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Spawn production and cultivation of two local edible fungal species in Kisangani (DRC) (case of *Pleurotus tuber-regium* and *Lentinus squarrosulus*)

Summary

This study focused on the cultivation of two local fungal species from spawn produced locally. The strains were isolated on PDA medium, then transplanted on sorghum-based seedling substrate. The production of fruit bodies was carried out on a substrate based on rice haulms mixed with sawdust of *Gilbertiodedron dewevrei* enriched with rice bran. In total, 4310g of *Pleurotus tuber-regium* fruit bodies and 894.22g of *Lentinus squarrosulus* were harvested after three successive emergences, the time between emergences being 3 to 4 days for *Lentinus squarrosulus* and 30 days for *Pleurotus tuber-regium*. This study makes it possible to produce spawn as well as mushrooms under local conditions, at a price that defies competition and is suitable for all budgets.

Keywords: local production of spown, *Pleurotus tuber-regium*, *Lentinus squarrosulus,* Kisangani

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Introduction

Among the non-timber forest products of the Democratic Republic of Congo (DRC) is the mushrooms which are consumed almost everywhere in the DRC. Mushrooms are also nutritional compared to other foods. According to Bram and Janna, 2007: while allowing food to vary, mushrooms provide some supplement rich in minerals, vitamins and even proteins. Studies carried out in particular in Benin, Malawi and Tanzania have shown that mushrooms often contribute essential nutritional value to diets;[1] which is an important food supplement in a country facing child malnutrition.

In Africa in general and in the DRC in particular, edible mushrooms are abundant in the wild and picking fruit bodies in the wild remains the only way to obtain them and represents an activity of great commercial value and involves thousands of rural women.[2] Studies in Tanzania have shown that mushroom harvesting is an alternative activity for farmers and contributes to improving socio-economic status by providing considerable income and food security. The picking of fruit bodies, which takes place during the rainy season, a favorable period for the formation of fruit bodies, remains the only means of supplying households, local markets and urban areas.[1,3-6]

In Yangambi (DRC), some farmers voluntarily cut trees and let them decompose for the development of fungi (*Schyzophyllum commune, Schyzophyllum commune, Auricularia spp, Marasmius buzungolo, Lentinus squarrosolus* and *Pleurotus spp*). The cultivation of such species of mushrooms would be essential to reduce the pressure on the forests, in order to generate substantial income for the local populations.

Of all the edible fungal species recorded in tropical Africa, very few are cultivated artificially. This difficulty is due, not only to the fact that mushroom cultivation is underdeveloped in the region, but also and above all, to the technology for the production of mycelium which is almost non-existent, or poorly mastered and which requires an efficient sterile laboratory. as well as specific knowledge.[7] Local production of the mycelium will not only help to facilitate the cultivation of local species, but above all to enhance and conserve them ex situ, but also and above all to make the mycelium of the various local edible species available, thus making it possible to sell it at a price that defies the competition and adapted to the realities of the environment, thus taking into account the local financial situation.

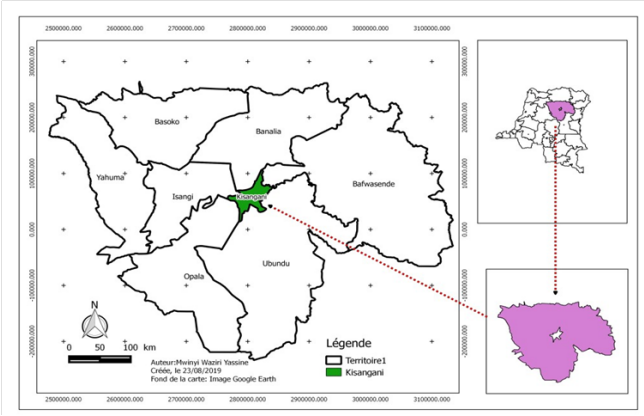
The main objective of this work is to cultivate the mushrooms *Pleurotus tuber-regium* and *Lentinus squarrosulus* from spawn produced locally. Its secondary objectives are: - to isolate the local strains, - to estimate the growth of mycelium on the culture medium, the sowing substrate as well as on the fruiting bales, - to determine the average durations of incubation, fruiting of fruit bodies and to evaluate the weight of fruit bodies harvested.

Materials and methods

Study site

This work was carried out in the mycology laboratory of the Faculty of Sciences of the University of Kisangani in Kisangani in the province of Tshopo in the Democratic Republic of Congo.

The city of Kisangani enjoys an equatorial climate of the Af type according to the Köppen classification. It is a hot and humid climate with high and constant temperature all year round. There are low thermal amplitudes and the temperature oscillates around 24 to 26°C, rainfall is abundant and distributed unevenly in two seasons during the year. The first is the rainiest, from September to November and the second, relatively rainy, from March to May. The two periods are separated by two other intermediate periods of low rainfall. Rainfall reaches 1800mm and there is no absolute dry season. The height of the rains of the driest month is more than 60 mm. The relative humidity of the air varies between 80 and 90% and the insolation is 1972 hours or 45% of the total radiation. It is therefore a continental equatorial climate par excellence (Mambani in Ongambo, 2014). The city of Kisangani is located in the central basin at 25°11' East longitude; at 0°31' North latitude and its altitude varies between 376 and 460m.[8]



Methods

The PDA (potato dextrose and agar) agar medium was obtained from the cooking filtrate of 200g of potato in 1000ml of distilled water, mixed with dextrose sugar (20g) and agar agar (20g), then increased to 1000 ml according to the methodology proposed by Dibaluka[9] is chosen to obtain the pure culture. After heating to homogenize the mixture, it was divided into test tubes and meticulously sealed with a cotton ball. After sterilization at 121°C for 30 minutes 1 atm, the tubes were cooled in a tilted position to room temperature. The pure culture was obtained from the spores of two strains of saprotrophic edible fungi (*Pleurotus tuber-regium* and *Lentinus squarrosulus*) collected locally in the botanical garden of the Faculty of Sciences (Stanyslas Lisowsky). The piece of each specimen of these two fungi was glued to the opening of the tube (the hymenial side facing the PDA medium) and secured with parafilm tape.

The sowing substrate was prepared from cereal, sorghum. The cereal was soaked in water and then boiled in an electric stove for 25 minutes, ensuring that the grains did not burst and still retained some hardness. These grains were then mixed with extinguished heat (1%) in order to obtain a neutral pH. After cooking, the grains were placed in glass bottles which were filled to 1/3 of their volume and closed with a lid under which a cotton ball was placed, then covered with aluminum foil. before sterilizing them in an autoclave at 120°C, under a pressure of 1 atmosphere, for 1 hour.[10-30]

The inoculation of the sowing substrate with mycelium from the pure culture (contained in the agar medium) was carried out under the current of laminar flow (aseptic conditions). Equipment for collecting and transferring the inoculum sterilized by the flame of a gas lamp. The inoculated jars were finally incubated in the dark, in a sufficiently ventilated cabinet. We used rice straw enriched with rice bran as a fruiting substrate according to the proportion in Table 1 below.

Table 1 Proportion of fruiting substrates

Substrate	Weight (g)	Proportion (%)
Rice haul	6000	68
Sawdust	1764	20
Rice bran	882	10
Lime	176	2
Total	8822	100

Fruiting substrates were prepared and packaged in small recyclable plastic bags. This waste consisted of paddy straw and sawdust from Gilbertiodedron dewevrei selected from the agricultural and agro-industrial waste of the city of Kisangani according to their water retention capacity. The waste was soaked in water for 24 hours, drained and fermented under a tarpaulin for at least 10 days, then composted for 30 days. The water content of the fruiting substrate was 65%.

Fruiting bundles were made by filling heat-resistant and lined plastic bags (19 x 28 cm). These bales contained 600 g of substrate, the composition of which is given in Table 1. They were then pasteurized in an autoclave for 1 h 30 min at a temperature of 120°C, and under a pressure of 1 atm. The sprouting was carried out under a laminar flow (aseptic condition), at a rate of 5% seed spawn relative to the mass of substrate.

All inoculated bags were incubated in cabinets (total darkness) at 29°C. Standardization of incubation conditions was ensured by moving the bags randomly inside the cabinet, at least twice a week until the substrate was completely invaded by the mycelium and the appearance of primordia.

The balloons with primordia are moved to a fruiting hut whose walls are made of mats and the floor is lined with fired bricks and in which the conditions favoring fruiting will be obtained, in particular low temperature, subdued light and high humidity thanks to a regular watering of the soil once or twice a day depending on climatic conditions while allowing good air circulation, in particular by maintaining a ventilation space between the wall and the roof.

Table 2 Average growth rate of mycelium on different medium for seed production

Fungi species	PDA	Seedling medium (sorghum).	Start of Mycelial growth (day)	Incubation time (day)
Lentinus squarrosulus	0,25±0,10 cm	0,63±0,17 cm	3-4	14
Pleurotus tuber-regium	0,24± 0,11 cm	0,75±0,29 cm	3-4	14

Table 3 Estimation of the duration of incubation, the emission of fruit bodies and the time between emergences

Fungi species	Mycelial growth (Average speed in cm/day)	Incubation time (day)	Sclerotium formation (day)	Sporophore emission (day)	Time between lifts
Lentinus squarrosulus	0,9±0,54 cm	25	-	3-4	4-6
Pleurotus tuber-regium	0,9±0,33 cm	27	14	7-10	30

We evaluated the average growth rate of the mycelium, in particular on the PDA, the seedling substrate as well as on the fruiting bundles. The fruiting times were calculated, the average weights of the fruit bodies of the two species were measured per sachet and per harvest. Three harvests (emergence) per sachet were carried out during the experiment.

The analysis for the comparison of means was carried out using harvest data. An ANOVA test (Analysis of variance) was used to compare the production means, a Tukey test to carry out a multiple comparison of the means.

Results and discussion

Production of the STARTER

The production of the mayor seed was carried out on the PDA medium and the estimate of the average growth rate of the mycelium on the PDA, the seedling medium (sorghum) is given in Table 2 below.

The growth of the mycelium begins three to four days after isolation in the form of a white spot. These spots begin to develop in the form of a measurable filament two days later and its average speed is slow on the PDA, 0.25 cm for the species *Lentinus squarrosulus* and after a maximum of 14 days, the tube is completely invaded by the mycelium. There is an increase in this on the seeding substrate and can reach 0.75 cm for *Pleurotus tuber-regium*. Incubation takes place in a dark but ventilated cabinet with a temperature of 27°C and lasts a maximum of 14 days.

Of all the cereals we have experimented with, sorghum is the best to use as a seedling medium, it does not stick and separates easily when the jars are shaken at the time of incubation (after every two or three days). The production of the seed takes at least a month, from the isolation of the strain, until the production of the seed itself (blank of the first generation). This process takes two months in total to produce the third generation white.

Average duration of incubation, fructification of fruit bodies and mycelial growth on the fruiting bundle

The average speed of growth on the fruiting bundle, the duration of the incubation as well as the emission of fruit bodies is given in table 3 below.

The mycelial growth on the fruiting substrate is 0.9 cm for both species, therefore higher compared to the PDA medium and the seedling substrate. The incubation period for *Lentinus squarrosulus* and *Pleurotus tuber-regium* is 25 and 27 days respectively. Unlike *Lentinus squarrosulus, Pleurotus tuber-regium* begins by producing sclerotium 14 days later before seeing the formation of primordium 7 to 10 days later. The time between emergence is 4 to 6 days for the species *Lentinus squarrosulus* when it is regularly watered while it is necessary to wait thirty days for *Pleurotus tuber-regium* before harvesting a second time, with the same watering effort and in the same fruiting condition as *Lentinus squarrosulus*. It should also be noted that, for Pleurotus tuber-regium, after formation of the sclerotium, the species fructifies when the substrate is buried after having removed the sachets and regular watering (two to three times a day depending on the climate). This is not necessarily the case for *Lentinus squarrosulus*, which fructifies on display, such as by burying the fructification substrate (gobetage).

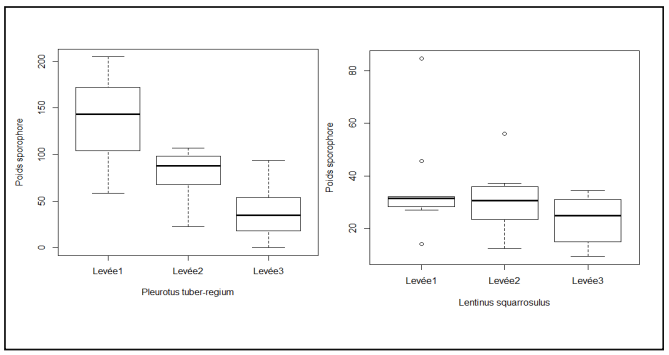


Figure 1 Average weights of fruit bodies of the different species per emergence.

Average weight of fruit bodies harvested

For the two species, we carried out three lifts in total for each species with a respective total weight of 4310g for *Pleurotus tuber-regium* and 894.22g for *Lentinus squarrosulus*, the averages of which are given in figure 1 below.

The average weights for the first lift are greater for both species but still remain higher for *Pleurotus tuber-regium* than for *Lentinus squarrosulus*. The weight decreases during different lifts and after a statistical test, the difference is significant for P. tuber-regium (p.value < 0.05), this significant difference is observed even after a multiple comparison test (Turkey). For the species *Lentinus squarrosulus*, there is no significant difference between the means.

Conclusion and suggestion

This study focused on the cultivation of two local edible fungal species *Pleurotus tuber-regium* and *Lentinus squarrosulus* from spawn produced locally. Two local strains were successfully isolated on PDA medium, then transplanted on sorghum-based seedling substrate. The production of fruit bodies was carried out on a substrate based on rice haulms mixed with sawdust of *Gilbertiodedron dewevrei* enriched with rice bran. Three harvests (lifts) were carried out during which 4310g of *Pleurotus tuber-regium* sporophore and 894.22g of *Lentinus squarrosulus* were harvested. This study gives the possibility of producing local mushrooms from spawn produced locally. It constitutes a means for the valorization of local species as well as a possibility of producing spawn locally for the benefit of the urban and peasant population in the city of Kisangani.

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Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. De Kesel A, Malaisse F, Edible Wild food: Fungi. In: Malaisse F, *How to live and survive in Zambezian Open Forest (Miombo Ecoregion)*. Gembloux (Belgium): Presses Agronomiques de Gembloux; 2010. 41-56 p.
2. Oghenekaro E, Akpaja O, Samuel JO. Continental J Applied Sciences. 2010;5:25–30.
3. Oei P. *Mushroom cultivation; appropriate technology for Mushroom growers*. 3rd Edn. Leiden, The Netherlands: Backhuys Publishers; 2003. 429 p.
4. De Kesel A, Kasongo B, Degreef J. Guide des Champignons Comestibles du Haut Katanga (RD Congo). *Abc Taxa*. 2017;17:290.
5. Diansambu MI, Dibaluka MS, Lumande KJ, et al. Culture des trois espèces fongiques sauvages comestibles du Groupement de Kisantu (RD Congo) sur des substrats ligno-cellulosiques compostés. *Afrique SCIENCE*. 2015;11(3):241-261.
6. Conférence régionale des élus (CRÉ). Culture de champignons sur billots et souches. Guide technique. Gaspésie- Îles-de-la-Madelaine; 2008. 23 p.
7. De Kesel A, Codjia, Jean TC. Guide des Champignons comestibles du Benin, Bibliothèque Nationale (BN). République du Bénin; 2002. 274 p.
8. Ndamiyehe C. Utilisation de données de télédétection 3D acquises par drone pour la caractérisation des conditions de croissance dans un dispositif de suivi de la dy-namique forestière (Yoko) en République démocratique du Congo. Mémoire Master, Unikis ; 2016. 70 p.

9. Dibaluka MS. Etude des macromycètes de la cité de Kimvula et de ses environs (Bas Congo/RDCongo): Diversité et Productivité en forêt claire, ethno mycologie etmiseen cultured'espècessa protrophes comestibles. Thèse de doctorat inédite Fac DesSc Universitéde Kinshasa; 2012. 468 p.
10. Adisco. Culture de champignon Pleurotes à petite échelle; 2007. 4-23 p
11. Awono A, Manirakiza D, Henri Owona H. Etude de base de la filière miel, Gnetum et *Dacryodes edulis* dans les provinces du Bas Congo et de Kinshasa (RDC). GCP/RAF/408/EC, Mobilisation et Renforcement des capacités des petites et moyennes entreprises impliquées dans les filières des produits forestiers non ligneux en Afrique centrale. CIFOR; 2008.
12. Degreef J, De Kesel A. The Edible Fungi of Tropical Africa annotated database; 2017.
13. Dibaluka MS, Lukoki FL, De Kesel A, et al. Essais de culture de quelques champignons lignicoles comestibles de la région de Kinshasa (R.D. Congo) sur divers substrats lignocellulosiques. *Biotechnol Agron Soc Environ*. 2010;45(3):417-422.
14. Dibaluka MS. Inventaire des macromycètes de la forêt de lac de Ma Vallée (Kinshasa) et essai de mise en culture des quelques espèces comestibles. Mémoire (DEA) inédit, Département de Biologie, Faculté des Sciences, Université de Kinshasa; 2005. 75 p.
15. Eyi Ndong H, Jerome D, De Kesel A. Champignons comestibles des forêts denses d'Afrique centrale, Taxonomie et identification. *Abc Taxa*. 2011;24(2):149-156.
16. FAO. Champignons comestibles sauvages. Vue d'ensemble sur leurs utilisations et leur importance pour les populations. Viale delle Terme di caracalla 00100 Rome, Italie; 2006. 170 p.
17. Isikhuemhen OS, Moncalvo JM, Nenid F, et al. Mating compatibility and phylogeography in *Pleurotos tuberregium*. *Mycological Research*. 2000;104(6):732-737.
18. Kahindo M. *Inventaire des Produits Forestiers végétaux non ligneux et leur commercialisation dans la ville de Kisangani* (RD congo). Mémoire DES innédit, Fac/SC, UNIKIS; 2007. 113 p.
19. Mushagalusa GN, Mondo JM, Masangu GB, et al. Effets de doses croissantes d'additifs sur la productivité de deux souches de Pleurotus (Jacq) P.Kumm. Sous la technique de gobetage et sur substrats locaux en R.D du Congo. *Tropicultura*. 2017;35(2):102–109.
20. Nyakabwa M. *Phytocénose de l'écosystème urbain de Kisangani*. Thèse de doctorat Inédit Université de Kisangani; 1882. 418 p.
21. Oei P. La culture des champignons à petite échelle (Pleurotes, Shütakes et Auricularia). Wageningen. Pays-Bas: Fondation Agromisa, CTA; 2005. 86 p.
22. Oei P. *Mashrooms cultivation with special emphasis on appropriate techniques for developing countries*. Tool Publications Leidens: The Netherlands ; 1996. 274 p.
23. Oei P. La culture des champignons. Guide technique. Amsterdam, Pays–Bas: CTA, TOOL, FGRET ; 1993. 318 p.
24. Okhuoya JA, Isikhuemhen OS. *Mushroom cultivation: The Nigerian Experience*. In. A. Broderick, T. Nair. (Eds.) Proceedings of the 3rd International conference on Mushroom Biology and Mushroom Products & AMGA's 26th National Mushroom Industry Conference: Sydney; 1999. 380-389 p.
25. Okhuoya JA, Isikhuemhen OS, Evue GA. *Pleurotus tuberregium* (Fr.) Sing.: Sclerotia and sporophore yield during cultivation on sawdust of different woody plañís. *The International Journal of Mushroom Sciences*. 1998;2(2):41-46.
26. Oso BA. *Pleurotus tuberregium* from Nigeria. *Mycologia*. 1977;69(2):272-279.
27. Rammeloo J, Walleyn R. The edible fungi of Africa south of the Sahara: a literature survey. *Scripta Botanica Belgica*. 1993;5:1-62.
28. Toirambe B. Analyse de l'état de lieux du secteur Produits forestiers non ligneux et leur évaluation à la contribution de la sécurité alimentaire en République Démocratique du Congo. Projet FAO GCO/RAF/398/GER-Renforcement de la sécurité alimentaire en Afrique Centrale à travers la gestion durable des produits forestiers non ligneux. Union Mondiale pour la Nature UICN ; 2006. 92 p.
29. Wasseige D, Devers R, de Marcken R, et al. Les forêts du bassin du Congo: état des Forêts 2008. Luxembourg, Office des publications de l'Union européenne; 2009. 426 p.
30. Zoberi MH. Some edible mushrooms from Nigeria. *Nigerian Field*. 1973;38:81-90.