Cultivation of *Lentinus squarrosulus* Mont. and *Lentinus sajor-caju* Fr. on agroforestry wastes under field conditions

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Abstract
Lentinus sajor-caju and L. squarrosulus are two important wild edible saprotrophic mushrooms used in tropical Africa. However, they have not yet been commercially cultivated and their uses depend largely on the environmental conditions. In order to improve the management of agricultural wastes and contribute to insure food security in Cameroon, a study was performed to assess the production of these mushrooms on different agroforestry wastes in rural farm conditions during the dry season.

Fragments of young wild sporophores were used to produce the spawns used to cultivate fruiting bodies on corn cobs, coffee parchment, rice husk, sawdust and mixture of wood sawdust with rice husks. For spawn production, mycelia growth was successful on all substrates with wood sawdust having the highest volume, colonizing 841.21 cm^3 and 836.78 cm^3 for L. squarrosulus and L. sajor-caju respectively after 30 days. Fruiting of the mushrooms started 94 days after inoculation. The best biological efficiency was obtained on corn cobs and sawdust for L. sajor-caju and L. squarrosulus respectively. This study supported the fact that native edible Lentinus can be produced in farm condition mainly on wood base substrate.

Keywords: Edible mushrooms, organic wastes, spawns of production, fruiting bodies, mushroom farm.

Introduction

Edible mushrooms are part of Non-Timber Forest Products (NTFP) exploited as food or medicine. Hence, they are nutritionally, socially and economically important organisms for local population especially in tropical Africa.[4,5,14] In the central Africa region, there are 300 species of edible mushrooms documented. They include ectomycorrhizal, termite associated and saprotrophic species.[9]

The saprotrophic species include *Lentinus squarrosulus* and *L. sajor-caju* that are among the most popular edible and medicinal species in the tropical areas (Africa, Asia and South America) especially in Cameroon where they are well appreciated by locals in both forest and savanna areas where they are used as food or medicine.[8,15] These species have great nutritional value and possess some putative pharmaceutical properties.[1,11,16,17,20]

Despite the socio-economic potentials of these fungi, they are not yet commercially produced and locals still largely depend on the natural environment to obtain their fruiting bodies that grow seasonally and intermittently during the rainy season on dead wood. To make them available throughout the year, it is necessary to develop techniques of their cultivation not only in laboratory but mainly in field condition. This technique should also take into consideration suitable substrates derived from agro-forestry waste.

Many attempts of cultivation of these species have been done both in Africa and Asia; these include, works of Adejoye et al[2], Adesina et al[3], Diansambu et al[6], Dibaluka et al[7], Hussein et al[12] and that of Kupradi et al.[13] Most of these works focus mainly on the production of mycelia and fruiting bodies in laboratory conditions that are generally not accessible to local farmers working in rural environment. The aim of this study was to evaluate the effect of some common local agroforestry wastes from the western savanna highlands of Cameroon on the production process of *Lentinus squarrosulus* and *L. sajor-caju* mushroom under field conditions.

Material and Methods

Study area: This study was conducted at the Mbeng Adio Mushroom Farm situated at Banjah village around Bamenda town in the North-West region of Cameroon. The climate is tropical with a dry season from November to March and a rainy season from March to November with an average annual rainfall of 2145 mm/year and average temperature of 21.5°C. Materials used in the Mbeng Adio Mushroom Farm for the cultivation process are constituted mainly of locally available materials and recuperated used materials such as empty glass bottles with large opening (fig. 1) for spawn production.

Source of strains and pure culture: The fruiting body of *L. squarrosulus* was collected from Koumemgba (Noun division, West Region, Cameroon) on stump of *Mangifera indica* L. and that of *L. sajor-caju* from a stem of *Polyscias fulva* (Hiern) Harms in the University of Bamenda campus in Bambili (Mezam division, North-West region, Cameroon). The collected samples were identified based on their morphological characteristics according to Njouonkou

et al[15] and the mycelia cultured on potato dextrose agar (PDA). The mycelia obtained directly from the fruiting bodies was purified through a series of three replications on PDA.

Substrate origin and composition: The different substrates used were pounded corn cobs collected from IRAD (Institute of Agricultural Research for Development) Bambui station, wood sawdust from carpenter workshops in Bamenda, coffee parchment wastes collected from a coffee transformation factory in Malentouen and rice husks from Ndop rice company. The composition of each substrate is presented in table 1.

Spawn production: Two types of spawns were prepared, the grain spawn that was multiplied to produce production spawn on the various substrate mentioned in table 1. Hence, 5 Kg of healthy corn grains were soaked for 24 hours with tap water, then boiled for 15 minutes and drained. They were mixed with 150 g of lime and 22 g of sugar dissolved in 100 ml of distilled water and the mixture was homogenized. These grains were distributed in 10 cleaned 1 litter glass bottles filled to ¾ full and protected with a cover perforated at the center and secured with cotton plug and and covered with used paper to protect cotton. They were sterilized using a pressure pot for 1 hour at 1 atm and 120°C.

After cooling down, the bottles were transferred into clean sterilized inoculation boxes where mycelia plugs of both species from PDA media were aseptically inoculated on the grains. They were incubated at room temperature (20°C – 25°C) in the dark for mycelial growth for 30 days during which they were stirred every three days according to Dibaluka et al.[7]

For the production spawn, each substrate was prepared with ingredients shown in table 1. The mixture of each substrate was then introduced in 10 cleaned 1 liter glass bottles and proceeded for sterilization as for grain spawn. They were sterilized in double bottom drum on fire for 4 to 5 hours. After cooling, each bottle was inoculated at 10% with mycelia from grain spawn and incubated in the dark at room temperature for 35 days during which they were examined every 5 days to control mycelia growth and contamination. To evaluate the mycelia growth, the circumference of substrate colonized was measured every 5 days using a graduated ruler and the volume (V) of substrate colonized calculated using the following formula:

$$V = h \times \frac{\pi d^2}{4}$$

where V = volume of colonization (cm^3), h = height of mycelia evolution (cm) and d = diameter of the bottle (cm).

Production of fruiting bodies: This experiment started from the month of November 2016 at the beginning of the dry season using the different substrates mentioned in table 1; for each substrate, 5 black polypropylene bags were filled with 1200 g of substrate each and the bags were sterilized as mentioned above. After cooling, each bag was inoculated with 300g of spawn produced on corn cobs. They were then incubated in the dark for 60 days at room temperature varying between 21 and 30°C. After 60 days, the bags were exposed in moderated light intensity by opening windows that were previously protected with white net; this also allows ventilation of the room. Sterilized tap water was used to water the bags every 2 to 3 days until appearance of the first mushroom buttons. To allow the easy rise up of sporophores, the upper surface of the bags was scarified with clean blade.

The following parameters were assessed: incubation period i.e. the duration between inoculation and the appearance of the first fruiting bodies, the number and average weight of sporophores and the efficiency. The efficiency was calculated using the formula below.

$$\text{Efficiency} = \frac{\text{Wfm}}{\text{Wsp} + \text{Wsb}} \times 100$$

where Wfm = weight of fresh mushroom produced, Wsp = Weight of spawn and Wsb = Weight of substrate.

Results and Discussion

Mycelia of *Lentinus sajor-caju* and *L. squarrosulus* were successfully obtained on PDA medium and grain spawn on corn. The different substrates were gradually colonized by mycelia without significant difference of the speed of colonization (fig. 1). After 30 days, the best mycelia growth was obtained on wood sawdust with respectively 841.23 cm^3 and 836.78 cm^3 colonized by *L. squarrosulus* and *L. sajor-caju*. The lowest growth was recorded on corn cobs for *L. squarrosulus* and rice husk for *L. sajor-caju*. For coffee parchment and wood sawdust, the volumes colonized by mycelia of both species were more or less similar. *L. sajor-caju* had the highest growth on corn cob while *L. squarrosulus* had it on rice husk and on the mixture of rice husk and wood sawdust (fig. 2).

Also, according to these results, mixture of rice husk and wood sawdust increases the mycelia growth compared to its growth on rice husk alone. Adesina et al[3] found that rice bran as supplement increases the mycelia growth of *L. squarrosulus*. Hence according to our results, rice husk in adequate quantity in wood sawdust could upgrade mycelia growth of mushrooms. According to these results, wood sawdust is the best substrate for mycelia propagation of the two species. This can be due to the fact that these species are lignicolous and can grow better on wood that is their natural substrate.

Fruit bodies production on substrate: Table 2 gives some parameters of fruit bodies production on the various substrate. No sporophores were produced on rice husk and coffee parchment for the two species and only *L. sajor-caju*

was produced on corn cobs. The fruit bodies of both species were produced on wood sawdust and mixture of wood sawdust with rice husk (fig. 3). Coffee parchment and rice husk seem to be inappropriate substrate for the *Lentinus* production. Both have poor water retention capacities especially during the dry season and are poor in lignin; however these 2 waste products have been successfully used in production of *Pleurotus ostreatus* (Jacq.) P. Kumm.[18,19]

The incubation period varies from 94 days for *L. sajor-caju* on corn cobs and 108 days for *L. squarrosulus* on wood sawdust mixed with rice husk. The incubation period in this study was longer compared to the time obtained by Adesina et al[3] who got sporophores of *L. squarrosulus* on logs of *Spondia mombin* L. and *Citrus sinensis* (L.) Osbeck 28 days after inoculation of spawn. The longer period here could be attributed to the atmospheric condition of the dry season that is not favorable to fructification of many mushroom species.

For *L. sajor-caju*, the number of sporophores varies from 8 on corn cobs to 54 on the mixture of wood sawdust and rice husk. The average weight varies from 5.25 g/fruiting body on corn cobs to 10.56g /fruiting body on wood sawdust. *L. squarrosulus* produced 46 sporophores of average weight of 8.19 g/fruiting body on the mixture of wood sawdust and rice husk. On wood sawdust, it produced 61 basidiomata with average mass of 10.11 g/fruiting body. The biological efficiency varies from 0.56 % to 8.22 % for *L. sajor-caju* on corn cobs and *L. squarrosulus* on wood sawdust respectively.

Table 1
Composition of different substrates

Type	**Composition**	**Observations**
Maize cob	- 4 kg, of pounded corn cob; - 200 g of lime, - 800 g of maize flour; - 1 l of tap water for humidification.	For spawn production
	- 9.2 kg of pounded corn cob; - 300 g of lime, - 500 g of maize flour; - 2.5 l tap water for humidification	For fruit bodies production
Coffee parchment wastes	- 3 kg coffee parchment, - 250 g lime, - 400 g maize flour.	For spawn and For fruit bodies production; Humidified with tap water
Rice husk	- 4 kg rice husks, - 300 g lime, - 700 g maize flour.	
Wood sawdust	- 8 kg wood, - 500 g lime, - 1500 g maize flour.	
Wood sawdust and Rice husk	- 6 kg wood sawdust, - 2 kg rice husks, - 500 g lime, - 1500 g maize flour	

Table 2
Parameters of fruiting bodies production on various substrates

Species	**Substrate**	**duration of incubation**	**Number of fruit bodies**	**Average weight of fruit bodies**	**Biological efficiency**
L. sajor-caju	Coffee parchment	NG	NG	NG	NG
	Rice husk	NG	NG	NG	NG
	Corn cobs	94	8	5.25	0.56
	Wood sawdust	104	31	10.56	4.34
	WSD + RH	108	54	10.46	7.53
L. squarrosulus	Coffee parchment	NG	NG	NG	NG
	Rice husk	NG	NG	NG	NG
	Corn cobs	NG	NG	NG	NG
	Wood sawdust	101	61	10.11	8.22
	WSD + RH	103	46	8.15	5

NG: No growth; WSD: Wood sawdust; RH: Rice husk

Fig. 1: Spawn production with mycelia growing on substrate; a. Mycelia of *L. squarrosulus* on corn cobs, b. Mycelia of *L. sajor caju* on rice husk.

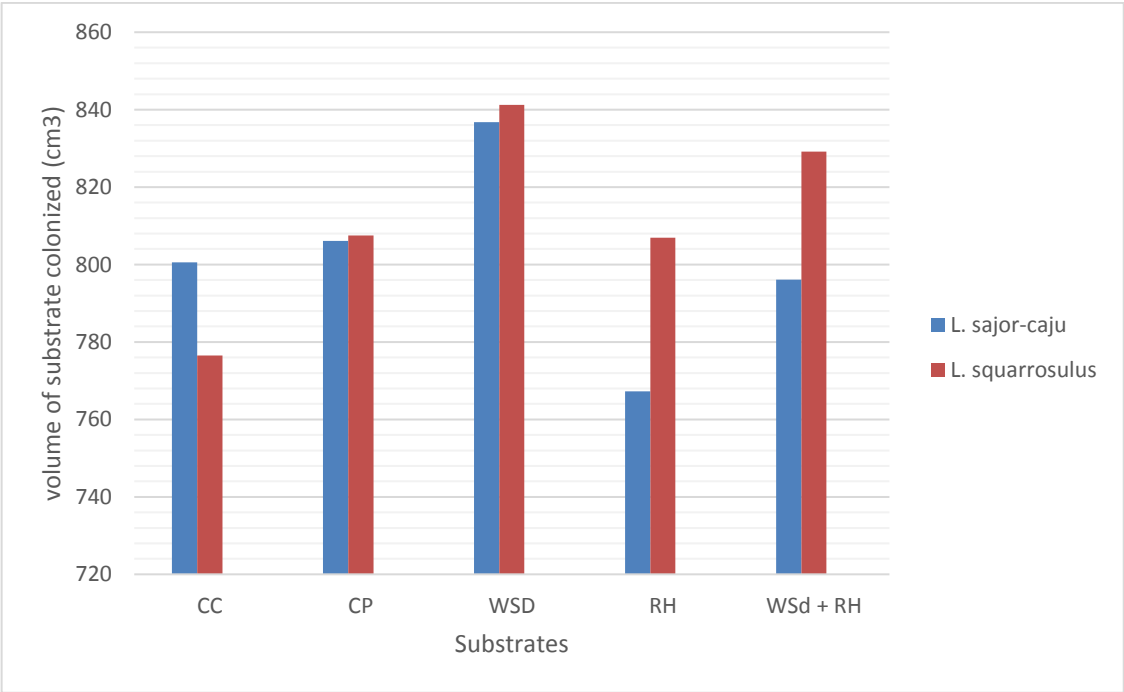

Fig. 2: Volume of substrate colonized after 30 days. (CC: Corn Cobs; CP: Coffee Parchment; WSD: Wood Sawdust; RH: Rice Husk; WSD + RH: Wood Sawdust and Rice Husk)

Globally the efficiency was low compared to the results of Diasambu et al[6] and Dibaluka et al[7] who obtained respectively 12 % and 20 % biological efficiency with *L. squarrosulus* on the wood sawdust of various local trees in laboratory conditions. However, they are more or less similar with the results of Adesina et al[3] who obtained efficiencies of 10.25 % and 5.85 % for *L. squarrosulus* cultivated on *S. mombin* and *C. sinensis* logs respectively, both supplemented with rice bran. In general, wood sawdust and mixture of wood sawdust and rice husk are the best substrates for the production of basidiomata of *L. squerrosulus* and *L. sajor-caju*.

As mentioned above, these mushrooms are wood inhabiting species; therefore, wood-based substrates are more suitable for their production. Nevertheless, supplementation of wood by some agricultural waste like rice husk could increase the efficiency and the quality of the production as Frimpong-Manso et al[10] found for *Pleurotus ostreatus*.

Fig. 3: Fruiting bodies growing on substrates: a. *Lentinus sajor-caju*; b. *Lentinus squarrosulus*

Conclusion

This study shows that *Lentinus sajor-caju* and *L. squarrosulus* can be cultivated under field conditions in tropical Africa even in the dry season. Wood sawdust supplemented with rice husk and wood sawdust are respectively the most efficient substrates for their cultivation.

However, studies have to be extended to other agroforestry waste products available in Cameroon for their efficiency in cultivation of *Lentinus* and mushroom in general. Also, in addition to substrates, studies are needed to find the best environmental conditions for qualitative and quantitative upgrading of their production.

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References

1. Acharya K., Ghosh S. and Saha T., Physicochemical characterization and antioxidant property of powdered basidiocarp of wild *Lentinus sajor-caju*, *International Journal of Chem Tech Research*, **10(1)**, 126-132 **(2017)**

2. Adejoye O. and Mesewonrun O., Growth and yield of *Lentinus squarrosulus* (M.) Singer a Nigerian edible mushroom as affected by supplements, *The Internet Journal of Nutrition and Wellness*, **8(2)**, 1-4 **(2008)**

3. Adesina F.C., Fasidi I.O. and Adenipekun O.C., Cultivation and fruit body production of *Lentinus squarrosulus* Mont. (Singer) on bark and leaves of fruit trees supplemented with agricultural waste, *African Journal of Biotechnology*, **10(22)**, 4608-4611 **(2011)**

4. Ayodele S.M., Akpaja E.O. and Adamu Y., Some edible medicinal mushrooms of Igala L and in Nigeria, their sociocultural and ethnomycological uses, *International Journal of Science Nature*, **2**, 473-476 **(2011)**

5. Boa E., Champignons comestibles sauvages. Vue d'ensemble sur leurs utilisations et leur importance pour les populations, *Produits Forestiers Non Ligneux*, **17**, 157 **(2006)**

6. Diansambu I.M., Dibaluka M.S., Lumande J.K. and Degreef J., Culture de trois espèces fongiques sauvages comestibles du Groupement de Kisantu (R.D. Congo) sur des substrats ligno-cellulosiques compostés, *Afrique Science*, **11(3)**, 241-261 **(2015)**

7. Dibaluka S.M., Lukoki F.L., De Kesel A. and Degreef J., Essais de culture de quelques champignons lignicoles comestibles de la région de Kinshasa (R.D. Congo) sur divers substrats lignocellulosiques, *Biotechnological Agronomic Social and Environment*, **14(3)**, 417-422 **(2010)**

8. Dijk H.V., Onguéné A.N. and Kuyper T.W., Knowledge and Utilization of Edible Mushrooms by Local Populations of the Rain Forest of South Cameroon, *Ambio*, **32(1)**, 19-23 **(2003)**

9. Eyi Ndong H.C., Degreef J. and De Kessel A., Champignons comestibles des forêts denses d'Afrique centrale Taxonomie et Identification, *Abc Taxa*, **10**, 262 **(2011)**

10. Frimpong–Manso J., Obodai M., Dzomeku M. and Apertorgbor M.M., Influence of rice husk on biological efficiency and nutrient content of *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer, *International Food Research Journal*, **18**, 249-254 **(2011)**

11. Gulati A., Atri N.S., Sharma S.K. and Sharma B.M., Nutritional Studies on Five Wild *Lentinus* Species from North-West India, *World Journal of Dairy & Food Sciences*, **6(2)**, 140-145 **(2011)**

12. Hussein J.M., Tibuhwa D.D., Mshandete A.M. and Kivaisi A.K., Successful domestication of *Lentinus sajor-caju* from an indigenous forest in Tanzania, *Journal of Applied Biosciences*, **108**, 10500-10506 **(2016)**

13. Kupradi C., Khongla C., Musika S., Ranok A., Tamaruay K., Woraratphoka J. and Mangkalanan S., Cultivation of *Lentinus squarrosulus* and *Pleurotus ostreatus* on Cassava Bagasse Based

Substrates, *International Journal of Agricultural Technology*, **13(6)**, 883-892 **(2017)**

14. Ndoye O., Awono A., Preece L. and Toirambe B., Marchés des produits forestiers non ligneux dans les provinces de l'Équateur et de Bandundu: présentation d'une enquête de terrain. *In* Quel avenir pour les forêts de la République démocratique du Congo?, Bruxelles, 68-70 **(2007)**

15. Njouonkou A.L., De Crop E., Mbenmoun M.A., Kinge T.R., Biyé E.H. and Verbeken A., Diversity of Edible and Medicinal Mushrooms Used in the Noun Division of the West Region of Cameroon, *Int. J. Med. Mushrooms*, **18(5)**, 387-396 **(2016)**

16. Omar N.A.M., Abdullah S., Abdullah N., Kuppusamy U.R., Abdulla M.A. and Sabaratnam V., *Lentinus squarrosulus* (Mont.) mycelium enhanced antioxidant status in rat model, *Drug Des. Devel. Ther.*, **9**, 5957-5964 **(2015)**

17. Sharma S.K. and Atri N.S., Nutraceutical composition of wild species of genus *Lentinus* Fr. from Nothern India, *Current Research in Environmental & Applied Mycology*, **4(1)**, 11-32 **(2014)**

18. Shimelis A., Optimization of coffee wastes for the cultivation of *Pleurotus ostreatus*, Master thesis School of Graduate Studies of Addis Ababa University, Addis Ababa, 48 **(2011)**

19. Thongklang N. and Luangharn T., Testing agricultural wastes for the production of *Pleurotus ostreatus*, *Mycosphere*, **7(6)**, 766-772 **(2016)**

20. Usha N., Shashikanth S. and Janardhana G.R., Structural elucidation of novel bioactive compound (2-chloro-1-(2-chlorocyclopropyl)-2-(4-nitrophenyl)ethanone) from basidiomycetous fungus *Lentinus squarrosulus*, *International Journal of Pharmacological Research*, **6(2)**, 62-67 **(2016)**.

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