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Article

Cultivation Trial of an Edible and Medicinal Mushroom Species, *Pleurotus Tuber-regium* (Rumph. ex Fr.) Singer 1951 (strain 190212) on Various Lignocellulosic Substrates

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Abstract: In Central Africa, mushrooms are critically important non-timber forest products (NTFPs), both nutritionally and economically. A strain of edible and medicinal lignicolous fungus, *Pleurotus* tuber-regium (Rumph. ex Fr.) Singer 1951 (strain 190212), isolated from tissue (sclerotia), on PDA medium, was tested on corn grain and sawdust seedling substrates and on palm oil male inflorescence (*Elaeis guineensis* Jacq.), ground corn (*Zea mays* L) stalks and grass (*Paspalum notatum* L) soaked for 24 hrs then drained for 24 hours, and unsoaked ground corn (*Zea mays* L) stalks. The highest mycelial growth rate recorded was about 0.9 cm on the PDA medium; 5.97 cm on the corn-based seedling medium and 11.95 cm on the sawdust-based seedling medium. Total mycelial invasion on the PDA medium was observed on day 10, day 14 on the corn-based seedling medium, and day 24 on the sawdust-based seedling medium. The onset of mycelial invasion was noticeable on day 3 of seeding for all treatments T_0 (control), T_1 (Final substrate based on soaked ground corn stalks), T_2 (Final substrate based on unsoaked ground corn stalks), and T_3 (Final substrate based on turf). Total invasion of mycelium was obtained at day 15 of incubation for treatments T_1 and T_2, at day 18 for treatment T3 and at day 24 for treatment T0. The results obtained on treatments T_1 and T_2 respectively (14.95±3.12% and 15.65±1.06%) of the maize stalk substrate, lead us to believe that the strain 190212 of *Pleurotus tuber-regium* species used has adapted and requires an improvement of the medium with nitrogen-rich additives such as soybean meal. This could achieve the theoretical yield of 20% or more, according to which a substrate can be considered better in producing sporophores.

Keywords: Cultivation, *Pleurotus tuber-regium*, DR Congo, fungus, substrate

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1. Introduction

In Central Africa, mushrooms are critically important non-timber forest products (NTFPs), both nutritionally and economically [1,2]. Although they have never ceased to be exploited by certain ethnic groups such as the Pygmies of Central Africa, NTFPs have recently been the subject of renewed interest even by urban populations [3].

The seasonality of the appearance of sporophores is therefore a limiting factor for their availability, which is often random and even limited to a few weeks per year in some regions, mainly during the rainy season [4]. Some species of *Pleurotus*, *Lentinus* and *Termitomyces* genera are found in small quantities in their natural environment because they often grow only in small numbers and can therefore only serve as subsistence food and not as a marketable product that can be a source of income for rural populations [5,6]. In nature, mushrooms have very specific requirements, which are difficult to reproduce or satisfy artificially. Therefore, mushroom cultivation is proving to be a profitable activity

for African farmers, as it provides fresh products in times of drought and outside harvesting areas that cannot be competed with by wild mushrooms that cannot be found in the forest, while at the same time transforming agricultural wastes into high quality food proteins [7].

Pleurotus tuber-regium has, in addition to its food qualities, a sclerotia which is dried, powdered and used in traditional medicine in many African countries, notably to treat anemia, stomach ache, hypertension [3, 8]. In Kinshasa city, several species of mushrooms are found and each species has its own particularities in their use, apart from their gastronomic side. However, the virtues of *Pleurotus tuber-regium* species are of obvious interest to the population of Kinshasa.

The main goal of this study was the valorization of NTFPs in the fight against hunger and food insecurity through the cultivation, in particular that of the 190212 strain of *Pleurotus tuber-regium* sepecies (Rumph. ex Fr.) Singer 1951 by making sporophores available and regular. To achieve this objective, we set as specific objectives to observe the behavior of the fungal strain 190212 of *Pleurotus tuber-regium* species (Rumph. ex Fr.) Singer 1951 on: agar nutrient medium, seedling media and on fruiting substrates.

2. Material and methods

2.1. Study area

This study was carried out in the laboratory of Systematic Mycology and Myciculture located in the Department of Biology, University of Kinshasa (Figure 1). Kinshasa city is located between 04°18' and 04°30' South Latitude and between 15°15' and 15°22' East Longitude. It is bordered to the East by the provinces of Mai-Ndombe, Kwilu and Kwango, to the South by the Province of Kongo Central, to the North and to the West by the Congo River, which constitutes the natural border with the Republic of Congo; its average altitude is 300 m.

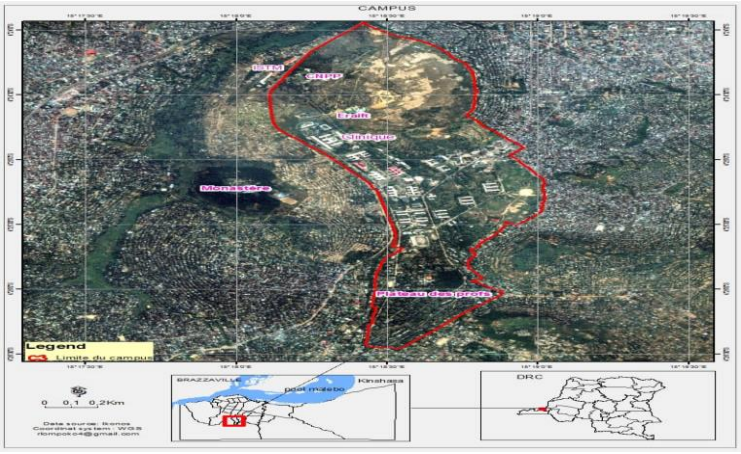

Figure 1. Map of Kinshasa city (Source: Geofirst development, 2021)

2.2. Methods

The tissue culture method (monoculture) was used to start the cultivation: isolation from a tissue (sclerotia: see Figure 2). The Potato Dextrose Agar (PDA) medium, which served as a support for the mycelium to obtain the mother culture, was prepared by referring to the formula proposed by the Mycothèque de l'Université Catholique de Louvain (MUCL) as described by Dibaluka [9]. It gives the proportions of the ingredients as follows: 200g of peeled sweet potato; 20g of dextrose and Agar-Agar in 1000 mL of demineralized water. We used a quarter of the above quantities, i.e. 50 g of sweet potato, 5 g of dextrose

and 5 g of Agar-Agar in 250 ml of distilled water. White seedlings (mother blank and final blank) were produced with reference to the technique developed by Dibaluka et al. [10].

Figure 2. Sclerotia of *Pleurotus tuber-regium* (Source: Batubenga, 2020)

To obtain the fruiting cultures, three final substrates divided into four treatments were used, enriched with 25% of the additives: sawdust, wheat bran and slaked lime.

The first was made from male palm inflorescences (Control), the second was made from soaked ground corn stalks, the third was made from unsoaked ground corn stalks and the fourth from grass. The different proportions are shown in Table 1 below.

Table 1. Proportions of ingredients in four final substrate treatments

Treatments	Ingredients	Proportion (g)	Proportion (%)
T_0	Male palm inflorescence	5500	75
	Sawdust	1100	15
	Wheat bran	586,7	8
	Slaked lime	146	2
	Moisture (%)	60	
T_1	Lawn	5500	75
	Sawdust	1100	15
	Wheat bran	586,7	8
	Slaked lime	146	2
	Moisture (%)	63	
T_2	Soaked ground corn stalks	4000	75
	Sawdust	800	15
	Wheat bran	480	8
	Slaked time	53	2
	Moisture (%)	61	
T_3	Soaked ground corn stalks	4000	75
	Sawdust	800	15
	Wheat bran	480	8
	Slaked lime	53	2
	Moisture (%)	59	

Legend:

T0: Final substrate of male palm inflorescences (Control); T1: Final substrate of soaked ground corn stalks; T2: Final substrate of unsoaked ground corn stalks; T3: Final substrate of grass.

The mixture obtained for each treatment was placed in plastic bags of 29 cm length and 18 cm width, which we doubled to increase their resistance to the heat of sterilization. These bags were filled with 500 g of substrate and were closed with a foam stopper wedged with a plastic ring of about 2.5 cm in diameter and 2 cm in height giving the shape of a neckline to the bag.

The bags thus closed were placed in the autoclave for sterilization at 120 °C for one hour under a pressure of one atmosphere. After sterilization, we let them cool down before spawning. The choice of the basic components or additives was justified by their availability (cost) and their richness in nutritive elements.

3. Results

3.1. Mycelial growth

The beginning of mycelial colonization of *Pleurotus tuber-regium* was observed within 48 hours on PDA (Potato-Dextrose-Agar) agar medium. A total invasion of the mycelium on the same medium was observed on day 10. The highest mycelial growth rate recorded was about 0.9 cm. Macroscopic characteristics of that mycelium revealed that the color was white with a velvety and then of cottony appearance (Figure 3). A total invasion of the mycelium on the corn-based seedling support was observed on day 14. The highest mycelial growth rate was around 5.97 cm. The spawn on corn was preserved for forty-three days at room temperature without deterioration (Figure 4). The spawn on sawdust was retained for 102 days at room temperature without damage. On the sawdust-based seedling medium, total mycelial invasion was observed on day twenty-four. The highest mycelial growth rate recorded was about 11.95 cm (Figure 5).

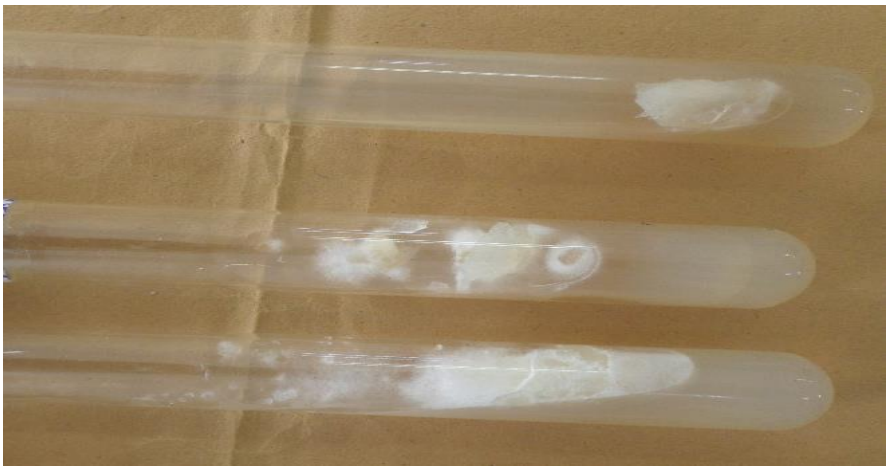

Figure 3. Mother culture on PDA

Figure 4. Seedling spawn on corn kernels (spawn)

Figure 5. Seedling white on sawdust (final wood)

3.2. Phenology of sporophore appearance on fruiting substrates

The beginning of mycelial invasion was noticeable on the 3rd day of spawning for all treatments (T_0, T_1, T_2 and T_3). The total mycelial invasion was obtained on day 15 of incubation for treatments T_1 and T_2, on day 18 for treatment T_3 and on day 24 for treatment T_0. After fruit induction, in the shed, there was hardly the appearance of primordia without sporophore development in all the substrate bags placed on the shelf. The same is true for the substrate made from the buried lawn (treatment T_3). For the bags of buried substrates (male palm inflorescences) and corn stalks (soaked ground and unsoaked ground, T_1 and T_2 respectively). The appearance of the primordia was followed by the development of sporophores in all the bags of the substrates in two lifts after 65 days of burial (Figure 6) as well as the appearance of sclerotia without development of sporophores on all the bags of the substrate made with *Paspalum notatum* (treatment T_3) (Figure 7). These two harvested lifts were used to calculate the average yield of the crop in sporophores.

Figure 6. Spreading of sporophores

Figure 7. Mature Sclerotia & Sclerotia in process of maturation

Table 2 shows the number of sporophore emergences and yields of Pleurotus tuber-regium strain 190212 grown on a substrate made of male oil palm inflorescences and on maize stalks (soaked ground and unsoaked ground), respectively T_0, T_1 and T_2.

Table 2. Sporophores collection of *Pleurotus tuber-regium* on different treatments

Treatments	NS	PS (g)	L_1 (g)	L_2 (g)	PTS (g)	Yield (%)	AY ± SD (%)
T_0	1(3)	500	19	19	38	7.6	9.2 ± 3.46
	2(04)	500	40	32	72	14.4	
	3(10)	500	20	17	37	7.4	
	4(15)	500	20	17	37	7.4	
T_1	1(07)	500	47	43	90	18	14.95 ± 3.12
	2(17)	500	41	39	80	16	
	3(18)	500	38	38	76	15.2	
	4(19)	500	31	22	53	10.6	
T_2	1(01)	500	41	33	74	14.8	15.65 ± 1.06
	2(06)	500	38	38	76	15.2	
	3(11)	500	41	36	77	15.4	
	4(18)	500	45	41	86	17.2	

Legend:

T_0: Final substrate based on male oil palm inflorescences (control substrate);

T_1: Final substrate based on soaked ground maize stalks; T_2: Final substrate based on unsoaked ground maize stalks; (1, 2, 3, ... n): trial index; NS: substrate bag number; PS: sporophore weight (in grams); L: emergence (in grams), where L1: first emergence; 1, 2, 3,... = number of emergence; TSP: total sporophore weight (in grams); Yield (%); AY: average yield (%); SD: standard deviation.

From Table 2, it was observed that the treatments (T_1 and T_2) of the corn stalk substrate gave average yields of 14.95 ± 3.46 % and 15.65 ± 1.06 % respectively. With a low yield for the treatment (T_0) of the palm oil male inflorescence substrate (control substrate) of 9.2 ± 3.46 % after harvesting two seedlings. The absence of treatment T_3 is explained by the absence of sporophore bloom on all substrate bags. The mean of the treatments (T_1 and T_2) of the corn stalk substrate are close to the theoretical yield of 20% according to which a substrate is considered better in the production of sporophores. The comparison of the average weights obtained per treatment is shown in Figure 8.

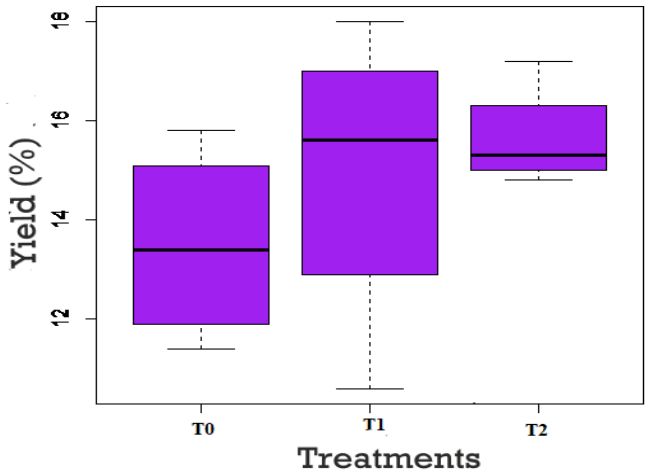

Figure 8. Average yield (%) per treatment during production

Multiple comparison tests of yield and treatment means reveal that at 95% there is no significant difference between the means according to the treatments ($p > 0.05$), this is explained by the burial technique which limits the amount of nutrients in the medium.

4. Discussion

The mycelium of *Pleurotus tuber-regium* isolated from the tissue (sclerotia) on PDA medium evolved normally. We observed the quality of the mycelium and its growth rate at this level. These results corroborate with those of Dibaluka [9], Dibaluka [11], Diansambu [12], Mbikulu [13] and Nsankisha [14]. The resulting stock culture on PDA medium was stored for 22 days at room temperature without spoilage.

Results on the seedling media showed good mycelium growth on all the corn kernel jars and sawdust. The spawn on corn was preserved for 43 days and 102 days for the sawdust-based spawn at room temperature without deterioration. The mycelium appeared as a whitish mat covering the corn kernels and sawdust. The results obtained at this stage show that both substrates can be retained to amplify the volume of mycelium

of *Pleurotus tuber-regium* strain 191202. These results agree with those of Oei [15], Dibaluka et al. [16], Dibaluka et al.[10], Dibaluka & Muambi [17], and Diansambu [12] who reported that the sawdust-based substrate material and mycelium retains its vigor and often purity beyond six months after being kept at room temperature, for more than six months or even more than a year when kept cold.

Mycelial growth of *Pleurotus tuber-regium* species was faster on T_1 and T_2 treatments while it was almost slow on T_3 treatment and much slower on T0 treatment. The beginning of the colonization of substrates by mycelium was perceptible at the 3rd day of seeding for all treatments (T_0, T_1, T_2 and T_3). A total invasion of mycelium was obtained at day 15 of incubation for treatments T_1 and T_2, at day 18 for treatment T_3 and at day 24 for treatment T_0. Previous studies have been conducted on *Pleurotus tuber-regium* species using the substrates of palm oil male inflorescences and stalks [14] and palm oil male inflorescences [13]. Their yields were much lower than ours, i.e. 0% (total invasion of substrates, production of sclerotia without sporophore bloom on all substrate bags) for Mbikulu [13] and 5.6% for Nsankisha [14] without sclerotia production.

On the other hand, the results obtained (42.25%), after the harvest of three seedlings by Mwinyi et al. [18], on a substrate made from rice straw enriched with sawdust and rice bran with a different strain (on the two treatments of the substrate made from maize stalks (14.95 ± 3.12% and 15.65 ± 1.06% respectively) after the harvest of two seedlings. This difference in yield is due to the difference in weight of the substrates (600 g for Mwinyi et al. [18] and 500 g for this study) and the number of emergences (3 emergences for Mwinyi et al. [18] and 2 emergences for this study). Therefore, we think that the substrates made of male inflorescences of the palm tree as we composed it did not have a suitable structure and/or adapted to the development of the strain 190212 of *Pleurotus tuber-regium* species that we tested. It is the same for the treatment of the substrate based on the lawn of which appearance of the primordia was done without blooming of the sporophores on all the bags of the substrate. On the other hand, the results obtained (42.25%), after the harvest of three yeasts by Mwinyi et al. [18], on a substrate made of rice straw enriched with sawdust and rice bran with a strain different from ours, are important than ours. This difference in yield is due to the weight of the substrates (600 g for Mwinyi [18] and 500 g for this study) and the number of lifts (3 lifts for Mwinyi et al. [18] and 2 lifts for this study). The observations made on *Pleurotus tuber-regium* show that the treatments (T_1 and T_2) of the maize stalk substrate gave average yields close to the theoretical yield of 20%, according to which a substrate is considered better in the production of sporophores. On the other hand, the average yield of the control treatment (T_0) is very low and far lower than the theoretical yield mentioned above.

5. Conclusion

The main goal of this study was to contribute to the valorization of NTFPs for the fight against hunger and food insecurity through the cultivation of edible mushrooms, in particular that of *Pleurotus tuber-regium* (Rumph. ex Fr.) Singer 1951 strain 190212, by making the sporophores available and regular. The findings obtained in this study are encouraging, as they demonstrate that the cultivation of the 190212 strain of *Pleurotus tuber-regium* on maize stalks is possible.

In view of the results obtained, the strain 190212 of the species *Pleurotus tuber-regium* performed well on both substrates (corn grain and sawdust based). Thus, these observations indicate that both substrates can be used to increase the volume of mycelium of *Pleurotus tuber-regium* strain 191202. The results of treatments T_1 and T_2 are 14.95±3.12% and 15.65±1.06% respectively on corn stalk substrate, suggesting that *Pleurotus tuber-regium* strain 190212 used in this study has adapted and requires improvement of nitrogen-rich

additives such as soybean meal, which would allow to reach the theoretical yield of 20% or more, according to which a substrate is considered better in sporophore production. We recommend, however, that further trials be conducted on this substrate while modifying the proportions of the ingredients, in order to improve the yield. The lawn remains and interesting substrate for the production of sporophores of *Pleurotus tuber-regium* by the sclerotia (preservative organ) which are produced there, once placed in a humid place.

For the justified use of the sclerotia as a nutraceutical, studies should be conducted on the analysis of its biochemical composition and secondary metabolites.

References

[1] Ndoye O, Awono A, Preece L, Toirambe B. Markets for non-timber forest products in the provinces of Equateur and Bandundu: presentation of a field survey. In: Croizer C. &Trefon T., eds. What future for the forests of the Democratic Republic of Congo? CTB, Brussels. 2007; 68-70.

[2] Batubenga R, Lukoki LF, Iteku BJ, Kanika KD, Mwambay KE, Bongo NG, Mukaya BC and Dibaluka MS (2021). Ethnomycological study of macromycetes used by the population of Mont-Ngafula, in Kinshasa, DRC. Open Access Research Journal of Science and Technology, 02(01):001-014.

[3] EyiNdong G, Degreef J, De Kesel A. Champignons comestibles de forêts denses d'Afrique centrale. Taxonomie et Identification. Abctaxa. 2011;10: 262.

[4] Boddy L, Buntgen U, Egli S, Gange AC, Heegaard E, Kirk PM, Mohammad A and Kauserud (2013). Climate variation effects on fungal fruiting. Fungal Ecology, 1-14

[5] Valverde ME, Hernandez-Perez and Paredes-Lopez (2015). Edible mushrooms: Improving human health and promoting quality life. International Journal of Microbiology, 2015:14.

[6] Koné NA, Yeo K, Konaté S and Linsenmair E (2013). Socio-economical aspects of the exploitation of *Termitomyces* fruit bodies in central and southern Côte d'Ivoire: Raising awareness for their sustainable use. Journal of Applied Biosciences, 70:5580-5590.

[7] Al-Obaidi JR, Jambari NN and Ahmad-Kamil EI (2021). Mycopharmaceuticals and nutraceuticals: promising agents to improve human xell-being nad life quality. Journal of Fungi, 7:503.

[8] Rammeloo J. & Walleyn R., 1993. The edible fungi of Africa south of the Sahara. Scripta Bot. Belg. 5: 1-62.

[9] Dibaluka M. Inventaire des macromycètes de la forêt du lac de Ma Vallée (Kinshasa) et essai de mise en culture de quelques espèces comestibles. Mémoire de DEA Inédit, Fac. Sces, Unikin. 2005; 75.

[10] Dibaluka S, Lukoki F, De Kesel A, Degreef J. Essais de culture de quelques champignons lignicoles comestibles de la région de Kinshasa (R.D. Congo) surdivers substrats lignocellulosiques. Biotechnol Agron. Soc. Environ. 2010; 14(3): 417-422.

[11] Dibaluka M. Etude de macromycètes de la cité de Kimvula et ses environs (Bas-Congo/RDC) : Diversité et productivité en forêt claire, ethnomycologie et mise en culture d'espèces saprotrophes comestibles. Thèse de doctorat Inédite. Fac Sces, Unikin. 2012; 469.

[12] Diansambu M., 2016. Compostage de déchets organiques solides en vue de leur utilisation pour la culture de champignons comestibles. Cas de Kisantu (Kongo-Central/République Démocratique du Congo). Aménagement et Gestion intégrés des Forêts et Territoires Tropicaux. Thèse de doctorat Inédite. ERAIFT, Unikin, 310p.

[13] Mbikulu N., 2019. Culture d'une espèce de champignons comestibles, *Pleurotus tuber-regium* (Rumph. ex fr.) Singer 1951 (souche 190212) sur les inflorescences mâles du palmier à huile (*Elaeis guineensis* Jacq.). Mém. de fin d'étude Inédit. Fac. Sces. Dép. Biologie. Unikin, 40p.

[14] Nsankisa M., 2019. Etude de l'espèce fongique *Pleurotus tuber-regium* (Rumph. ex Fr) Sing. : ethnomycologie (Mont-Amba /Kinshasa/ RDC) et mise en culture sur les inflorescences mâles et rafles du palmier à l'huile (*Elaeis guineensis* Jacq.). Aménagement et Gestion intégrés des Forêts et Territoires Tropicaux. Master professionnel en Techniques d'Aménagement Forestier (TAF). ERAIFT, Unikin, 63p.

[15] Oei P., 2005. La culture des champignons à petite échelle: pleurotes, shiitakes et auriculaires. 1ère édition. Wageningen: Fondation Agromisa, CTA.

[16] Dibaluka M.S., Muambi S., Taba K.M., Kayembe S., Kumbukama B., Kubadi J., 1999. Biodégradation des rafles de maïs par la culture d'une espèce de champignon comestible *Lentinus tigrinus* (Bull.) Fr. In Med. Fac. Landbouwn Univ. Gent 64/1 1999, 277-280.

[17] Dibaluka M & Muambi S., 1992. Recherche sur la culture des champignons utiles d'Afrique centrale: essai de culture de *Lentinus tuber-regium* (Fr.) Fr. Rev. Méd. Pharm. Afr. 8(2), 45-52.

[18] Mwinyi Waziri, Lebisabo Bungamuzi, Kanyama Joseph, Rammeloo Jan, NshimbaSeya Wa Malale & Degreef Jérôme., 2021. «Culture de *Pleurotus tuber-regium* (Fr.) Singer sur substrat ligno-cellulosique en République Démocratique du Congo», *Tropicultura* [En ligne], Volume 39 (2021), Numéro 1, URL : https://popups.uliege.be/2295-8010/index.php?id=1695.