (CRD) with six treatments and four replications as shown on Table 1.

Table 1 Various treatments and their identification marks

Treatments	Substrates	Composition
T1	Substrate 1	Eucalyptus on corn flour and CaCO ₃ (tied normally). In the ratio 69:30:1 respectively
T2	Substrate 2	Eucalyptus on rice bran and CaCO ₃ (tied with white thread). In the ratio 69:30:1 respectively
T3	Substrate 3	Corn cobs on corn flour and CaCO ₃ (tied with orange thread). In the ratio 69:30:1 respectively
T4	Substrate 4	Corn cobs on rice bran and CaCO ₃ (tied normally). In the ratio 69:30:1 respectively
T5	Substrate 5	Iroko on rice bran and CaCO ₃ (tied with yellow thread). In the ratio 69:30:1 respectively
T6	Substrate 6	Iroko on corn flour and CaCO ₃ (tied with blue thread). In the ratio 69:30:1 respectively

Harvesting

Harvesting was carried out when the fruiting bodies were matured. Harvesting was made three times for each bag of substrate. The process of harvesting involved the removal of the matured fruiting bodies from their substrate without any destruction on the substrate bag. The mature mushroom was held on their stipe below the pileus and close to the substrate level and was gradually pulled out. All fruiting bodies of a particular substrate bag were harvested at the same time since each bag had to be watered after harvest. Watering was done by immersing the bags in a bowl of water for 5 seconds. This was done to enable the substrate to have moisture that enables fruiting to occur again for harvest.

Morphological Data collection

Data was recorded on the following parameters;

Time from Stimulation to Primordia Initiation (days): Time required from stimulation to primordia initiation (days) was counted and recorded. Time from Primordial Initiation to Harvest (days): Time required from primordial initiation to harvest (days) was counted and recorded. Average Number of Primordial/Package: Number of primordial/package was counted and recorded. Average Number of fruiting Body/Package: Number of well-developed fruiting body was counted and recorded. Dry and pinheaded fruiting bodies discarded but tiny fruiting bodies included in counting. Average Number of Effective Fruiting Body/Package: Number of very well-developed fruiting body was counted and recorded. Tiny fruiting bodies discarded from counting. Average Weight of Individual Fruiting Body/Package: Average weight of individual fruiting body was calculated by dividing the total weight of fruiting body per package obtained from the balance reading by the total number of fruiting body per package. Dimension of Fruiting Body (pileus and stipe): Diameter of pileus (The mushroom pileus diameter was taken from one end of the pileus to the other passing through the centre of the pileus and measured in cm using a tailor's tape), thickness of stipe (The thickness of stipe was taken at the base

of the stipe where it attaches to the substrate from one end of the stipe base to the other passing through the centre and measured in cm using a tailor's tape) and length of stipe (This was measured in centimeters using a tailor's tape from the point where it was attached to the substrate to the point where the gills on the pileus start on the stipe). All these measurements were done on 3 randomly selected bags of fruiting bodies per treatment after harvest and then the average was calculated. Biological Efficiency was calculated as; Fresh weight of mushroom/Dry weight mushroom x 100.

Data Analysis

The data was analyzed using ANOVA and treatment means compared using Duncan's Multiple Range Test (Gomez & Gomez 1984). The graphical representation was done using Statgraphics Centurion XV, Sigma plot 2000 and excels 2007. A correlation between economic yield and fresh weight was determined and was expressed by the equation $y=1.144x-0.026(R^2=0.9224^{**})$ where y =economic yield and x =fresh weight of fruiting body (Bhattacharjya et al. 2014).

Results

Effect of substrates on early growth performance

The time from stimulation to primordia initiation ranged from 20.00-27.00days. All the treatments were statistically different. The lowest time from stimulation to primordia initiation was observed in T6 (20.00 days).The highest time from stimulation to primordia initiation was observed in T1 (27.00days). The time from primordia initiation to harvest ranged from 3.25-5.50days. The lowest time from primordia initiation to harvest was observed in T6 (3.25 days).The highest time from primordia initiation to harvest was observed in T1 (5.50days). Statistically, similar primordia initiation to harvest was shown in T6 (3.25days), T3 (3.25days), T1 (5.50) and T5 (4.75days) treatments. There was significant difference in the number of contaminated bags. T1 (75%) and T2 (100%) were the only treatments contaminated. These results are shown inTable2.

Table 2 Effect of substrates on early growth performance

Treatments	Stimulation to primordial initiation (days)	Primordial initiation to harvest (days)	Degree of colonisation after inoculation (%)	Contamination (%)
Corn cobs_corn flour T1	27.00±0.00 ^c	5.50±0.58 ^b	65.00±0.00 ^b	75.00±50.00 ^b
Corn cobs_rice bran T2			65.00±0.00 ^b	100.00±0.00 ^b
Iroko_corn flour T3	25.50±1.00 ^{b^c}	3.25±0.50 ^a	55.00±0.00 ^a	00.00±0.00 ^a
Iroko_rice bran T4	24.00±2.45 ^{abc}	4.25±0.96 ^{ab}	75.00±0.00 ^c	00.00±0.00 ^a
Eucalyptus_corn flour T5	22.00±4.69 ^{ab}	4.75±0.96 ^b	87.00±0.00 ^d	00.00±0.00 ^a
Eucalyptus_rice bran T6	20.25±2.63 ^a	3.25±0.96 ^a	95.00±0.00 ^e	00.00±0.00 ^a
F (p)	4.04 (0.02)	5.70 (0.0054)		20.20 (0.0000)

Means followed by same letter significantly different at 1% or 5% level of significance

Effect of substrate on Yield Attributes of oyster mushroom

The Mean Average number of fruiting body per packet, Mean Average number of effective fruiting body and Mean Diameter of pileus (cm) were statistically similar for each treatment but numerically varied. The Mean thickness of stipe (cm) was higher in T5 (12.08) and lowest in T6 (3.53). The later was statistically different from T1 (8.30) but statistically similar to T3 (4.60) and T4 (5.03). Finally, the Mean Length of stipe (cm) was highest in T1 (6.30) and lowest in T4 (4.03) as shown in Table 3.

Table 3 Effect of substrates on yield attributes of oyster mushroom

Treatments	Mean Average number of fruiting body per packet	Mean Average number of effective fruiting body	Mean Diameter of pileus (cm)	Mean Thickness of stipe (cm)	Mean Length of stipe (cm)
Corn cobs_corn flour T1	42.00±0.00 ^a	26.00±0.00 ^a	8.30±0.00 ^a	8.30±0.00 ^b	6.30±0.00 ^b
Iroko_corn flour T3	34.75±11.53 ^a	25.00±4.69 ^a	8.00±1.15 ^a	4.60±0.69 ^a	4.10±0.12 ^a
Iroko_rice bran T4	35.75±4.57 ^a	19.75±3.10 ^a	8.30±1.13 ^a	5.03±0.19 ^a	4.03±0.19 ^a
Eucalyptus_corn flour T5	43.25±24.86 ^a	26.25±12.39 ^a	7.98±1.24 ^a	12.08±2.57 ^c	5.18±1.75 ^{ab}
Eucalyptus_rice bran T6	43.00±13.49 ^a	23.25±5.12 ^a	9.48±0.99 ^a	3.53±1.43 ^a	5.73±1.95 ^{ab}
F (p)	0.36 (0.8325)	0.68 (0.6176)	3.4(0.2999)	26.55(0.0000)	2.89(0.0585)

Means followed by same letter significantly different at 1% or 5% level of significance

Effect of Substrates on Biological and Economic Yield (Kg)

The Average weight of individual fruiting body was statistically similar for all treatments. The Mean Biological yield varied statistically between treatments ranging from 0.23-0.47, with T5 (0.47) having the highest mean Biological yield, while T3 (0.29) had the lowest. The mean economic yield on the other hand was highest in T1 (0.43) and T5 (0.41) but lowest in T3 (0.28) as shown in Table 4 and Figure 2. Pictures of some of the cultivated mushrooms are shown Figure 3A and B.

Table 4 Effect of Substrates on Biological and Economic Yield

Treatments	Mean Fresh weight (kg)	Mean Dry weight (kg)	Average weight of individual fruiting body	Mean Biological yield	Mean Economic yield
Corn cobs_corn flour T1	0.45±0.00 ^b	0.08±0.00 ^{ab}	0.01±0.00 ^a	0.45±0.00 ^b	0.43±0.01 ^b
Iroko_corn flour T3	0.29±0.04 ^a	0.06±0.01 ^a	0.01±0.00 ^a	0.29±0.04 ^a	0.28±0.03 ^a
Iroko_rice bran T4	0.37±0.06 ^a	0.07±0.01 ^{ab}	0.01±0.00 ^a	0.37±0.06 ^{ab}	0.35±0.06 ^{ab}
Eucalyptus_corn flour T5	0.47±0.14 ^b	0.09±0.03 ^b	0.02±0.01 ^a	0.47±0.16 ^{ab}	0.41±0.15 ^{ab}
Eucalyptus_rice bran T6	0.36±0.11 ^{ab}	0.07±0.02 ^{ab}	0.01±0.01 ^a	0.36±0.11 ^{ab}	0.35±0.11 ^{ab}
F (p)	2.81(0.0634)	2.24(0.1133)	0.93(0.4702)	1.67(0.2090)	1.73(0.1957)

Means followed by same letter significantly different at 1% or 5% level of significance

Correlation analysis

A significant and positive correlation between economic yield and fresh weight was observed with the differently supplemented substrates (Fig.4). This suggests that economic yield dependent on fresh weight. The R^2 value indicated that 92% of economic yield of oyster mushroom (*P. ostreatus*) was attributed to the fresh weight of fruiting body.

Discussion

The highest time from stimulation to primordia initiation differed with the findings of Ahmed (1998) who found out that *Pleurotus ostreatus* took 23-27 days for initiation of fruiting bodies, unlike Yoshida et al. (1993) reported that the number of fruiting bodies was lower, but increased when the substrates were mixed with different supplements. Bhuyan (2008) in a same type of experiment on the effects of substrates on the yields of cultivated mushroom, found similar results.

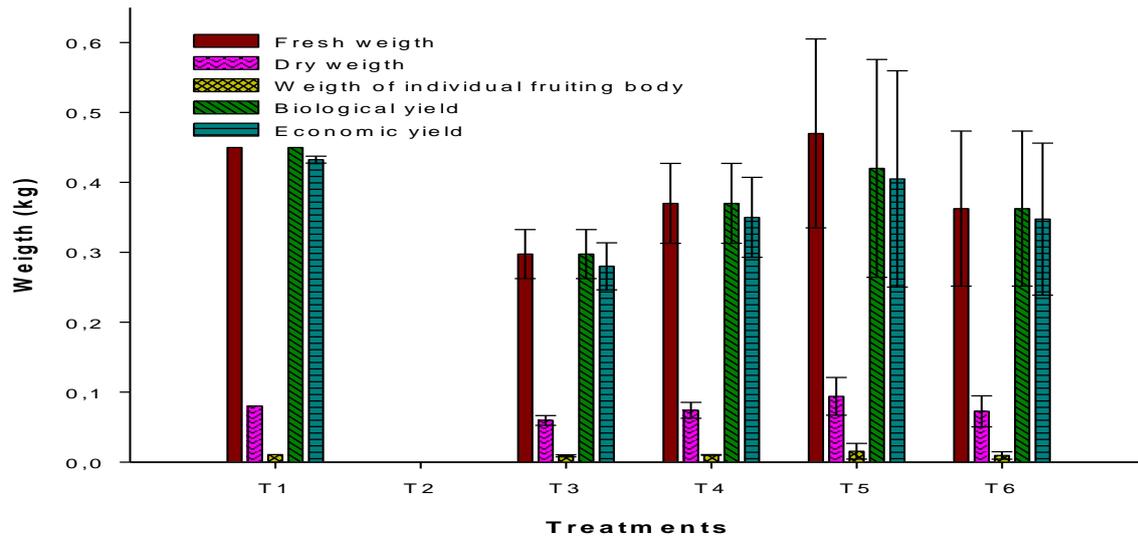


Fig. 2 – Effect of Different Substrates on Yield

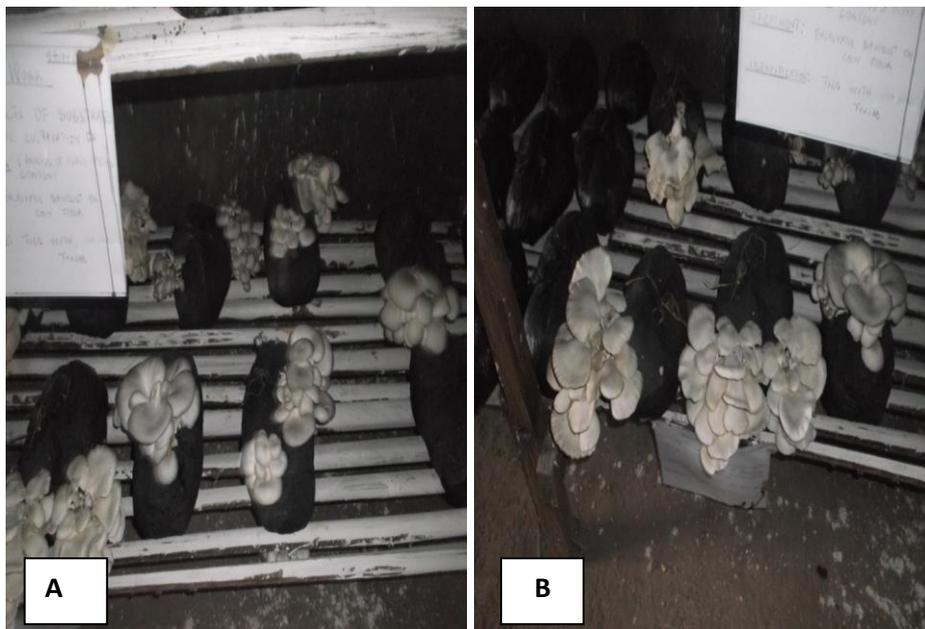


Fig. 3 – AB – Fruiting bodies of T₅ and T₆ respectively

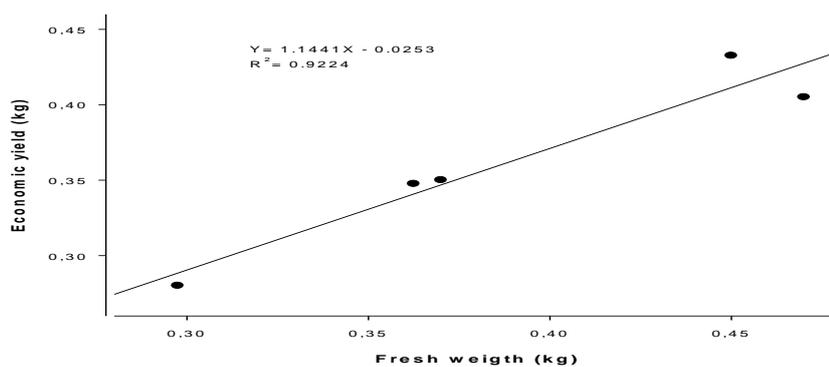


Fig. 4 – Functional Relationship between Economic and Fresh Weight as influenced by substrates

The results varied with the findings of Sarker et al.(2007) and Habib (2005),who reported that the stipe length of *Pleurotus spp.* on different substrate varied from 1.93-2.97cm and the diameter ranged from 0.74-1.05cm. The difference among the findings may be due to the difference in substrates and supplements used or the species varieties. The humidity of the bags in which mushrooms were cultivated was favourable, since this fungus was grown in late March and early April when the coming rains are abundant in this part of the country. The thickening of the mycelia in the bags, colonization of the bags was an indication for the bags to be opened for fruiting.

The result of the present study on the effect of substrates on biological and economic yield corroborates with Amin et al. (2007) who found the highest biological yield of 0.24kg/packet. He also found that the trend of economic yield corresponded with different supplements at different level. Payapanon et al. (1994) mentioned that suitable amount of supplements added to rice straw medium maximized economic yield of oyster mushroom at optimum production cost. For correlation study the strong association is due to the fact that; the higher the fresh weight, the larger the amounts of mushroom material for sale (economic yield) as these mushrooms are sold in kilograms when freshly harvested.

Eucalyptus on corn flour (T5) showed the best performance compared to other substrates used for the cultivation of *Pleurotus ostreatus* in terms of growth and yield parameters measured. It was followed by; corn cobs on corn flour (T1), eucalyptus on rice bran (T6), iroko on rice bran (T4), iroko on corn flour (T3), and finally corn cobs on rice bran (T2), which did not sprout due to one hundred percent contamination by brownish fungal growth, known as the *Trichoderma* disease of mushroom. High yields and absence of contamination risks with T5, makes it the most appropriate substrate for the cultivation of the oyster mushroom. Also, despite the 75% contamination in T1 it could also be one of the most appropriate substrate for cultivation provided that the *Trichoderma* disease of mushroom is controlled and care is taking to eliminate or minimize contamination. Eucalyptus sawdust and corn flour are relatively abundant in rural communities in the study area where resource poor farmers reside and it can therefore be used to cultivate *Pleurotus ostreatus*, providing a highly profitable agribusiness that produces not only nutritious and medicinal food products from different substrates, but also helps to dispose them in the environment in a friendly manner and conserving mushroom biodiversity in the forest.

Acknowledgements

This work was financed by the Research Modernization Grant from the Ministry of Higher Education, Cameroon. The management of Mbeng-Adio Mushroom cultivation center, Banjah Bamenda is thanked for use of facilities.

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