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Cultivation of an Edible Mushroom Species, *Pleurotus sajor – caju* (Fr.) Singer (1951) (*Pleurotaceae*) (strain 221114) on Various Lignocellulosic Substrates

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Abstract

The current food crisis and the continuing deterioration in the health of the populations of the DRC in general, and those of the province of Kongo Central in particular, are among the causes of severe pressure on natural environments, especially forests. In the long term, these risks creating scarcity or the disappearance of certain resources, including mushrooms. The main aim of this study is to contribute to the promotion of NTFPs in the fight against hunger and food insecurity through the cultivation of strain 221114 of the species *Pleurotus sajor-caju*, making sporophores available on a regular basis. To achieve this objective, we set ourselves the specific goals of determining the mycelial growth rate and comparing the yields obtained from strain 221114 of the species *Pleurotus sajor-caju* on fruiting substrates. The strain used in the culture was isolated in the Ngeba area, as shown in Figure 1 below, in the province of Central Kongo. The preparation of seedling media and the production of seedling whites, the preparation of final substrates, the addition of nutrients, incubation, fruit induction, and obtaining and harvesting sporophores were carried out at the Systematic Mycology and Myciculture Laboratory of the Life Sciences Department, Faculty of Science and Technology, University of Kinshasa, in room B28 and the department's experimental garden. The fungal material used was strain 221114 of *Pleurotus sajor – caju*. The plant material for the substrate consisted of rice straw, banana leaves, wood chips, sugarcane bagasse, and sawdust. Wheat bran and slaked lime were used as additives. The isolation method from tissue or tissue culture (known as monoculture) was used to start the culture. The best average sporophore yields were 35.01 ± 3.7% on rice straw, 28.67 ± 4% on wood chips, and 23.3 ± 2.4% on sugarcane bagasse, yields well above the theoretical yield of 20% at which a substrate is considered best and suitable for sporophore production.

Keywords: Culture, Substrate; *Pleurotus sajor-caju*; Growth; Yield.

INTRODUCTION

Today, given the socio-economic realities of various developing countries (insufficient or even non-existent income, scarcity of employment, chronic food insecurity, etc.), including the Democratic Republic of Congo (DRC), the population has developed a variety of lucrative activities, including the exploitation of NTFPs (Biloso & Lejoly, 2006). The current food crisis and the continuing deterioration in the health of the populations of the DRC in general, and those of the province of Kongo Central in particular, are among the causes of severe pressure on natural environments, especially forests. In the long term, this pressure risks creating scarcity or the disappearance of certain resources, including mushrooms (Biloso, 2008). However, in Africa, mushrooms are non-timber forest products of paramount importance, both nutritionally and economically (Ndoye et *al*., 2007). Studies on edible mushrooms in Africa, and particularly in the Democratic Republic of Congo, have often been the work of pioneers, with more than 300 species catalogued to date (Boa, 2006; Eyi Ndong et *al*., 2011).

These systematic studies are gradually building up an inventory of the diversity of edible mushrooms, but very few wild strains have been cultivated to date, particularly in Central Africa (Dibaluka et *al*., 2010; Diansambu et al., 2015; Batubenga et *al.*, 2021b, Mwinyi et *al*., 2021).

Many species of wild mushrooms are harvested as their sporophores appear in their natural environment during the rainy season (Mwinyi et *al*., 2021). However, the seasonality of sporophore appearance is therefore a limiting factor for their availability, which is often unpredictable and even limited to a few weeks per year in certain regions, mainly during the rainy season (Batubenga, 2020). Very few species are known and consumed fresh in large urban areas such as the city of Kinshasa, where they are often poorly preserved (moldy) or poorly presented (lots of debris and sand in the sporophores), with the risk of poisoning consumers if a

poisonous specimen is inadvertently included (Batubenga et *al*., 2021a). The cultivation of edible mushrooms provides populations with food that is safe to consume in all seasons and yields a high production per cultivated area (Dibaluka, 2005).

As a result, mushroom cultivation is proving to be a profitable activity for African farmers, as it provides fresh produce during periods of drought and outside of harvest seasons that cannot be competed with by wild mushrooms, which are then unavailable in the forest, while also transforming agricultural waste into high-quality dietary protein.

The cultivation protocol presented is based on the techniques recommended by Oei (1993; 2003), which have been implemented for more than ten years using local strains of edible lignicolous mushrooms. It makes it possible to recycle various types of lignocellulosic waste and has the advantage of being easily transposed to a farming environment without requiring significant financial investment.

In the province of Central Kongo, particularly in the Ngeba area, several species of mushrooms are found, each with its own specific uses, apart from its gastronomic value.

The main objective of this study is to contribute to the promotion of NTFPs in the fight against hunger and food insecurity through the cultivation of *Pleurotus sajor – caju* strain 221114 by making sporophores available on a regular basis. To achieve this goal, we set ourselves the specific objectives of determining the mycelial growth rate and comparing the yields obtained from strain 221114 of the species *Pleurotus sajor – caju* on fruiting substrates.

The hypothesis of this study is that the mycelial growth and sporophore yield of *Pleurotus sajor – caju* strain 221114 vary significantly depending on the nature of the lignocellulosic substrate used.

Study Environment

The strain was isolated in the Ngeba area, as shown in Figure 1 below, in the province of Central Kongo, Democratic Republic of Congo, on National Road No. 16 (R.N.16). The Ngeba area is located between 04°9' south latitude and 15°2' east longitude. Its average altitude is 210 meters, more precisely at the Kisantu Botanical Garden. The preparation of seedling media and the obtaining of seedling whites, the preparation of final substrates, the larding, incubation, fruiting induction, and the obtaining and harvesting of sporophores were carried out at the Laboratory of Systematic Mycology and Myciculture located at the University of Kinshasa, Faculty of Science and Technology, Life Sciences Department, in the city province of Kinshasa.

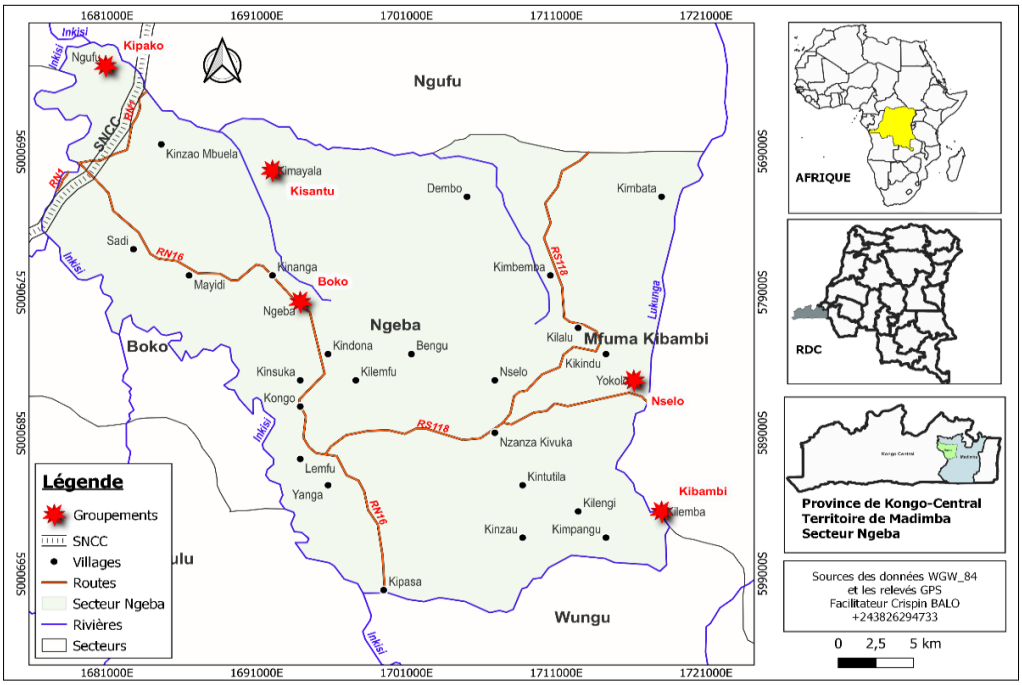

Figure 1. Map of Central Kongo Province, showing the Ngeba area. Source: shp DRC, coordinate system. Dabum: WGS 84. Arc GIS version 10.5.

MATERIAL

The fungal material used in this study consists of strain Psa 221114 of the *species Pleurotus sajor – caju* (see Figure 2). The strain was identified based on the morphological characteristics of the sporophores according to the criteria described by Eyi Ndong et al.

(2011). No molecular analysis was performed to confirm the identification of this strain.

The spawn was obtained from corn kernels, rice (paddy), and sawdust. We used rice straw, banana leaves, sugarcane bagasse, wood chips, and sawdust as fruiting substrates, as illustrated below. Wheat bran and slaked lime were used as additives to the fruiting substrate in the different proportions indicated in Table 1 below.

Figure 2. Espèce *Pleurotus sajor – caju.*

Figure 3. Plant material used as substrate. A. Rice straw (*Oryza sativa* L); B. Banana leaves (*Musa sp*). C. Wood chips; D. Sawdust; E. Sugarcane bagasse. (Source: Batubenga, 2022).

Table 1. Proportions of ingredients in five final substrate treatments.

Traitement	Ingrédients	Proportion (en %)	Teneur en eau (%)
T_1	Rice straw	80	
	Wheat bran	18	65
	Slaked lime	2	
T_2	Banana leaves	80	
	Wheat bran	18	63
	Slaked lime	2	
T_3	Wood chips	80	
	Wheat bran	18	68
	Slaked lime	2	
T_4	Sugarcane bagasse	80	
	Wheat bran	18	71
	Slaked lime	2	
T_5	Sawdust	80	
	Wheat bran	18	60
	Slaked lime	2	

Legend: S1: Final substrate based on rice straw; S2: Final substrate based on banana leaves; S3: Final substrate based on wood chips; S4: Final substrate based on sugarcane bagasse; S5: Final substrate based on sawdust.

METHODS

The isolation method from tissue or tissue culture (known as monoculture) was used to start the culture. PDA (Potato Dextrose Agar) agar medium was used as a substrate for the mycelium to obtain the mother or starter culture, prepared according to the formula proposed by the Mycothèque de l'Université Catholique de Louvain (MUCL) and reproduced by Batubenga et al., 2021b; Dibaluka (1999, 2010); Diansambu, 2015), which gives the proportions of the ingredients as follows: 200 g of peeled sweet potato; 20 g of dextrose and agar-agar in 1000 ml of demineralized water. We used a quarter of the above quantities, i.e., 50g of sweet potato, 5g of dextrose, and 5g of agar-agar in 250ml of distilled water in a 500ml Erlenmeyer flask. The seed whites (mother white and final white) were produced on corn kernels, paddy rice, and sawdust. Wheat bran and slaked lime were used as additives, referring to the technique developed by Dibaluka et al. (2010) and Batubenga et al. (2021b).

To obtain fruiting cultures, five final substrates were used, enriched with 20% additives: wheat bran and slaked lime. The first is made from rice straw (Oryza sativa L), the second from banana leaves (Musa sp), the third is made from wood chips, the fourth from sugarcane bagasse (Saccharum officinarum L), and the fifth from sawdust.

The straw and banana leaves were cut into small pieces of about 3 to 4 cm using a machete, then ground using a locally made cassava husk grinder, then soaked in tap water for 24 hours, as were the wood chips, sugarcane bagasse, and sawdust. After removing them from the water and draining them for 24 hours, they were then mixed with the additives (slaked lime and wheat bran) according to the different proportions indicated in Table 1 below.

The mixture obtained for each treatment was placed in plastic bags measuring 29 cm long and 18 cm wide, which we doubled up to increase their resistance to sterilization heat. These bags were filled with 500 g of substrate. The bags were closed with a foam plug secured using a plastic ring approximately 2.5 cm in diameter and 2 cm high, giving the bag a neck shape.

The sealed bags were placed in an autoclave for sterilization. Sterilization was carried out at 121°C for 1 hour under a pressure of approximately 1 bar. After sterilization, we left them to cool before larding. The inoculation was carried out under aseptic conditions in an inoculation box, next to the flame of an alcohol lamp, using a tablespoon, using the seed (seedling white) on sawdust at a rate of 50 grams, or 10% of the weight of the substrate (as illustrated in Figure 4). After inoculation, incubation was carried out in the dark, in a ventilated cabinet, at room temperature until the fruiting substrate was completely colonized by the mycelium and primordia appeared.

Figure 4. Lardage performed on the bundles, next to the flame of an alcohol lamp. (Source: photo Batubenga, 2023).

The sporophore yield was calculated using the formula proposed by Oei (1993) as follows:

$$Rendement\ (\%) = \frac{PFS}{PS} \times 100\ (Rd \geq 20\ \%)$$

Where:
FWS = Fresh weight of sporophores (in g); WSS = Wet substrate weight (in g).

If the Yield (Yield) is less than (<) the consensus yield of 20% after harvesting three flushes, the substrate cannot be used efficiently for the production of a fungal species. If the Yield (Yield) is greater than or equal to (≥) the consensus yield of 20% after harvesting three crops, the substrate can be used efficiently for the production of a fungal species.

Each experimental treatment included five independent replicates corresponding to five bags of substrate.

Incubation was carried out at a temperature of approximately 25 to 28°C, in room B28, in a ventilated cabinet and in the dark until the substrate was completely colonized by the mycelium. Relative humidity was not controlled and constitutes a limitation of the study.

Statistical analyses were performed using one-way analysis of variance (ANOVA) to compare the average yields obtained on different substrates, followed by Tukey's test for multiple comparisons. The significance threshold was set at 5%.

RESULTS AND DISCUSSION

Phenology of sporophore emergence on fruiting substrates

The growth of mycelium from *Pleurotus sajor – caju* strain 221114 was noticeable on the second day of inoculation on all substrates. Total invasion of the

substrate by the mycelium was observed from the 18th to the 24th day on rice straw, banana leaves, and wood chips, from the 23rd to the 27th day on sugarcane bagasse, and from the 30th to the 31st day on sawdust. Fruit buds appeared on the 29th day on rice straw and banana leaves, on the 30th day on wood chips and sugarcane bagasse, and on the 37th day on sawdust. This difference in the colonization of the substrates by the mycelium and the appearance of fruiting buds is thought to be due to the difference in texture between the substrates used in these treatments, as well as their chemical composition. Unlike the substrates based on rice straw, wood chips, and sugarcane bagasse, which were completely colonized, the substrates based on banana leaves and sawdust were only partially colonized.

The emergence of the first sporophores, constituting the first flush, was observed between the third and fourth day of fruiting induction after the appearance of fruiting buds, and the interval between the two flushes was 14 to 22 days depending on the substrate.

Table 2. Harvesting of Pleurotus sajor – caju sporophores on different substrates.

Substrats	NB	PB (g)	L1 (g)	L2 (g)	L3 (g)	L4 (g)	PTS (g)	RU (%)	RM ± SD (%)
Rice straw	**1**	**500**	62	57	51,2	20	190,2	**38,04**	**35,01 ± 3,7**
	2	**500**	49	56,9	33	31,89	170,79	**34,158**	
	3	**500**	62,6	37,1	24	21,1	144,8	**28,96**	
	4	**500**	94	44,15	28	22,8	188,95	**37,79**	
	5	**500**	62,3	41	47,5	29,8	180,6	**36,12**	
Banana leaves	**1**	**500**	24,66	23,56	11,09		59,31	**11,86**	**15,4 ± 2,5**
	2	**500**	39,78	24,84	9,058		73,67	**14,73**	
	3	**500**	42,12	28,87	10,8		81,79	**16,35**	
	4	**500**	44,98	28,13	5,096		78,2	**15,64**	
	5	**500**	58,37	21,3	14,65		94,32	**18,86**	
Wood chips	**1**	**500**	61,25	37,35	11,36	28,6	138,56	**27,71**	**28,67 ± 4**
	2	**500**	88,9	33,25	17,98	22,2	103,15	**32,46**	
	3	**500**	45,56	38,3	24,32	19,57	107,75	**25,55**	
	4	**500**	97,8	29,7	21,5	17,6	107,5	**33,32**	
	5	**500**	63	21,39	27,4	9,8	105,19	**24,31**	
Sugarcane bagasse	**1**	**500**	98,9	28,37	9,28		136,55	**27,31**	**23,3 ± 2,4**
	2	**500**	57,6	33,18	18		108,78	**21,75**	
	3	**500**	59,1	24,38	24		107,48	**21,49**	
	4	**500**	41,38	40,35	28		109,73	**21,94**	
	5	**500**	78,05	21,41	21,41		120,87	**24,17**	
Sawdust	**1**	**500**	39,9				39,9	**7,98**	**6,96 ± 2,2**
	2	**500**	19,4				19,4	**3,88**	
	3	**500**	48,75				48,75	**9,75**	
	4	**500**	38,37				38,37	**7,67**	
	5	**500**	28,2				28,2	**5,64**	

Legend:
NB: Bag number of the bale; PB: Bale weight (in grams); L: Lift (in grams), with L1: first lift; 1, 2, 3, ... = number of lifts; PTS: Total weight of sporophores (in grams); UR: Unit yield (in %); AVY: Average yield (in percentage); SD: Standard deviation; %: percentage.

Analysis of Figure 2 shows that Pleurotus sajor – caju strain 221114 cultivated on different substrates yielded the highest average sporophore yields of 35.01 ± 3.7% on rice straw, 28.67 ± 4% on wood chips, and 23.3 ± 2.4% on sugarcane bagasse, as well as low yields of 15.4 ± 2.5% on banana leaves and 6.96 ± 2.2% on sawdust. Statistical analyses of these results using the ANOVA test show that there is a significant difference at 95% between the average yields of the different strains tested (p=3.54e-10*** < 0.05).

TukeyHSD multiple comparison test shows significant differences between the best average yields in sporophores obtained between banana leaves and sugarcane bagasse (p= < 0.001); rice straw and bagasse (p= < 0.001); sawdust and sugarcane bagasse (p= < 0.001); sawdust and rice straw (p= < 0.001); rice straw and banana leaves (p=0.00 < 0.05); wood chips and sawdust (p= < 0.001); banana leaves and wood chips (p= < 0.001); banana leaves and rice straw sawdust (p= < 0.001). No significant differences were observed between wood chips and sugarcane bagasse (p=0.12 > 0.05); sawdust and banana leaves (p=0.09 > 0.05). No physical-chemical analysis of the substrates (pH, nitrogen content, C/N ratio) was performed.

This constitutes a limitation of this study and does not allow us to explain precisely the differences in yield observed between substrates.

With regard to the yields obtained in this study, we note that the yields obtained on rice straw (35.01 ± 3.7%), 28.67 ± 4% on wood chips, and 23.3 ± 2.4% on sugarcane bagasse are much higher than those obtained on banana leaves (15.4 ± 2.5%) and sawdust (6.96 ± 2.2%). The same observation also applies to the yields obtained by Diansambu (2019) ((11% for 7 crops on sawdust; 11% for 6 crops on soaked sawdust plus male oil palm inflorescences; 8% in 4 seedlings; 6% in 3 seedlings for the Kis 0312 Lc strain of Lentinus cladopus); (10% for 4 seedlings on sawdust; 6% for 4 seedlings on soaked sawdust plus male oil palm inflorescences for the unreferenced strain of Marasmius buzungolo); (18% for 7 crops on sawdust; 15% for 5 crops; 8% in 6 crops; 6% in 3 crops for the Pleurotus cystidiosus strain); (18% for 5 crops on sawdust; 15% for 4 crops, for the Pleurotus sajor-caju strain)); Batubenga et al., (2021b) (9.2 ± 3.46%; 14.95 ± 3.12%; 15.65 ± 1.06%) yields obtained from the cultivation of Pleurotus tuber – regium).

Furthermore, the results obtained (42.25%), after harvesting three crops by Mwinyi et al (2021), on a substrate made from rice straw enriched with sawdust and rice bran with a strain of Pleurotus tuber-regium are similar to those obtained on rice straw (35.01 ± 3.7%); Lumba et al., (2026) (12.53 ± 2.12% and (10.05 ± 1.25%) of Pleurotus sajor – caju (exotic strain 1259);

These results suggest that the physical and nutritional properties of the substrates strongly influence the growth and sporophore production of strain 221114 Pleurotus sajor – caju. However, substrates made from straw, wood chips, and sugarcane bagasse yielded average yields greater than 20%, which is generally considered acceptable under artisanal edible mushroom production conditions (Oei, 2023), according to which a substrate is considered better and more suitable for sporophore production.

CONCLUSION

The results obtained in this study are encouraging, as they have enabled the development of lignocellulosic substrates (rice straw, wood chips, and sugarcane bagasse) to assess the adaptability of Pleurotus sajor – caju strain 221114 in artificial culture, compared to others under the same culture conditions. They demonstrated that *Pleurotus sajor – caju* strain 221114 can be cultivated on rice straw, wood chips, and sugarcane bagasse is possible. The main objective of this study was to contribute to the promotion of NTFPs in the fight against hunger and food insecurity through the cultivation of local edible mushrooms, in particular strain 221114 of the species *Pleurotus sajor – caju*, by making sporophores available on a regular basis.

Based on the results obtained, we can say that Pleurotus sajor – caju strain 221114 performed well on all three substrates (rice straw, wood chips, and sugarcane bagasse), and these can be used for the production of sporophores of this strain. The results for substrates made from banana leaves and sawdust, 15.4 ± 2.5% and 6.96 ± 2.2% respectively, suggest that the Pleurotus sajor-caju strain 221114 used in this study has adapted and requires an improvement in nitrogen-rich additives such as soybean meal, which will enable the theoretical yield of 20% or more to be achieved, according to which a substrate is considered to be better and more suitable for sporophore production. The main limitations of this study concern the lack of physical and chemical characterization of the substrates and the lack of precise control of the environmental conditions of cultivation.

The adaptation of other strains of local species should be considered by comparing their yields with other species already adapted to cultivation. It would also be possible to grow crops on complex substrates based on the simple substrates used in this study in order to further increase yields.

Future studies should include chemical analysis of the substrates and evaluation of nitrogen-enriched mixed substrates in order to optimize production yields.

Competing Interests: The authors declare that there are no competing interests.

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