Characterization and Domestication of Wild Edible Mushrooms from Selected Indigenous Forests in Burundi

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Abstract

In Burundi, minimum work has been done to comprehensively identify and commercialize high yielding local mushrooms. The previous studies carried out on mushroom cultivation have focused on exotic strains. This is the first study undertaken on domestication of wild edible mushrooms from Burundi indigenous forests. Nine samples were collected from four protected areas and characterized using phenotypic and molecular markers. Germoplasm isolation through tissue culture techniques, spawn production and cultivation studies were also undertaken. Mushroom samples were identified as *Pleurotus citrinopileatus*, *Lentinus squarrosulus*, *Hypholoma fasciculare*, *Laetiporus sulfureus*, *Macrolepiota dolichaula*, *Trametes polyzona*, *Amanita zambiana*, *Lactarius delicious* and *Amanita verna*. Spawn production was successful in six of the nine collected species. Fruiting body production was successful for *Pleurotus citrinopileatus*, *Lentinus squarrosulus*, *Hypholoma fasciculare* and *Trametes polyzona*. Mushroom yield and biological efficiency of domesticated species varied among species and ranged from 15.3 to 30.6% and 41.2% to 81%, respectively. *Macrolepiota dolichaula* and *Laetiporus sulfureus* remained at the secondary mycelium stage while *Amanita zambiana*, *Lactarius delicious* and *Amanita verna* did not develop even the mother spawn. Burundi indigenous forests harbour wild edible mushrooms with potential for domestication. More research should be conducted to domesticate them for food and nutritional security.

Keywords: Domestication, wild edible mushrooms, germplasm, spawn, Burundi indigenous forests.

Introduction

In Burundi, mushrooms are harvested and consumed at certain times of the year and are abundant in markets during the rainy seasons (Buyck 1994). Burundi has important forest ecosystems rich in edible fungi, among which, some are saprophytic with potential of being domesticated (Degreef et al. 2016). Burundi mountainous forests, namely Kibira National Park and Bururi Forest Reserve are rich in saprophytic mushrooms, whereas low altitude open forests such as the forest reserve of Rumonge are endowed with ectomycorrhizal mushrooms (Buyck 1994). Several studies have been carried out on wild edible mushrooms in Burundi; the results of which helped to raise public awareness of the existence of a rich and unique mycoflora in Burundi (Buyck 1994, Buyck and Nzigidahera 1995, Nzigidahera 2007, Degreef et al. 2016). Likewise, Degreef et al. (2016) reported that wild edible mushrooms constitute an interesting and under-

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exploited resource in Burundi and Rwanda, and suggested a sustainable gathering of ectomycorrhizal species in miombo woodlands and cultivation of saprotrophic species from mountain forests.

Mushrooms grow seasonally in the forest and the rhythm of their appearance and availability for harvest is dictated by weather conditions (Buyck 1994). During the rainy season, people living around the forests harvest wild edible mushrooms in large amounts and consider them as important sources of food and income. On the other hand, few mushroom farmers currently depend on cultivation of exotic mushrooms, for which strains are expensive with difficult access, making cultivated mushrooms expensive, irrespective of preferences of local people.

Therefore, the domestication of saprophytic wild edible mushrooms from Burundi's forest ecosystems is a very important way of solving the aforementioned problems. Apart from increasing the production of traditionally preferred and high nutritional mushrooms for the improvement of food security, it will as well increase farmers' income throughout the year since mushroom cultivation is practiced regardless of the seasons. Thus, this study presents, for the first time, the domestication studies of wild edible mushrooms from indigenous forests in Burundi.

Materials and Methods
Study sites
Samples were collected from the following protected areas: Kibira National Park, Rumonge Forest Reserve, Makamba Protected Landscape, and Ruvubu National Park (Cankuzo side) (Figure 1).

Sample collection
Fresh fruiting bodies were harvested and immediately examined following the protocols by Chang (2008), Haq (2009) and Tibuhwa et al. (2010). Photographs were taken before and after harvesting the fruiting body from its substrate. The mushroom collection was conducted from March to May 2012 and from November 2012 to February 2013. Local guides comprising mushroom harvesters and guardians of the nature reserves of the National Institute for the Environment and Nature Conservation (INECN, actually renamed OBPE = Burundi Office for Environment Protection) helped in the sample collection (Figure 2).

Mushrooms domestication
Tissue culture technique
In order to produce the mother spawn, tissue culture was done using the modified method that was described in Stamets and Chilton (1983) and Hussein et al. (2016). Fresh fruit bodies collected from the field were thoroughly pre-washed in sterile distilled water followed by surface disinfection using ethanol (70%, v/v). A small inner part tissue was aseptically taken from a young and healthy fruit body and inoculated in the petri dish containing "AVOINE" culture medium whose composition is described in INRA (1995) as follows: i) wheat flour: 20 g; ii) sugar: 5 g; iii) yeast: 2 g; iv) agar: 20 g; v) antibiotic chloramphenicol: 1 capsule and distilled water to adjust the volume to 1 litre. The inoculated petri dishes were incubated at 25 °C in a half-dark and air-conditioned room. Mushroom cultures were isolated and purified by growing them on fresh medium. The pure cultures were preserved in the refrigerator at 4 °C and subculturing done monthly as described in Mshandete (2011) and Juma et al. (2015).
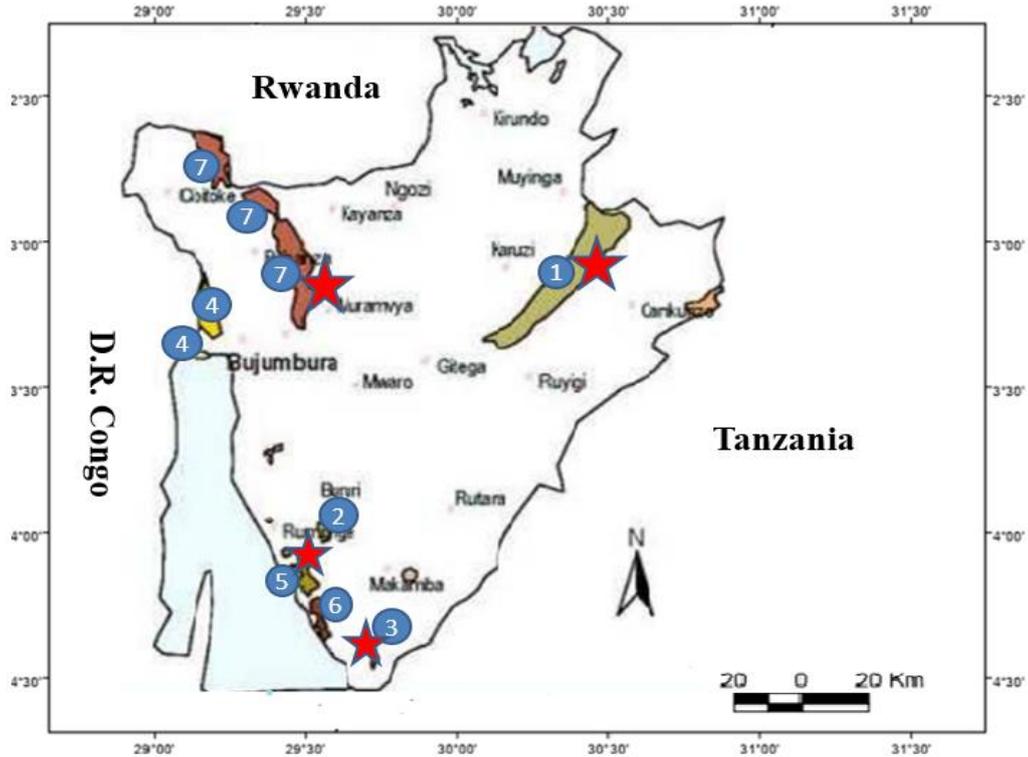
Legend: 1 = Ruvubu National Park; 2 = Forest Nature Reserve of Bururi; 3 = Protected Landscape of Makamba; 4 = Rusizi National Park; 5 = Forest Nature Reserve of Rumonge; 6 = Forest Nature Reserve of Kigwena, and 7 = Kibira National Park; ★ = Study sites

Figure 1: A map of Burundi's protected areas showing the study sites (Adapted from UICN/PACO, 2011).

Figure 2 (a & b): Collection of WEM in Kibira National Park (Photo 2012).

Spawn production

Spawn production was done using the modified method described by Mshandete and Cuff (2008). Intact sorghum grains were brought from Jabe market in Bujumbura town and soaked in tap water for 12 hours and parboiled for 20 minutes. After draining excess water, 1% (w/w) of calcium carbonate ($CaCO_3$) was added and properly mixed with the grains before spreading them out on a clean wire net under the sun heat for 30 minutes. The grains were then packed in clean mayonnaise glass bottles to ¾ of their volume and sterilized in an autoclave at 121 °C for 30 minutes. Thereafter, each cooled bottle of sterilized grains was aseptically inoculated with a piece of pure culture of about 2 cm x 2 cm from a fully colonized petri dish. All the inoculated glass bottles were then incubated at 25 °C in an air-conditioned room. The mycelium development was daily monitored through direct observations until the complete colonization of the bottles and the contaminated bottles immediately removed. The mycelial colonization time was recorded starting with the inoculation day until the complete colonization.

Substrate preparation

Mushrooms were cultivated on the cottonseed crack substrate purchased from RAFINA (Agricultural Products Processing and Refining Company), an industry specialized in cotton oil production in Burundi. The substrate was first soaked in tap water (10 kg of substrate in 20 litres of water) for 12 hours for moisture absorption, then spread on wire net under the sun heat (3 hours) to drain the excessive water, packed into transparent and heat resistant polypropylene bags (1 kg of moist substrate), sterilized in autoclave (12 °C, 1 hour) and cooled at room temperature (25-30 °C) as detailed in Kiyuku and Bigawa (2013). Inoculation and incubation were performed according to the technique of holes described by Olivier et al. (1991). Three teaspoons of mushroom spawn were inoculated into the bagged substrates in each of the three holes. Four replications were performed per treatment. After inoculation, the bags were placed on the shelves in the incubation room located in the cellar of the building of soil physics laboratory at the University of Burundi. The room was regularly cleaned and disinfected with diluted Dettol, a disinfectant and antiseptic solution whose active ingredient was chloroxylenol.The mycelia development was daily monitored through direct observations until the complete colonization of the substrates. Frequent contaminants such as *Trichoderma* and *Rhizopus* were also checked and the contaminated bags immediately displaced to a distant shelf to avoid contamination of the neighbour bags (chain contamination). Depending on the species of the grown SWEM, it took 24-29 days of incubation to have the substrates completely colonized by the mycelium.

Fruiting body production

The fruiting body production was carried out using the method described by Olivier et al. (1991), Oei (1993) and Kiyuku and Bigawa (2013). After complete colonization, the bags containing the spawned substrates were moved to the greenhouse of the Faculty of Agriculture and Bio-engineering (FABI) for fruiting body production. The greenhouse was relatively cooler (22-26 °C), more humid (relative humidity of 85-95%), ventilated and with more light, a requirement for fruiting initiation (Kiyuku and Bigawa 2013). Two fruiting techniques were used as per Olivier et al. (1991), namely fruiting on shelves and fruiting by casing soil. The substrates on the shelves were watered twice per day whereas the ones in casing soil were watered once per day using a watering can.

Mushroom harvesting and yield determination

Mushrooms were harvested in 4 flushes for a period of 3 months. The harvesting was done manually by twisting slowly the fruit bodies at their base. The yield was determined flush by flush for each of the domesticated species, after weighing the harvested mushrooms on each substrate bag. Finally the total mushroom yield

(MY) was determined for each species by adding together the weights of 4 flushes and by applying the following formula (Morais et al. 2000):

MY = [Weight of fresh mushrooms harvested (g) / Fresh substrate weight (g)].

The biological efficiency (BE) was also calculated as per Royse et al. (2004):

BE = [Weight of fresh mushrooms harvested (g) / dry substrate weight (g)] x 100.

The dry substrate weight was determined by heating 100 g of fresh substrate at 105 °C for 24 hours in a drying oven (Royse et al. 2004).

Identification and analysis

The morphological identification of mushrooms was performed with fresh specimens using the "Provisional macroscopic key to the edible mushrooms of tropical Africa" developed by De Kesel (2011). Molecular identification was performed within the Biosciences Eastern and Central Africa-International Livestock Research Institute (BecA-ILRI) hub. After sun drying, deoxyribonucleic acid (DNA) was extracted from the samples using both the Dellaporta et al. (1983) protocol modified to be fit with mushrooms and the Qiagen's DNeasy Plant Mini Kit. After purification and quality assessment, the resulting DNA was then amplified by Polymerase Chain Reactions (PCR) and sequenced at the BecA-ILRI Segolip unit. Sequences were assembled and manually examined for errors control using CLC Main Workbench software and the amplified regions were aligned using CLUSTAL W (Thompson et al. 1994) in MEGA version 6 (Tamura et al. 2013). The aligned sequences were then interrogated in NCBI for identity with known sequences of the NCBI's GenBank database.

Results

Mushroom species identified

A total of 9 species of mushrooms were collected and identified from four protected areas namely the Kibira National Park, the Forest Natural Reserve of Rumonge, the Protected Landscape of Makamba and the Ruvubu National Park where the study was conducted (Table 1). The results from molecular identification (Table 2) showed that the collected mushroom samples were *Amanita zambiana*, *Pleurotus citrinopileatus*, *Amanita verna*, *Lactarius delicious*, *Lentinus squarrosulus*, *Hypholoma fasciculare*, *Macrolepiota dolichaula*, *Trametes polyzona* and *Laetiporus sulfureus*. *Macrolepiota dolichaula*, *Pleurotus citrinopileatus*, *Amanita verna*, *Hypholoma fasciculare*, *Trametes polyzona* and *Laetiporus sulfureus* were obtained exclusively from the Kibira National Park. *Lentinus squarrosulus* was found only in the Mwange palm grove in Rumonge while *Lactarius delicious* and *Amanita zambiana* were recorded almost in all the protected areas that were visited.

Mushroom domestication

Tissue culture isolation incubation period varied from species to species and it ranged from 6-8 days (Figure 3 a). Spawn production incubation period was 12-15 days (Figure 3 b). Substrate incubation period varied from one species to another and ranged from 24 to 29 days (Figure 3c) with *Pleurotus citrinopileatus* exhibiting the shortest colonization time of 24 days, while *Trametes polyzona* the longest time of 29 days (Table 2).

Two other species, namely *Macrolepiota dolichaula* (Ikigogo) and *Laetiporus sulfureus* (Nkundamazi 2) remained at the stage of secondary mycelium on sorghum while *Amanita zambiana* (Rerya), *Lactarius delicious* (Mwate) and Amanita *verna* (Urwerakare) did not even form the mother spawn.

Table 1: Mushroom species obtained in selected forests, sites and substrates

Scientific Name	Vernacular Name	Collection Site	Substrate
Amanita zambiana Pegler & Piearce	Rerya	Nyamirambo and Nkayamba (RNFR), Mungongo (PLM), Kigamba (RNP)	Dead leaves and moss carpet under *Brachystegia* sp. trees
Pleurotus citrinopileatus Singer	Ubuzirantete	Teza (KNP)	Decaying wood
Amanita verna (Bull.) Lam.	Urwerakare	Teza (KNP)	Decaying grass near a decaying wood trunk
Lactarius delicious (Fries) S. F. Gray	Mwate	Nyamirambo and Nkayamba (RNFR), Mungongo (PLM), Kigamba (RNP)	Decaying leaves (litter)
Lentinus squarrosulus Mont. (Singer)	Rumonge 6	Palm grove of Mwange (Rumonge)	Decaying palm trunks
Hypholoma fasciculare (Huds.:Fr) P. Kumm	Ubunyagashiru	Teza (KNP)	Decaying stump of *Eucalyptus*
Macrolepiota dolichaula (Berk. & Broome) Pegler & R.W. Rayner	Ikigogo	Bugarama (KNP)	Decaying grass under *Eucalyptus* trees
Trametes polyzona (Pers.) Just	Nkundamazi 1	Teza (KNP)	Decaying wood
Laetiporus sulfureus (Bull.: Fr.) Murrill	Nkundamazi 2	Teza (KNP)	Decaying wood

RNFR = Forest Natural Reserve of Rumonge; KNP = Kibira National Park; PNR = Ruvubu National Park; PPM = Protected Landscape of Makamba.
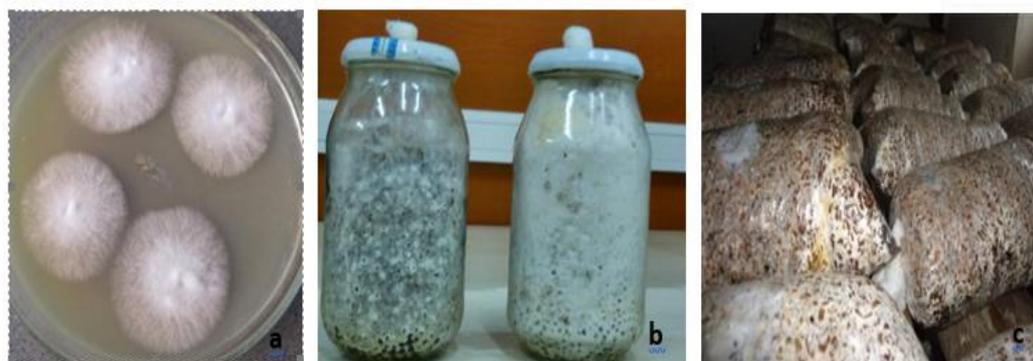

Figure 3: (a) Primary mycelium of *P. citrinopileatus* (b) Secondary mycelium on sorghum grains, and (c) colonized cottonseed crack substrate.

Table 2: Results from molecular identification of collected samples

Sample No.	Description from NCBI- BLAST	M S	T S	Q C (%)	E-V	% ID	Accession
1	*Amanita zambiana* strain RET-094-7 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence.	821	821	95.4	0	99	JX844753.1
2	*Pleurotus citrinopileatus* strain FPCMC 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	1160	1160	100	0	99	JX429936.1
3	*Trametes polyzona* voucher BKW004 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	554	554	99.6	0	99.64	JN164978
4	*Lactarius delicious* voucher JN2001-064 (GENT) 28S ribosomal RNA gene, partial sequence.	656	656	99	0	96.79	KF133305
5	*Lentinus squarrosulus* strain TTHF1-6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	223	223	100	0	100	MN398967.1
6	*Hypholoma fasciculare* voucher HFJAU0101 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	1092	1092	100	0	96.52	MN258649
7	*Macrolepiota dolichaula* strain HKAS34018 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence.	1064	1064	95	0	97.02	DQ411537.1
8	*Amanita verna* strain A44 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence.	144	144	92	0	100	MK512066
9	*Laetiporus sulfureus* strain CBS 343.69 large subunit ribosomal RNA gene, partial sequence.	584	584	100	0	100	MH878467

MS = Max score; TS = Total score; QC = Query cover; E-V = E- Value; ID = Identity; NCBI: National Centre for Biotechnology Information.

Four species successfully produced fruiting bodies, i.e., *Pleurotus citrinopileatus* (Ubuzirantete), *Lentinus squarrosulus* (Rumonge 6); *Trametes polyzona* (Nkundamazi 1) and *Hypholoma fasciculare* (Ubunyagashiru) (Figure 4).
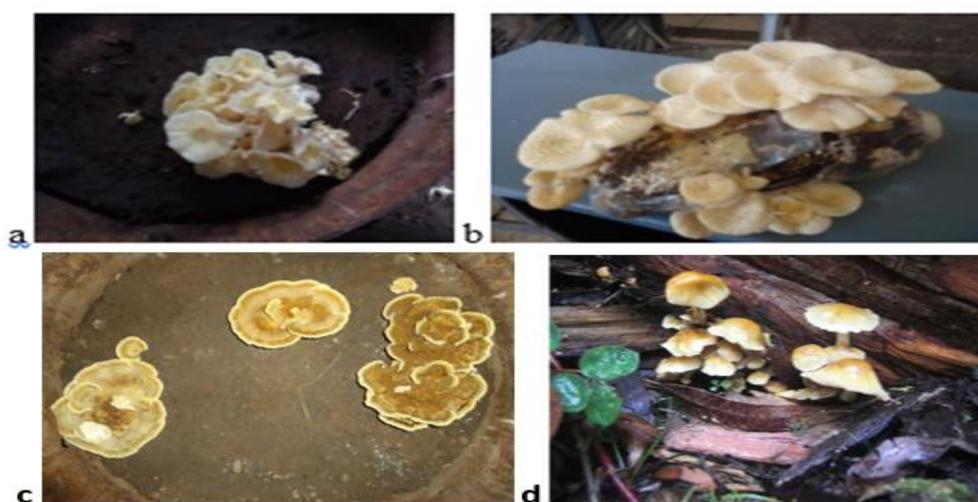

Figure 4: Successfully domesticated species (a) *Pleurotus citrinopileatus*-substrate was buried in the soil (b) *Lentinus squarrosulus*-substrate was put on the shelves (c) *Trametes polyzona*-substrate was buried in the soil (d) *Hypholoma fasciculare*- sample from the field.

Discussion

Mushroom identification results

The molecular identification showed that collected mushrooms were *Amanita zambiana*, *Pleurotus citrinopileatus*, *Amanita verna*, *Lactarius delicious*, *Lentinus squarrosulus*, *Hypholoma fasciculare*, *Macrolepiota dolichaula*, *Trametes polyzona* and *Laetiporus sulfureus*. The molecular identification results (Table 2) obtained are reliable with regards to the percentage of identity. Indeed, for all the studied mushroom sequences interrogated in NCBI database, the BLAST search results showed that the percentage of identity ranged between 96.52 and 100%. Juma et al. (2016) reported a percentage of identity of ≥ 97% for ITS and ≥ 85% for LSU during the study on identification of Tanzanian edible mushrooms. Likewise, Hussein et al. (2014) reported a percentage of identity ≥ 92% for ITS and 97% for LSU data set for eight wild edible mushrooms from Tanzania. These findings support the range of the identity percentage reported in this study. Mushroom identification using molecular genetics techniques is more reliable and accurate due to its capability to differentiate even those species closely related and morphologically looking similar (Muruke et al. 2002, Lian et al. 2008, Fonseca et al. 2008).

Mushroom species successfully domesticated

In the domestication trials, four species, namely *Pleurotus citrinopileatus*, *Trametes polyzona*, *Lentinus squarrosulus* and *Hypholoma fasciculare* produced fruit bodies (Figure 4). *Macrolepiota dolichaula* and *Laetiporus sulfureus* successfully colonized the substrate but did not fruit, while *Amanita zambiana*, *Lactarius delicious* and *Amanita verna* did not produce even the mother spawn. Apart from *Lentinus squarrosulus*, the other three species successfully domesticated were harvested from the Kibira National Park, which is known to be rich in saprophytic wild edible mushrooms; this corroborates the report by Buyck (1994). Several studies have previously reported successful domestication and/or

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cultivation of *Lentinus squarrosulus* (Kadiri and Arzai 2004, Dibaluka et al. 2010, Adesina et al. 2011, De Leon et al. 2013), *Pleurotus citrinopileatus* (Liang et al. 2009, Musieba et al. 2011, Musieba et al. 2012, Shevale and Deshmukh 2016), *Trametes polyzona* (Lueangjaroenkit et al. 2018) but no literature was found about domestication and/or cultivation of *Hypholoma fasciculare.* This may be due to the fact that this small tuft mushroom is considered as poisonous (Cortez and Silveira 2007) However, data concerning its toxicity are controversial (Badalyan et al. 1995) and this can explain the fact that local villagers around the Kibira National Park especially Batwa communities like this small mushroom. This shows that some species can be eaten in a region and not in another region. This corroborates the report by Degreef et al. (2016) who observed that some species (*Agaricus* spp., *Macrolepiota africana*, *Marasmius* spp) commonly eaten in neighbouring countries are not consumed in Burundi and Rwanda despite their abundance in the region. Even though *Macrolepiota dolichaula* and *Laetiporus sulfureus* produced only spawn but did not fruit in the experimental conditions of this study, there are some literatures reporting successful domestication of *Macrolepiota dolichaula* (Rizal et al. 2016) and *Laetiporus sulfureus* (Pleszczynska et al. 2012). This suggests that further studies are needed to optimize their domestication conditions such as temperature, relative humidity, substrate variation and supplementation.

Mycelia colonization rate
Mycelial growth tests for the domesticated mushrooms revealed a colonization rate of 24-29 days on cottonseed crack substrate (Table 3). This period is longer compared to the exotic strains of the most cultivated oyster mushrooms in Burundi (*Pleurotus ostreatus* HK51 and *Pleurotus sapidus* P969) for which mycelial colonization period lasts between 14 and 21 days in Bujumbura depending on the type of substrate. However, this colonization rate compares favourably with some previous studies carried out on mushroom domestication. For instance, Hussein et al. (2016) reported a similar colonization rate (24-28 days) for *Lentinus sajor-caju* and *Pleurotus conchatus* (Tanzanian domesticated mushrooms) to fully colonize the substrate made of 70% banana leaves and 30% shred wood. Dibaluka et al. (2010) reported a mycelial colonization rate of 28-30 days for *Lentinus squarrosulus* and *Pleurotus cystidiosus* cultivated on sawdust and stems of *Cyperus papyrus*. Likewise, Adesina et al. (2011) reported a mycelial colonization rate of 23 days for *L. squarrosulus* while Liang et al. 2009 reported a rate of 22-28 days for *P. citrinopileatus* on different substrates.

Table 3: Duration of mycelial colonization of domesticated species on cottonseed crack substrate

Vernacular name	Scientific name	Mycelial colonization time (in days)				
		Substrate bag 1*	Substrate bag 2*	Substrate bag 3*	Substrate bag 4*	Mean
Ubuzirantete	*Pleurotus citrinopileatus*	24	25	22	25	24
Nkundamazi 1	*Trametes polyzona*	30	29	29	28	29
Rumonge 6	*Lentinus squarrosulus*	27	25	26	26	26
Ubunyagashiru	*Hypholoma fasciculare*	26	25	24	25	25

*Four replications were performed per species.

Mushroom yields and biological efficiency

In this study, the fresh mushroom yields obtained from *P. citrinopileatus*, *L. squarrosulus*, *T. polyzona* and *H. fasciculare* cultivated on cottonseed cracks ranged between 15.3 and 30.6% (Table 4) and can be considered as satisfying according to Oei (2003). These results show that mushroom yields varied according to the mushroom species. *P. citrinopileatus* exhibited the highest yield (30.6%) followed by *L. squarrosulus* (21.3%) and *T. polyzona* (20.9%), while *Hypholoma fasciculare* exhibited the lowest one (15.3%). This agrees with the findings of Mshandete and Cuff (2008) who reported that mushroom yields were directly related to the mushroom species used in their experiment. The yield range of 15.3-30.6 % is consistent with the findings of previous studies.

Manirakiza et al. (2014) recorded a yield of 18-40% for 3 strains of *Pleurotus ostreatus* cultivated on elephant grass supplemented with 10-40% of ground avocado stones or not supplemented. Musieba et al. (2012) recorded a yield of 21.36% and 39.77% for *P. citrinopileatus* cultivated on rice straw and bean straw respectively. The yield obtained in this study for *L. squarrosulus* is close to that of Adesina et al. (2011) who obtained a yield of 20.5% for the same species cultivated on *Spondias mombin* supplemented with rice bran. Mshandete and Cuff (2008) reported a yield of 290 g/kg wet substrate (29%) for *Pleurotus flabellatus* cultivated on non-composted sisal decortication residues, whereas the composted one gave a yield of 371 g/kg wet substrate (37.1%) for the same species.

Table 4: Yield and biological efficiency of domesticated species cultivated on cottonseed crack substrate

Species	Flush 1 (g/kg)	Flush 2 (g/kg)	Flush 3 (g/kg)	Flush 4 (g/kg)	Total yield* (g/kg)	Yield in %	BE (%)
Pleurotus citrinopileatus	159.2	76.3	51.1	19.2	305.8	30.6	81
Lentinus squarrosulus	130	56.4	21.2	5	212.6	21.3	62.5
Trametes polyzona	120.5	70.2	15	3	208.7	20.9	52.2
Hypholoma fasciculare	100.3	60	13.2	0	153.5	15.3	41.2

All values are the means of four replicates. *Fresh weight after adding together the weights of four flushes.

The results of mushroom harvesting (in 4 flushes) showed that the mushroom yield decreased from the first flush to the last one. This may be due to the decrease of the amount of nutrients in the substrate. This is consistent with the findings of Musieba et al. (2012), Raymond et al. (2012), Shevale and Deshmukh (2016) and Hussein et al. (2016) who reported a decrease in yield from one flush to another. In this study, the biological efficiency ranged between 41.2% and 81% (Table 4). This means that, generally, these mushrooms species were able to utilize efficiently the nutrients present in the substrate for their development, even though the efficiency differed from one species to another. The highest biological efficiency was recorded for *P. citrinopileatus* (81%) followed by *L. squarrosulus* (62.5%) and *T.*

polyzona (52.2%), while the lowest was recorded for *Hypholoma fasciculare* (41.2%). This range is consistent with the biological efficiency range of 43.4-98.5% obtained by Mamiro and Mamiro (2011) when growing *P. ostreatus* on rice straws mixed with various amounts of *Leucaena leucocephala*, maize cobs and banana leaves. The high yield and biological efficiency for *P. citrinopileatus* could be explained by the ability of oyster mushrooms to grow on a wide range of lignocellulosic agricultural waste because they have a strong enzyme profile helping them to breakdown most complex organic macromolecules into simple absorbable forms (Shevale and Deshmukh 2016). The lowest yield and biological efficiency for *H. fasciculare* could be explained by the fact that

it is very small and is mainly a wood decaying mushroom.

Even though the mushroom yields obtained in this study were satisfying, it was noted by simple observations that for all the four species, the fruit bodies of domesticated mushrooms were smaller than those harvested under natural conditions (data not shown). This may have led to a lower yield after domestication compared to the expected yield on ideal substrate. This was a preliminary study on the possibilities of domestication of indigenous mushrooms and did not explore all the aspects of mushroom production. More studies should be done on determination of mushroom size, cropping period and yields comparison on assorted and/or complemented substrates. Some physiological factors may have caused the prolonged incubation period and the decrease of the mushroom yield. These may include temperature, humidity, type of substrate and adaptation to the artificial environment as well as the strain genotype (Hussein et al. 2016). For this study, the temperature and the type of substrate may have contributed to the decrease of mushroom yields for *Pleurotus citrinopileatus*, *Trametes polyzona* and *Hypholoma fasciculare*. This study was conducted in Bujumbura where the temperature ranges between 23 and 26 °C for most time of the year whereas these three species were harvested from the Kibira mountain forest, in the Teza zone where the temperature ranges between 16.7 and 18.2 °C. *Lentinus squarrosulus* was harvested on decaying oil palm trunks in Rumonge region. Here the temperature could not be involved because Rumonge and Bujumbura have similar altitude and temperature. The decrease of mushroom yield after domestication may be due to the types of substrates since the domesticated mushroom was grown on cottonseed cracks while it was found growing naturally on the oil palm trucks. Petre and Teodorescu (2012) reported that the substrate plays an important role in mushroom

cultivation by insisting on the fact that some species of mushroom require specific micro-environment including complex nutrients for the mycelia growth and fructification. This study recommends domestication optimization trials including diversification and supplementation of the substrates in order to optimize the yields and reduce colonization time.

Among the five remaining species, two of them also from the Kibira National Park (*Macrolepiota dolichaula* and *Laetiporus sulfureus*), successfully colonized the cottonseed crack substrate but there were no fructification. Hussein et al. (2016) reported similar findings in the domestication of *Pleurotus conchatus*; the substrate was fully colonized but no fruiting body appeared even after cold shock induction. Also, Marino et al. (2003) had previously reported a failure of *Lentinula edodes* isolate to adapt to tropical regions and to form primordia. These results confirm that these species may have specific requirements in terms of substrate and climatic conditions needed for fruiting. Further fructification trials are to be carried out in high altitude conditions to simulate their natural climatic conditions. More domestication trials on assorted substrate types with characteristics close to the natural substrates such as composted *Eucalyptus* sawdust are recommended.

Three species, i.e., *Amanita zambiana*, *Lactarius delicious* and *Amanita verna*, did not produce even the mother spawn. The literature shows that the genus *Amanita* and *Lactarius* are ectomychorizal and not saprophytic. They were harvested from the open forest of Rumonge (RNFR) dominated by *Brachystegia* trees that are known for mycorrhizal association. Fungi from ectomycorrhizal association cannot be cultivated *in vitro* (Oei 1993, Tibuhwa 2013). This observation corroborates that of Buyck (1994) who reported that the RNFR is one of the few remnants of open forests with dominant trees

associated with many ectomycorrhizal fungi whereas saprophytes are almost non-existent. Degreef et al. (2016) reported that domestication of edible ectomycorrhizal fungi remains a challenge for mushroom growers worldwide and that success has only been limited to a few temperate species by planting appropriate mycorrhiza tree seedlings inoculated with specific fungal strains.

Conclusion

This study revealed that some wild edible mushrooms from Burundi's natural forests have the potential for being domesticated. Optimal exploitation of this aspect could contribute to the promotion of these poorly known or neglected food resources in Burundi. For the four species that have been successfully domesticated, it is recommended that their growing conditions be optimized for yield improvement. In the case where two species successfully colonized the substrate but did not fruit, further studies are to be undertaken to find out their optimum growth conditions in order to grow them successfully in vitro.

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Competing interests

The authors declare that they have no competing interests.

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