The Nelson Mandela AFrican Institution of Science and Technology

NM-AIST Repository **https://dspace.mm-aist.ac.tz**

Life sciences and Bio-engineering Research Articles [LISBE]

2024-01-15

Successful domestication of Neonothopanus Hygrophanus (Mont.) De Kesel & Degreef and Lentinus Squarrosulus Mont., indigenous saprophytic edible mushrooms from Kibira National Park in Burundi

Nteziryayo, Vincent

CABI Agriculture and Bioscience

https://doi.org/10.1186/s43170-023-00212-z

Provided with love from The Nelson Mandela African Institution of Science and Technology

Nteziryayo *et al.*
CABI Agriculture and Bioscience (2024) 5:7
https://doi.org/10.1186/s43170-023-00212-z

CABI Agriculture and Bioscience

RESEARCH

Open Access

Successful domestication of *Neonothopanus Hygrophanus* (Mont.) De Kesel & Degreef and *Lentinus Squarrosulus* Mont., indigenous saprophytic edible mushrooms from Kibira National Park in Burundi

Vincent Nteziryayo[1,2*], Anthony M. Mshandete[1,3] and Donatha D. Tibuhwa[1]

Abstract

Background Mushroom cultivation in Burundi provides an excellent way to diversify agricultural production although domestication of mushroom species is at an infancy stage. The country is endowed with indigenous forests that harbour a wide diversity of mushrooms with potential for domestication. This study was undertaken to explore opportunities for domestication of saprophytic wild edible mushrooms from the Kibira National Park (KNP) in Burundi.

Methods Samples of *Lentinus squarrosulus* Mont. and *Neonothopanus hygrophanus* (Mont.) De Kesel & Degreef were collected from the field, and tissue cultured on Potato Dextrose Agar (PDA) medium. Spawn production and development was performed on sorghum grains and lignocellulosic substrates respectively and the parameters of mycelial growth and mushroom yield were determined.

Results The germplasm of *L. squarrosulus* and *N. hygrophanus* was successfully isolated with an average tissue culture incubation time of 6.4 ± 0.54 days and 7.6 ± 0.54 days for *L. squarrosulus* and *N. hygrophanus*, respectively. Spawn production incubation time on sorghum grains was 12.6 ± 0.89 days and 14.8 ± 0.83 days for *L. squarrosulus* and *N. hygrophanus*, respectively. For full colonization of lignocellulosic substrates, spawn production time ranged between 19 ± 1 and 21 ± 1 days for *L. squarrosulus* and between 17 ± 1.22 and 18 ± 1.22 days for *N. hygrophanus*. Both species successfully produced fruiting bodies and mushrooms yielded at a rate of 18.24 ± 9.76 to 22.85 ± 9.16% for *L. squarrosulus* and 12.66 ± 8.95 to 15.3 ± 8.94% for *N. hygrophanus* which is here reported for the first time to be successfully domesticated. For both species, the cottonseed hulls substrate comparatively showed the best yield followed by the combination of maize cobs/soybean straws (MC + SBS) (50:50), the combination of rice straws/soybean straws (RS + SBS) (50:50) and the maize cobs substrate respectively, while the rice straw showed the least. The combinations of MC + SBS (50:50) and RS + SBS (50:50) showed a yield close to that of cottonseed hulls.

Conclusions For the first-time, this study presents successful domestication of *N. hygrophanus* and *L. squarrosulus* from KNP. It is concluded that the substrates combinations used in the study give good yields, and therefore recommended for use as cost-effective and efficient alternative substrates.

*Correspondence:
Vincent Nteziryayo
vincent.nteziryayo@yahoo.fr; nteziv83@gmail.com
Full list of author information is available at the end of the article

© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Keywords Tissue culture, Germplasm isolation, Mycelial growth, Substrates, Mushroom yield

Introduction

Food and nutritional security remain a great challenge especially among rural communities in developing countries. The exploration of natural resources such as mushrooms is considered to be one of the avenues to address these challenges (Adedokun et al. 2016). In Africa, over two thirds of the population rely on forest products, either in the form of subsistence or as a cash income derived from a wide range of non-wood forest products, including edible mushrooms (Rammeloo and Walleyn 1993; Tibuhwa 2013; Sileshi et al. 2023). Mushrooms are considered as a relatively low-cost alternative to animal proteins, a source of food and income and raw material in local traditional medicine practice (Osarenkhoe et al. 2014). The appearance of the majority of edible mushrooms in the tropical region is restricted to the rainy season, corresponding to the period of food shortage. Mushrooms are then an important option for people's survival during the periods of food shortage (Boa 2004).

The decline in natural forest areas and the restricted access to National Parks has led to decrease in the consumption of wild species in Burundi, even though a number of species hold potential for cultivation (Degreef et al. 2016). Despite the fact that more than 300 edible mushroom species have been inventoried in tropical Africa, very few have been domesticated (Dibaluka et al. 2010). Currently, mushroom cultivation in Africa is limited mainly to the importation of fungal mycelia from Europe, while excellent indigenous edible species are completely neglected (Buyck 1994). During the rainy season, Burundian people living around the forests harvest wild edible mushrooms in large quantities and consider them as an important source of food and income (Buyck 1994, Buyck and Nzigidahera 1995, Nteziryayo et al. 2022). This is particularly the case at the beginning of the rainy season, which is a difficult period corresponding to food shortages when the crops from the previous season are almost exhausted and the new crops are not ready (Nteziryayo et al. 2022).

The study by Degreef et al. (2016) revealed the presence and diversity of saprophytic wild edible mushrooms in mountain forests of Burundi and most of them are probably good candidates for cultivation. Recently, Nteziryayo et al. (2022) documented the local knowledge and the use of different mushroom species by the communities living around KNP and Natural Forest Reserve of Bururi. The results of the study revealed once again that these mountainous forests are endowed with a reach and diversified mycoflora and most of the collected species were identified as saprotrophic with a high potential for domestication (Nteziryayo et al. 2022). Nevertheless, the study revealed that some of the edible species that people were used to harvest and consume in past years are no longer found in the wild due to increasing of urbanization and deforestation and suggested an urgent action for preserving existing mushrooms from extinction (Nteziryayo et al. 2022). Apart from preserving the studied mushroom species germplasm, mushroom domestication constitutes an important contribution to the improvement of food and nutritional security in the country.

Indeed, the mushroom industry in Burundi has a significant potential to support food and nutritional security and income generation. Nevertheless, minimum work has been done to comprehensively identify, domesticate and commercialize high yielding mushroom strains from indigenous forests (Nteziryayo et al. 2019). Most of the previous studies that have been carried out on edible mushrooms in Burundi have focused mainly on exotic strains, essentially oyster mushrooms. The only study by Nteziryayo et al. (2019) dealt with domestication of four species from selected indigenous forests that are presently performing well. However, the high mountainous forests of Burundi were not deeply explored on this respect, and opportunities exist for domestication of saprophytic wild edible mushrooms from the KNP. *Lentinus squarrosulus* Mont. and *Neonothopanus hygrophanus* (Mont.) De Kesel & Degreef are among the edible species that were found and harvested in the KNP and are known to be saprophytic.

Recently, *Lentinus squarrosulus* gained a lot of attention in view of increasing reports on its successful artificial cultivation (Nwanze et al. 2005a, b; Ahmad et al. 2013; De Leon et al. 2013; Leon et al. 2017; Osibe and Chiejina 2015). To the best of our knowledge, there is no report about successful domestication of *Neonothopanus hygrophanus* (Mont.) De Kesel & Degreef, except a study by Dibaluka et al. (2010) who tried to cultivate *Nothopanus hygrophanus* (Mont.) Singer ex Pegler, which resulted in production of spawn on maize grains only, without fruiting on the used substrates. Therefore, the objective of this study was to explore opportunities for

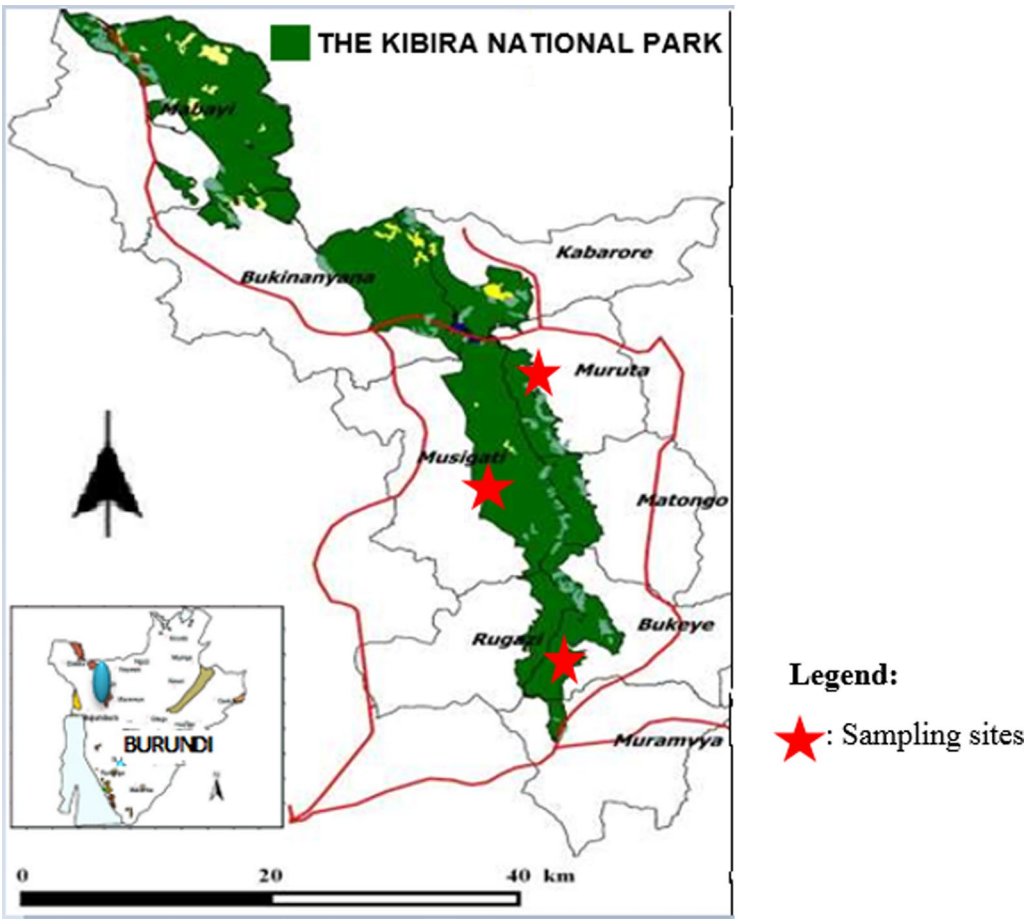

Fig. 1 A map showing the KNP and the sampling sites (Adapted from Nzigidahera & Habonimana (2016))

domestication of *Neonothopanus hygrophanus* and *Lentinus squarrosulus*.

Materials and methods

Study site

The mushroom samples were collected from the Kibira National Park (KNP) (Fig. 1). The park is situated in the northwest part of the country and has an area coverage of approximately 400 square kilometres. It is characterized by mountainous landscape covered by dense tropical rainforest. The Park forms an important water catchment area, with several rivers originating from its forested hills, which makes it a home to a wide variety of flora and fauna, including rare and endemic species and unique mushrooms as well as providing essential water resources to nearby communities.

Sample collection and morphological identification

Mushroom fruiting bodies were harvested and examined in fresh conditions as explained in Tibuhwa et al. (2008), Hussein et al. (2018). Photographs were taken before and after harvesting the fruiting body from its substrate. The mushroom collection was conducted from 2018 to 2019. Local guides comprising mushroom harvesters and guardians of the Burundi office for Environment Protection (OBPE) helped in the sample collection. Specimens of each species were morphologically identified by comparing with other published literature such as Eyi Ndong et al. (2011), Degreef and De Kesel (2017), De Kesel et al. (2017), Tibuhwa (2023).

Tissue culture

Pure mycelial culture was produced through tissue culture using the modified method that was described in Dhouib et al. (2005), Mshandete and Cuff (2008) and Haq (2009). Fresh fruit bodies of the studied mushrooms collected from the field were thoroughly pre-washed in sterile distilled water followed by surface disinfection using ethanol (70% v/v). A small fragment of inner tissue was aseptically cut from a young and healthy fruit

Table 1 Composition of different substrate formulations, with 1% lime in each one

Base substrate	Final substrate formulation	Label
Cottonseed hulls (control)	990 g Cottonseeds hulls + 10 g lime	CSH
Maize cobs	990 g Maize cobs + 10 g lime	MC
Rice straw	990 g Rice straw + 10 g lime	RS
Soybean straw	Not used as a single substrate	SBS
Maize cobs + Soybean straw (50:50)	495 g Maize cobs + 495 g Soybean straw (50:50) + 10 g lime	MC + SBS
Rice straw + Soybean straw (50:50)	495 g Rice straw + 495 g Soybean straw (50:50) + 10 g lime	RS + SBS

body using a sterile scalpel and inoculated in a 9 cm petri plate containing PDA culture medium (manufactured by the company "OXOID Ltd" from Basingstoke Hampshire in England). The inoculated petri plates were incubated at 25 °C in a half-dark and air-conditioned room of the Microbiology laboratory of the Faculty of Agriculture and Bioengineering, University of Burundi. 5 replicates were done for each species. Incubation time was recorded from the first day of tissue culture until the full colonization of the petri plates.

Spawn production

Spawn was produced using the method described by Mshandete and Cuff (2008) with slight modifications. Intact sorghum grains were brought from Gasenyi market in Bujumbura town and soaked in tap water for 18 h and parboiled for 20 min. 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 45 min. The grains were then packed in clean mayonnaise glass bottles to ¾ of their volume and autoclaved at 121 °C for 30 min. Thereafter, each cooled bottle of sterilized grains was aseptically inoculated with a piece of pure culture of about 2 x 2 cm from a fully colonized petri plate. All the inoculated bottles were incubated at 25 °C in a half-dark and air-conditioned room. Five (5) replicates were done for each species. The mycelium development was daily monitored through direct observations until the complete colonization of the sorghum grains. Incubation time was recorded, from the inoculation day until full colonization of the bottles.

Substrate preparation and inoculation

Three types of substrates locally available in Burundi the cottonseed hulls (CSH), maize cobs (MC) and rice straw (RS) were used for cultivating the domesticated mushroom species. Soybean straws (SBS) were chosen as a supplement for MC and RS substrates. CSH were purchased from RAFINA (Agricultural Products Processing and Refining Company), an industrial company specialized in cotton oil production in Burundi. MC, RS and SBS were collected from agricultural farms of Gihanga (Bubanza Province). Before use, they were ground using a grinder machine. MC and RC were used singly or mixed with SBS at a rate of 50:50. CSH were used singly as a control because they are popular used substrates in Burundi that have been reported by several researchers as a good substrate for mushroom cultivation (Li et al. 2001, Zhou et al. 2011, Xu et al. 2016). Details of the substrate compositions and treatments are presented in Table 1.

Each substrate was first soaked in tap water for 12 h for moisture absorption, then spread on wire net under the sun heat for five (5) hours to drain the excessive water. A palm squeeze test was then performed to ensure the right amount of moisture in the substrates. It consisted of pressing a small amount of wet substrate between palm fingers. When there are no water drops between palm fingers, the substrate moisture was considered suitable for mushroom cultivation. This was followed by substrate packaging into transparent heat resistant polypropylene bags weighing 1 kg of moist substrate. The packed substrates were then autoclaved at 121 °C for 1 h and then allowed to cool 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 in Olivier et al. (1991). Three teaspoons of mushroom spawn were inoculated into the bagged substrates in each of the three holes. After inoculation, the bags were placed on the shelves in the incubation room located in the cellar of the soil physics laboratory at the University of Burundi. The room was regularly cleaned and disinfected with diluted Dettol (5% v/v). The mycelium development was daily monitored through direct observations until complete colonization of the substrates. Five replications were performed per treatment (i.e. 5 bags per each treatment). In total, 25 substrate bags were used to fully conduct this experiment.

Fruiting body production

The fruiting body production was done as per 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 (22–27 °C) with relative humidity of 80–90%, well ventilated and with more light which is 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 in casing soil. After harvesting one flush of mushrooms from the substrate bags on shelves, the polypropylene bags covering the colonized substrate were completely removed and the substrates were then buried into casing soil. Briefly, a roofing bag was placed on the shelf in the greenhouse and then a thin layer of soil was spread on it. Thereafter, the substrates were placed aligned on it and then another soil layer of about 2–3 cm thick was added for covering the substrates.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.

Data collection and analysis

The mycelial growth and mushroom development were monitored daily and data were collected on the followings parameters: (1) incubation time i.e., the number of days from inoculation to full colonization, (2) number of sporophores for each substrate bag and flush by flush, (3) the total weight of sporophores harvested on all substrate bags and for all the flushes, (4) the mushroom size defined as total weight of sporophores/number of sporophores, and (5) the total mushroom yield.

Mushrooms were harvested in 3 flushes for a period of 2 months. The yield was determined flush by flush for each treatment and for each domesticated species, after weighing the harvested mushrooms on each substrate bag. The total mushroom yield (MY) was then determined for each treatment by adding together the weights of different flushes and by applying the following formula (Morais et al. 2000):

$$\text{MY (\%)} = \left[\text{Weight of fresh mushrooms harvested (g)} / \text{Fresh substrate weight (g)}\right] * 100$$

The data were analyzed using a Statistical Package for Social Sciences (SPSS) program, version 20.0 (2020). Means (± standard deviations) were determined for colonization time and mushroom yield, and their comparison for different substrates was performed using the Duncan Test.

Results

Morphological characterization

The two domesticated mushroom species were morphologically characterized and identified as *Lentinus squarrulosus* Mont. and *Neonothopanus hygrophanus* (Mont.) De Kesel & Degreef, (Fig. 2).

Lentinus squarrulosus, is a saprotrophic species with sporophores in tufts on dead wood. The cap (2–9 cm diameter), is at first convex and narrowly depressed in the centre then infundibuliform, fleshy, flexible, tough with age, brittle when dry; the margin is entire, curved then inflexed, straight and lobed, acute, becoming eroded jagged, without veil; the pileipellis is creamy-white or fawn, sometimes ochracerous, light brown, dry, radially striated, scaly to squarrose, squames innate to detached, concentric, concolorous or brownish, sometimes completely washed by rain. The st*ipe* is central, eccentric or sublateral, grouped at base, cylindrical, attenuated downwards, curved, solid, white, sometimes brown-stained at base, irregularly squarrose when young, sometimes becoming glabrous or sub-smooth, without ring. The Hymenophore *has* decurrent lamellae, arcuate, tight, narrow (2–3 mm high), rarely forked, slightly interveined at base, lamellulae in subregular series (3–4/lamella), white then creamy-white; the flesh is fibrous, elastic in cap, tough and hard in stipe, white to creamy. The odour is relatively strong, pleasant. The taste mild, pleasant, sometimes slightly pungent (Fig. 2a). *Lentinus squarrosulus* can be confused with *L. cladopus* Lév., which has a completely smooth cap and with some oyster mushrooms, notably *Pleurotus pulmonarius* (Fr.) Quél., which however has no squams on cap and whose lamellae are decurrent up to the base of the stipe as described in Degreef and De Kesel (2017).

Neonothopanus hygrophanus is characterized by its bitter taste and its distinctive bioluminescent properties. Sporophores are found in groups on dead wood. In young stages it is moist and sticky, convex or bell-shaped which expand to flat, dry and smooth as it matures. The cap is firstly white, and soon becomes scattered with brownish to reddish, finally blackish spots. The lamellae are decurrent, broad, fairly thick, spaced and the stipe is short, lateral, solid, glabrous and white. The gills are initially white or cream-colored, but gradually turn brownish as the spores mature. It is unique and fascinating by its bioluminescence where by the entire mushroom, including the cap, gills, and stem, emits a greenish glow in the dark due to the presence of a bioluminescent pigment called luciferin (Fig. 2b). Further elaborate description and illustration can be found in Tibuhwa (2023), Fig. 51.

Neonothopanus hygrophanus can be confused with some *Pleurotus*, especially *P. flabellatus* Sacc., but they differ from it in that they have a sweet taste, a fleshier context and a cap generally not spotted (Degreef and De Kesel 2017).

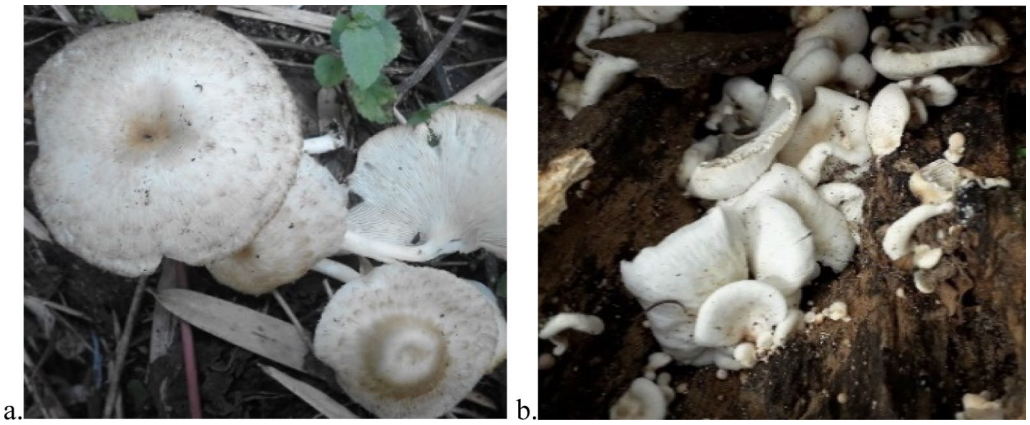

Fig. 2 The studied mushroom species in their natural habitat: (**a**) *Lentinus squarrosulus* (**b**) *Neonothopanus hygrophanus*

Domestication results

The germplasm of *N. hygrophanus* and *L. squarrosulus* was successfully isolated on PDA culture medium. Average incubation time for tissue culture was 6.4 ± 0.54 days for *L. squarrosulus* and 7.6 ± 0.54 days for *N. hygrophanus* while incubation time for spawn production on sorghum grains was 12.6 ± 0.89 days for *L. squarrosulus* and 14.8 ± 0.83 days for *N. hygrophanus* (Table 2). Mycelial colonization on lignocellulosic substrates ranged between 19 ± 1 and 21 ± 1 days for *L. squarrosulus* and between 17 ± 1.22 and 18 ± 1.22 days for *N. hygrophanus* (Table 3). The yield for *L. squarrosulus* ranged between 18.24 ± 9.76 and 22.85 ± 9.16%, while that of *N. hygrophanus ranged between* 12.66 ± 8.95 and 15.3 ± 8.94% (Table 3).

Discussion

This study is reporting for the first time the successful domestication of *Neonothopanus hygrophanus*, except for the study by Dibaluka et al. (2010) on *Nothopanus hygrophanus* (a homotypic synonym of *Neonothopanus hygrophanus*), which isolated the germplasm and produced only the spawn on maize grains. Unlike *Neonothopanus hygrophanus*, several studies have previously reported for the successful domestication or cultivation of *Lentinus squarrosulus* (Nwanze et al. 2005a, b; Dibaluka et al. 2010; Adesina et al. 2011; Ahmad et al. 2013; De Leon et al. 2013; Leon et al. 2017; Osibe and Chiejina 2015; Nteziryayo et al. 2019), which aroused a lot of attention and interest for this mushroom species (Dibaluka et al. 2010). The limited studies on domestication trials for *Neonothopanus hygrophanus* might be due to the

Table 3 Effect of different lignocellulosic substrates on the incubation time, number of fruiting bodies, mushroom size and yield of the studied mushroom species

Species name	Substrate	Average incubation time (days)*	Total nr. of sporophores**	Total weight of sporophores (g)	Mushroom size (g/fruit body)	MY (%)
L. Squarrosulus	CSH	20.4 ± 1.81[a]	31	228.5	7.3	22.85 ± 9.16[a]
	MC	21 ± 1[a]	29	190.6	6.57	19.06 ± 9.67[c]
	RS	20 ± 1[a]	27	182.4	6.75	18.24 ± 9.76[c]
	MC + SBS	19 ± 1.58[b]	31	211.2	6.8	21.12 ± 8.58[b]
	RS + SBS	19 ± 1[b]	30	203	6.76	20.3 ± 8.55[b]
N. hygrophanus	CSH	17 ± 1.58 [d]	42	153	3.64	15.3 ± 8.94[d]
	MC	17 ± 1.22[d]	40	132.5	3.31	13.25 ± 8.93[e]
	RS	17 ± 1.41[d]	37	126.6	3.4	12.66 ± 8.95[e]
	MC + SBS	18 ± 1.22[c]	39	150.4	3.85	15.04 ± 9.8[d]
	RS + SBS	18 ± 1[c]	40	148.4	3.7	14.84 ± 9.14[d]

[*] Five replications were performed for each species; [**]the number of sporophores and the total weight of sporophores were determined from 3 flushes; *nr.*=number

[a,b,c,d,e] Show that there are 4 and 5 different groups of means for incubation time and MY(%) respectively. Means with different superscript letters in the same column are significantly different at $P < 0.05$ according to the Duncan test

Table 2 Incubation time (in days) for tissue culture germplasm isolation and spawn production i.e. from the tissue culture day to full colonization of the PDA culture medium for germplasm isolation and from the inoculation day to full colonization of the sorghum grains in mayonnaise bottles by the mycelium of the studied species

Species name	Incubation time for germplasm isolation on PDA culture medium (days)*	Incubation time for spawn production on sorghum grains (days)*
Lintinus squarrosulus	6.4 ± 0.54^{a}	12.6 ± 0.89^{a}
Neonothopanus hygrophanus	7.6 ± 0.54^{b}	14.8 ± 0.83^{b}

*Five replications were performed for each species and results were expressed as "Mean ± SD"

[a, b] Show that there are 2 different groups of means in each column. Means with different superscript letters in the same column are significantly different at $P < 0.05$

lack of interest in the species because of its small size and bitter taste. However, people living around Kibira National Park consume this mushroom after subjecting it to a pre-treatment referred to as "*Gusabura*" in order to eliminate the bitter taste prior to its consumption (Nteziryayo et al. 2022).

Results from this study showed that *L. squarrosulus* colonized the PDA medium and the sorghum grains more rapidly than *N. hygrophanus.* In contrast, *L. squarrosulus* colonized the different lignocellulosic substrates more slowly than *N. hygrophanus*! The incubation time for germplasm isolation on PDA medium was 6.4 ± 0.54 days for *L. squarrosulus* and 7.6 ± 0.54 days for *N. hygrophanus*. Nteziryayo et al. (2019) reported an incubation time ranging from 6 to 8 for tissue germplasm isolation during domestication of four local mushroom species. De Leon et al. 2017 reported also an incubation period of 6 days for mycelial growth of *Lentinus squarrosulus* on different indigenous culture media including potato sucrose gelatin. In the same line, Hussein et al. (2016) reported a period of 4 days for *L. sajor-caju* and *P. conchatus* to fully colonize the petri plate and 7 days for *Pleurotus umbrosus* on the same culture medium (PDA). The incubation time for spawn production on sorghum grains in this study was 12.6 ± 0.89 days for *L. squarrosulus* and 14.8 ± 0.83 days for *N. hygrophanus*. This is in line with the findings of Nteziryayo et al. (2019) who reported an incubation period ranging between 12 and 15 days for spawn production on mayonnaise bottles containing sorghum grains substrate. These findings do not correlate with those of Dibaluka et al. (2010) who reported a duration of 18 to 21 days for *L. squarrosulus* and *Nothopanus hygrophanus* on maize grains. This could be due to difference in terms of substrate type (sorghum versus maize) and incubation conditions (25 °C in a half-dark and air-conditioned room versus 27°–29 °C and in completely dark place (closed box). Incubation time on different lignocellulosic substrates ranged between 19 ± 1 and 21 ± 1 days for *L. squarrosulus* and between 17 ± 1.22 and 18 ± 1.22 days for *N. hygrophanus*. This result was confusing at the first sight given that *Lentinus squarrosulus* was more rapid than *N. hygrophanus* for tissue culture and spawn production. However, it is well known that mycelial growth rate depends on the type of substrate. Meanwhile, the findings on colonization rate are similar or close to other findings previously reported (Liang et al. 2009; Adesina et al. 2011; Hussein et al. 2016; Nteziryayo et al. 2019). In contrast, Dibaluka et al. (2010) reported a relatively long time of 28–30 days for *Lentinus squarrosulus* and *Pleurotus cystidiosus* to fully colonize the substrates made of sawdust and stems of *Cyperus papyrus*.

In this study, the yield for *L. squarrosulus* ranged between 18.24 ± 9.76 and 22.85 ± 9.16%. This range is consistent with the findings reported in previous studies for the same species (Dibaluka et al. 2010; Adesina et al. 2011; Nteziryayo et al. 2019). The yield for *N. hygrophanus* ranged between 12.66 ± 8.95 and 15.3 ± 8.94%. No literature was found about successful cultivation of *N. hygrophanus* to allow comparison. But in general, the mushroom yields obtained in this study are satisfying according to the yield range close to or above 15–20% (Oei 1993).

Based on the results obtained in this research, the farming of these well-adapted mushroom species appears to be a positive and potential option that will enhance the living standards of people and promote food security and nutrition in Burundi. Indeed, these yield, although lower, are close to the range of yields reported for oyster mushrooms cultivated on different substrates : 18–40% for 3 strains of *Pleurotus ostreatus* cultivated on elephant grass supplemented or not with ground avocado stones (Manirakiza et al. 2014); 21.36–39.77% for *P. citrinopileatus* cultivated on rice straw and bean straw (Musieba et al. 2012), 29–37.1% for *Pleurotus flabellatus* cultivated on composted or non-composted sisal decortication residues (Mshandete andCuff 2008).

Apart from health benefits which one may get from direct consuming mushrooms, grown mushrooms can be sold and become a good source of income. The financial analysis shows that mushroom cultivation and trade can be a veritable business. Tibuhwa (2023) gives an estimated cost analysis and financial benefits for a small-scale oyster

mushroom farm in Tanzania. Taking an example of a small farm of 2 square meters, it shows that one would need a capital estimated of TZS 220,000–280,000/= depending on the location of the farm (village or urban) and can earn up to TZS 600,000–1,000,000. This corresponds to a profit range of 380,000–720,000 for a period between 2–3months depending on the growing area whereby urban areas sell the cultivated mushrooms at good prices compared to the country side. The most interesting thing is that mushrooms have a high yield per unit of land, usually requiring a small area of around 2–8 square meters for significant earnings in a short span of time, apart from the fact that it can be done as a part time job (Tibuhwa 2023).

Concerning substrate performances, the study showed that, for both species, the cottonseed hulls gave the best yield than other used substrates while the rice straw showed the lowest yield (Table 3). Cottonseed hulls are already the most used substrates in Burundi for mushroom cultivation. Previous studies have shown that cottonseed hulls have advantages as a substrate material due to their high water-holding capability and nitrogen content which leads to high mushroom yield (Li et al. 2001; Zhou et al. 2011; Xu et al. 2016). The mixture of substrates i.e., maize cobs + soybean straws (50:50) and rice straws + soybean straw (50:50) showed a total yield close to the one obtained on cottonseed hulls for both species. Royse and May (1982) reported that proteinaceous materials such as ground pigeon pea and soybean stimulate high fruit yield. Moreover, previous studies reported that rice straw (Bano and Srivastava 1962; Jandaik and Kapoor 1974; Khanna and Garcha 1982) and maize cobs (Bhatti et al. 1987) were suitable substrates for cultivations of oyster mushrooms. Furthermore, supplementation of main substrates with nutrients sources or combination of two or more substrates were reported to increase the yields (Jadhav et al. 1998). Likewise, in this study, the substrate mixtures were found to be good alternative to the cottonseed hulls. Indeed, cottonseed hulls in Burundi are very competitive for mushroom cultivation due to their performance in terms of mycelial growth rate and yield. This led to the decrease of their supply and the gradual increase of their prices year by year. To overcome this challenge, studies are being undertaken at the Faculty of Agriculture and Bioengineering to find out alternative substrates with high potential to replace the cottonseed hulls. This study established that the combined substrates maize cobs/soybean straws (50:50) and rice straws/soybean straw (50:50) perform comparatively well as the cottonseed hulls (Table 3), and hence recommend them as good alternative substrates.

Conclusion

In this study, *N. hygrophanus* and *L. squarrosulus* were successfully domesticated and the parameters of their mycelial growth and yields were determined. Owing to the high competition on the cottonseed hulls as the best but expensive substrate, combination of the two substrates, i.e., maize cobs/soybean straws (50:50) and rice straws/soybean straw (50:50) are hereby recommended as alternative substrates. Therefore, we recommended their use as cost-effective and efficient alternative substrates.

Abbreviations

CSH	Cotton seed hulls
DAAD	German Academic Exchange Service
FABI	Faculty of Agriculture and Bio-engineering
KNP	Kibira National Park
MC	Maize cobs
MY	Mushroom yield
OBPE	Burundi office for Environment Protection
PDA	Potato Dextrose Agar
RAFINA	Agricultural Products Processing and Refining Company
RS	Rice straw
SBS	Soybean straws

Acknowledgements

Authors wish to acknowledge the German Academic Exchange Service (DAAD) that funded this study. The University of Dar-es-Salaam, Department of Molecular Biology and Biotechnology and the University of Burundi, Microbiology Laboratory hosted the first author and provided all the facilities for the research. Elias Niyongabo and Désiré Nimpagaritse are acknowledged for their support during the sample collection and morphological identification. Dieudonné Sinzotuma particularly helped during the cultivation step.

Author contributions

Author contributions: VN, DDT and AMM conceived and designed the study; VN performed the experiments, the data collection and analysis and drafted the manuscript. DDT and AMM revised the manuscript draft. All authors read and approved the final manuscript.

Funding

This study was funded by the German Academic Exchange Service (DAAD).

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

The authors agreed to publish the article in the journal.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Department of Molecular Biology and Biotechnology, University of Dar-es-Salaam, P. O. Box 35179, Dar-es-Salaam, Tanzania. [2]Faculty of Agriculture and Bio-engineering (FABI), Microbiology Laboratory, University of Burundi,

P. O. Box 2940, Bujumbura, Burundi. [3]School of Life Sciences and Bio- engineering, Nelson Mandela African Institution of Science and Technology, P.O.Box 447, Arusha, Tanzania.

Received: 14 July 2023 Accepted: 29 December 2023
Published online: 15 January 2024

References

Adedokun OM, Kyalo M, Gnonlonfin B, Wainaina J, Githae D, Skilton R, Harvey J. Mushroom: molecular characterization of indigenous species in the Niger Delta Region of Nigeria. Eur J Hortic Sci. 2016;81(5):273–80.

Adesina FC, Fasidi IO, Adenipekun OC. Cultivation and fruit body production of *Lentinus Squarrosulus* Mont. (Singer) on bark and leaves of fruit trees supplemented with agricultural waste. Afr J Biotechnol. 2011;10:4608–11.

Ahmad R, Al-Shorgani NKN, Abdul Hamid A, Wan Yusoff WM, Daud F. Optimization of medium components using response surface methodology (RSM) for mycelium biomass and exopolysaccharide production by *Lentinus Squarrosulus*. Adv Biosci Biotechnol. 2013;4:1079–85.

Bano Z, Srivastava HC. Studies in the cultivation of *Pleurotus* sp. on paddy straw. Food Sci. 1962;12:363–8.

Bhatti MA, Mir FA, Siddiq M. Effect of different bedding materials on relative yield of oyster mushroom in the successive flushes. Pak J Agric Res. 1987;8:256–9.

Boa E. Wild edible fungi: a global overview of their use and importance to people. In: Non-wood forest products 17. Rome: FAO Publications. 2004. p. 157.

Buyck B, Nzigidahera B. Ethnomycological notes from western Burundi. Belg J Bot. 1995;128:131–8.

Buyck B. Ubwoba: Les champignons comestibles de l'Ouest Du Burundi. Publication agricole no 34. Bruxelles: AGCD; 1994. p. 123.

Degreef J, Demuynck L, Mukandera A, Nyirandayambaje G, Nzigidahera B, De Kesel A. Wild edible mushrooms, a valuable resource for food security and rural development in Burundi and Rwanda. Biotechnol Agron Soc Environ. 2016;20:1–12.

Degreef J, De Kesel A. The edible fungi of tropical Africa annotated database. 2017. www.EFTA-online.org . Accessed 7 Jul 2023.

De Kesel A, Kasongo B, Degreef J, Congo RD. Champignons comestibles du Haut-Katanga. Abc Taxa. 2017;17; p. 290.

De Leon AM, Guinto LJZG, De Ramos PDV, Kalaw SP. Enriched cultivation of *Lentinus squarrosulus* (Mont.) singer: a newly domesticated wild edible mushroom in the Philippines. Mycosphere. 2017;8(3):615–29. https://doi.org/10.5943/mycosphere/8/3/9.

De Leon AM, Reyes RG, Dela Cruz TEE. *Lentinus squarrosulus* and *Polyporus grammocephalus*: newly domesticated, wild edible macrofungi from the Philippines. Philipp Agric Sci. 2013;96:411–8.

Dhouib A, Hamza M, Zouari H, Mechichi T, H'midi R, Labat M, Martínez MJ, Sayadi S. Autochthonous fungal strains with high ligninolytic activities from Tunisian biotopes. Afr J Biotechnol. 2005;4:431–6.

Dibaluka MS, Lukoki FL, De Kesel A, Degreef J. Essais de culture de quelques champignons lignicoles comestibles de la région de Kinshasa (R.D. Congo) sur divers substrats lignocellulosiques. Biotechnol Agron Soc Environ. 2010;14(3):417–22.

Eyi Ndong H, Degreef J, De Kesel A. Champignons comestibles des forêts denses d'Afrique centrale. Taxonomie et identification Abc Taxa. 2011;10; p. 255.

Haq IU. Biology and molecular characterization of *Volvariella* spp. PhD thesis, University of Agriculture of Faisalabad. 2009; p. 127.

Hussein J, Tibuhwa DD, Mshandete AM, Kivaisi AK. Successful domestication of *Lentinus sajor-caju* from indigenous forest in Tanzania. J Appl Biosci. 2016;108:10507–18.

Hussein J, Tibuhwa DD, Tibell S. Phylogenetic position and taxonomy of *Kusaghiporia Usambarensis* gen. et sp. nov.(Polyporales). Mycology. 2018;9(2):136–44.

Jadhav AB, Bagal PK, Jadhav SW. Biochemical changes in different agro-residue to oyster mushroom cultivation. J Maharashtra Agric Univ. 1998;23(1):22–4.

Jandaik CL, Kapoor JN. Studies on cultivation of *Pleurotus sajor-caju* (Fr.). Singer Mush Sci. 1974;9(1):667–72.

Khanna P, Garcha HS. Utilization of paddy straw for cultivation of *Pleurotus* species. Mush Newslett Trop. 1982;2(1):5–9.

Kiyuku P, Bigawa S. Production de *Pennisetum* sp. et son utilisation pour la culture de *Pleurotus Ostreatus* Au Burundi. VertigO Revue Electron Sci Environ. 2013;17:1–16.

Li XJ, Pang YZ, Zhang RH. Compositional changes of cottonseed hull substrate during *P. Ostreatus* growth and the effects on the feeding value of the spent substrate. Bioresour Technol. 2001;80:157–61.

Liang Z, Wu C, Shieh Z, Cheng S. Utilization of grass plants for cultivation of *Pleurotus Citrinopileatus*. Int Biodeterior Biodegrad. 2009;63:509–14.

Manirakiza E, Bigawa S, Ndayishimiye J. Effet de Pennisetum sp. enrichi au broyat de noyaux d'avocats sur le rendement des souches de Pleurotus ostreatus (2125, 2153 et 969). Bull Sci Inst Natl Environ Conserv Nat. 2014;13:60–5.

Morais MH, Ramos AC, Matos N, Santos Oliveira EJ, Note. Production of shiitake mushroom (*Lentinus edodes*) on ligninocellulosic residues. Food Sci Technol Int. 2000;6:123–8.

Mshandete AM, Cuff J. Cultivation of three types of indigenous wild edible mushrooms: Coprinus cinereus, Pleurotus flabellatus and Volvariella volvocea on composted sisal decortications residue in Tanzania. Afr J Biotechnol. 2008;7(24):4551–62.

Musieba F, Okoth S, Mibey RK, Wanjiku S, Moraa K. Suitability of locally available substrates for cultivation of the Kenyan indigenous golden oyster mushroom (*Pleurotus Citrinopileatus* Singer). Am J Food Technol. 2012;7(10):650–5.

Nteziryayo V, Tibuhwa DD, Kiyuku P, Muvunyi R, Masharabu T. Characterization and domestication of wild edible mushrooms from selected indigenous forests in Burundi. Tanz J Sci. 2019;45:417–30.

Nteziryayo V, Tibuhwa DD, Mshandete AM. Indigenous knowledge and utilization of wild mushrooms in communities around Kibira and Bururi mountain forests in Burundi. Asian J Mycol. 2022;5(2):202–19. https://doi.org/10.5943/ajom/5/2/9.

Nwanze PI, Khan AU, Ameh JB, Umoh VJ. The effect of spawn grains, culture media, oil types and rates on carpophore production of *Lentinus squarrosulus* (Mont.) singer. Afr J Biotechnol. 2005;4:472–7.

Nwanze PI, Khan AU, Ameh JB, Umoh VJ. The effect of the interaction of various spawn grains with different culture medium on carpophore dry weights and stipe and pileus diameters of *Lentinus squarrosulus* (Mont.) singer. Afr J Biotechnol. 2005;4(7):615–9.

Nzigidahera B, Habonimana B. Etude des tendances de la biodiversité, des espèces et des écosystèmes fournissant les services écosystémiques: formulation des indicateurs pour mesurer, suivre et rapporter la tendance de la biodiversité Au Burundi. Bujumbura: CEBioS, OBPE; 2016.

Oei P. La culture des champignons. Paris: Gret; 1993. p. 318.

Olivier JM, Laborde J, Poitou N, Houdeau G. La culture des champignons. Paris: Armand Colin; 1991. p. 160.

Osarenkhoe OO, Okhuoya AJ, Theophilus DA. Ethnomycological conspectus of west African mushrooms: an awareness document. Adv Microbiol. 2014;4:39–54.

Osibe DA, Chiejina NV. Assessment of palm press fibre and sawdust-based substrate formulas for efficient carpophore production of *Lentinus Squarrosulus* (Mont.) Singer. Mycobiology. 2015;43:467–74.

Rammeloo J, Walleyn R. The edible fungi of Africa south of the Sahara: a literature survey. Scripta Bot Belg. 1993;5:1–62.

Royse DJ, May B. Use of isozyme variation to identify genotypic classes of *Agaricus brunnescens*. Mycologia. 1982;74:93–102.

Sileshi GW, Tibuhwa DD, Milambo A. Underutilized wild edible fungi and their undervalued ecosystem services in Africa. CABI Agric Biosci. 2023;4(3):1–20.

Tibuhwa DD. Wild mushroom-an underutilized healthy food resource and income generator: experience from Tanzania rural areas. J Ethnobiol Ethnomed. 2013;9(1): 49. https://doi.org/10.1186/1746-4269-9-49.

Tibuhwa DD. Mushrooms of the Serengeti national park: a field guide and technical manual of mushroom cultivation in communities surrounding the Serengeti National Park as alternative crop to solve human-wildlife conflicts. Dar es Salaam: Dar es Salaam University Press (DUP); 2023. p. 225.

Tibuhwa DD, Buyck B, Kivaisi AK, Tibell L. *Cantharellus fistulosus* sp. nov. from Tanzania. Cryptogamie Mycol. 2008;29:129–35.

Xu F, Li Z, Liu Y, Rong C, Wang S. Evaluation of edible mushroom *oudemansiella canarii* cultivation on different lignocellulosic substrates. Saudi J Biol Sci. 2016;23:607–13.
Zhou Q, Gong WQ, Xie CX, Yuan X, Li YB, Bai CP, Chen SH, Xu N. Biosorption of methylene blue from aqueous solution on spent cottonseed hull substrate for *Pleurotus ostreatus* cultivation. Desalina Water Treat. 2011;29:317–25.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.