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Suitability of Locally Available Substrates for Cultivation of the Kenyan Indigenous Golden Oyster Mushroom (*Pleurotus citrinopileatus* Singer)

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ABSTRACT

The growth and yield performance of indigenous *Pleurotus citrinopileatus* on selected locally available substrates were determined as a prelude to its domestication. Seven substrates namely bean straw (*Phaseolus vulgaris*), sawdust of African mahogany (*Khaya anthotheca*), rice straw (*Oryza sativa*), maize cobs (*Zea mays*), wheat straw (*Triticum aestivum*), sugarcane bagasse (*Saccharum officinarum*) and banana leaves (*Musa* sp.) were tested for their suitability for production of the indigenous *Pleurotus citrinopileatus*. Each treatment had 9 plastic bags each containing 1 kg of fresh weight of substrate, each spawned with 50 g of indigenous oyster mushroom, *Pleurotus citrinopileatus*. The treatments were arranged in a completely randomized design. Data was collected on days to pinning, fruiting body yield (fresh weight) and biological efficiency. Data collected was subjected to analysis of variance using minitab version 14. Mean separation was done using Tukey test and effects declared significant at 5% level. The substrates were significantly different ($p<0.05$) in biological efficiency; yield and days to pinning. The best performance was obtained from the bean straw substrate. Maximum yield (397.71 g kg^{-1} wet substrate) and biological efficiency of 148% were obtained from bean straw at spawn rate of 5%. This study recommends bean straw as a new substrate for cultivation of *Pleurotus citrinopileatus* at spawn rate of 5% under local conditions which is being reported for the first time in Kenya.

Key words: Indigenous, golden oyster mushroom, *Pleurotus citrinopileatus*, substrates, cultivation, yield, Kenya

INTRODUCTION

Mushroom cultivation in Kenya began in 1970s with *Agaricus bisporus* as the first commercially cultivated species. Different species of *Pleurotus*, *Auricularia* and *Lentinula edodes* are also being grown today but at a comparable low rate. These species except for *Pleurotus* are dependent on spawn importation. Kenya being a tropical country is rich with mycodiversity which are still in the wild and remain unharnessed for their nutraceutical value (Wambua, 2004).

Wild mushrooms have been a delicacy in Kenya for many years and are meat substitute especially for the rural population. The uncontrolled harvesting of mushrooms throughout the year

and destruction of forest habitat to create land for settlement, agriculture and firewood has resulted into loss of germplasm of these fungi (Palapala *et al*., 2006). Apart from providing food, cultivation of mushrooms is a method of conservation (Onyango *et al*., 2011a). There is a need to develop cultivation techniques for indigenous mushrooms for adaptation by local farmers.

During the routine survey to collect *Pleurotus* mushrooms useful for cultivation, strain improvement and systematic breeding programme by Kenya Industrial Research and Development Institute staff in Kenya's Kakamega forest, an interesting wild species of *Pleurotus citrinopileatus* was found growing on dead logs and branches of indigenous trees namely *Antiaris toxicaria* (Pers.) Lesch. (Moraceae), *Polyscias fulva* (Hiern) Harms (Araliaceae), *Ficus thonningii* Bl. (Moraceae) (Musieba *et al*., 2011). In order to produce and conserve the germplasm of this native mushroom, several substrates were tested for their efficiency in cultivation this mushroom.

MATERIALS AND METHODS

The study was conducted at the Mushroom Pilot Plant facility of the Kenya Industrial Research and Development Institute (KIRDI) between October and December, 2011. *Pleurotus citrinopileatus* previously collected from Buyangu forest reserve within the Kakamega forest and phonetically characterized by the author of this manuscript and maintained as pure culture at mushrooms culture bank at KIRDI was used for this study. During this study, all stock mushroom cultures were maintained on Potato Dextrose Agar (PDA) medium for one month at 5°C in a refrigerator. Subcultures were made routinely monthly. Pure cultures were kept on slants and plates at 25°C for 10 days.

Spawn preparation: Procedure by Isikhuemhen *et al*. (2000) was followed with slight modification for preparation of mushroom spawn. Briefly, spawn of the *P. citrinopileatus* was prepared with intact wheat grains which were bought from Nyamakima market, Nairobi, Kenya. One kilogram of wheat grains were washed thoroughly and boiled in 2 L of water until semi soft. The grains were left for 10 min in hot water to allow moisture absorption. Water was drained and the grains spread on a mesh to cool. The grains were mixed with 1% (w/w) $CaCO_3$ to adjust the pH and 100 g of the wet grains put in 500 mL conical flasks. The mouth of the conical flasks were tightly plugged with cotton wool and covered with aluminium foil. All prepared flasks were transferred to the autoclave and sterilized at 121°C for 20 min. The flasks were allowed to cool down before aseptically inoculating them with ten 1 cm^2 pieces of mycelia taken from 10 day old cultures of *P. citrinopileatus*. This was done in a laminar flow. The inoculated grains were incubated in a dark room at 25±2°C until the mycelia fully colonized the grains.

Substrate preparation: Plastic bag technology was used in this experiment with treatments replicated 9 times and arranged in a completely randomized design. Seven substrates namely bean straw (*Phaseolus vulgaris*), sawdust African mahogany (*Khaya anthotheca*), rice straw (*Oryza sativa*), maize cobs (*Zea mays*), wheat straw (*Triticum aestivum*), sugarcane bagasse (*Saccharum officinarum*) and banana leaves (*Musa* sp.) were tested. All the substrates were compared to wheat straw because it the most commonly employed substrate for *Pleurotus* cultivation (Philippoussis *et al*., 2003).

The substrates were collected from a rural farm in Kangundo, Kenya and a local jaggery in Industrial area, Nairobi, air dried and chopped into pieces of 4-5 cm long using a motorized chaff cutter. The chopped substrates were wetted separately on cemented floor and allowed to drain

overnight. One kilogram of the substrate was then packed into heat resistant polythene bags with a diameter of 12 cm and a length of 18 cm. The bags were closed with a twist-tie and a piece of cotton wool plugged at the neck to allow for gaseous exchange and pasteurized at 70°C for 2 h. After cooling, they were spawned individually at the rate of 5% under a laminar air flow, labeled and incubated in the dark at 25±2°C for 8-21 days to allow complete spawn run. The bags were transferred onto horizontal wooden racks in a humid mushroom growing room with a 12 h light/ 12 h dark photoperiod at 23±2°C to induce basidiome formation. The cotton plugs were removed and the bags slit at the sides using a sharp blade. The production rooms were watered twice a day to increase humidity and induce fruit body formation. The temperature and relative humidity readings of the production blocks were recorded daily. After each flush mushrooms were harvested and the production blocks watered again to promote development of another flush. Harvesting was done by hand and weighing done immediately. Date of each harvest was recorded. Total number of flushes or breaks produced per bag was also noted. The distribution of yield per flush was tabulated to observe changes in the yield over the course of multiple flushes.

The number of days from inoculation to total colonization of the substrates, first primordial formation and first flush were recorded. Mushroom yields were also weighed. Duration of time from inoculation to final harvest was also recorded.

The biological efficiency (ratio of fresh mushrooms produced/substrate dry weight, expressed in percentages) was calculated using the formula outlined by Onyango *et al.* (2011b). Total wet weight of the mushroom was recorded during the four flushes. Yield (fresh weight of harvested mushrooms/substrate fresh weight, expressed in percentages) was also recorded. Also considered was the production period and number of crops. Bioconversion was determined by substrate dry weight loss after mushrooms harvest.

All the experimental units were discarded after 62 days. Data obtained were analyzed statistically by Minitab Release 14 statistical software (Minitab Inc, Pennyslania, USA). An Analysis of Variance (One way ANOVA) and Tukey test were used to indicate the significant differences between the mean values ($p<0.05$).

RESULTS

The mean yield of *P. citrinopileatus* on different agricultural wastes and their biological efficiency are given in Table 1 and 2. After four flushes, yields of mushroom from bean straw were markedly higher than all the substrates (Table 1). No harvestable produce was recorded from saw dust.

Though all substrate recorded successful mycelia colonization and fruiting, bean straw presented the shortest period of colonization (8 days) and the highest biological efficiency of 148%. Rice straw followed with an efficiency of 98%, sugarcane bagasse: 78%, wheat straw: 41%, banana leaves: 16% and maize cobs: 5%. The biological efficiency of the control substrate was 40% (Table 2).

The highest mushroom weight in different flushes for all the substrates was in the first flush, except for sugarcane bagasse and banana leaves.

Table 1: Effect of substrates on yield of *Pleurotus citrinopileatus*

Substrate	Sawdust	Maize cobs	Banana leaves	Wheat straw	Sugarcane bagasse	Rice straw	Bean straw
Yield (g)*	0.00^{a}	28.28^{a}	61.26^{ab}	109.59^{b}	177.14^{c}	213.62^{c}	397.71^{d}

Means followed by the same letter in the row are not significantly different at $p<0.05$, *Fresh weight after four flushes

Table 2: Biological efficiency of substrates for *Pleurotus citrinopileatus*

Substrate	Biological efficiency (%)
Saw dust	0.00[a]
Maize cobs	5.17[a]
Banana leaves	16.89[ab]
Wheat straw	41.11[b]
Sugarcane bagasse	78.66[c]
Rice straw	98.88[c]
Bean straw	148.78[d]

Means followed by the same letter in the column are not significantly different at $p<0.05$

Table 3: Days for completion of spawn running, fruiting bodies formation and pinhead formation of *Pleurotus citrinopileatus* on different substrates at the spawn rate of 5%

Substrate	Completion of spawn running (days)	Pinhead formation (days)	Fruiting body formation (days)
Bean straw	8	13	17
Rice straw	13	17	21
Sugarcane bagasse	13	17	21
Wheat straw	13	24	27
Banana leaves	21	23	27
Maize cobs	21	28	33
Saw dust	21	31	61

Pinhead formation was found to vary between 13 days and 31 days. Faster pinning occurred on bean straw (Table 2). By using bean straw, the number of days until production decreased (Table 3).

Whereas mycelia colonization of all substrates was complete at the end of spawn running period, the sawdust bags showed obvious thin incomplete colonization even up to the end of incubation period (Table 3).

DISCUSSION

Bean straw emerged as the most suitable substrate for cultivation of the indigenous *Pleurotus citrinopileatus*. Bean straw showed the shortest colonization period and the highest biological efficiencies and yields. Substrate composition and structure is important for vegetative growth and fruiting bodies development (Mshandete, 2011). Compared to the control, using bean straw reduced the number of days until production by 10 days resulting into shorter crop cycles for growers and minimizing the exposure of the production substrate to pest infestations, especially sciarid (*Lycoriella mali* [Fitch]) flies. A study has shown that the sciarid fly may complete its life cycle in 25 days at 21°C, while 35 to 38 days are required at 18°C (Royse, 2002).

Timely disposal of spent substrate may help to minimize the build up of fly populations on a mushroom farm (Royse, 2002). The superiority of bean straw as a growth medium for the efficient mycelial growth of *Pleurotus* sp. further reaffirms the previous findings regarding its suitability as a growth medium for the propagation of mycelia of most wild mushrooms we have cultivated including *Pleurotus* (Kimenju *et al*., 2009). Bean straw is an ideal medium due to its nutrient content that can fulfill the nutritional needs of this mushroom.

In all the cases, first flush fruit bodies gave much more yield than second and subsequent flushes. There was decrease in mushroom yield in subsequent flushes. These results agree with

those of Kimenju *et al.* (2009). Sawdust performed the least amongst the substrates tested. Sawdust presented the longest spawn running time exposing the substrate for a longer time to competitors such as weed molds and bacteria. Further, the mycelia density in sawdust was observed to be thin and scanty which implies that the nutrients present in the substrate was not sufficient to nourish a vigorous and luxuriant growth. In the wild however *P. citrinopileatus* was found growing naturally in Kakamega forest on the dead logs and branches of indigenous trees namely *Antiaris toxicaria* (Pers.) Lesch. (Moraceae), *Polyscias fulva* (Hiern) Harms (Araliaceae), *Ficus thonningii* Bl. (Moraceae) suggesting that if nutritionally improved sawdust could be a suitable candidate as a substrate. Thus there is a need to reinvestigate the appropriate formulation of saw dust-based medium. For instance, in a side experiment for spawn production, addition of wheat bran at the rate of 5% to sawdust, stimulated luxuriant mycelia growth and early fructification.

CONCLUSION

This study recommends bean straw as a new substrate for cultivation of the indigenous *Pleurotus citrinopileatus* at spawn rate of 5% which is being reported for the first time in Kenya. This breakthrough in growing this mushroom at our pilot plant is a major incentive for growing wild mushrooms and will catalyze their adoption by farmers. If properly harnessed, this species can be used as direct source of protein and bioactive compounds for the people while generating livelihood, contributing to food availability and promoting environmental protection in the countryside.

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