DOMESTICATION OF INDIGENOUS EDIBLE MUSHROOMS USING THE OPEN-NURSERY SYSTEM

Joseph Mwafaida[1]*, Miguel Okoko[1], Emma Kabugu[2] and James H.P. Kahindi[1]
[1] Department of Biological Sciences, Pwani University, PO Box 195-80108, Kilifi, Kenya
[2] Department of Dietetics and Nutrition, Pwani University, PO Box 195-80108, Kilifi, Kenya
* Corresponding Email: j.mwafaida@pu.ac.ke

Abstract

***Kaya* forests are pockets of natural rainforests in the coastal regions of Kenya. For generations, the more than 50 Kaya forests have sustained communities living around them with food, medicine and fuelwood. Mushrooms have seasonally been harvested from these forests but the harvests have dwindled with time because of population pressure and frequent disturbance on the breeding grounds. This study was intended to domesticate edible mushrooms species collected from Kaya Chivara in Kilifi using the open-nursery system. Spawn from six (6) indigenous edible mushrooms species were prepared and cultured on plots under a baobab (*Adansonia digitata*) tree canopy using a complete randomized block design. *Hakoranyani*, (*Rusulla sp), Mwasi* (*Tylopilus sp)*, *Mbwate (Agaricus sp), Choga nyama* (*Agaricus sp*). *Mhama* (*Rusulla sp)*, *Choga sikiro reruhe* (*Pleurotus sp*) were evaluated against a commercial mushroom, the *Pleurotus spp* in this trial. Moisture was regularly maintained and daily harvest (g) entries were made for each replicated plot. Cumulative weekly harvest (g) for each species were then computed for the four-week harvest season. Commercial mushroom, the *Pleurotus* species had an average yield of 360g per week *Chogasikiro reruhe* yieded 315g, *Mwasi* (300g), *Muhama* (260g), *Hakoranyani* (249g), *Mbwate* (191g) and *Choga Nyama* at 120g. There was significant statistical difference ($p < 0.05$) among the species in production. *Choga sikiro reruhe, Mwasi, Muhama* and *Hakoranyani* adapted well to open nursery system outside the forests. Interestingly, contamination by *Trichoderma* spp. was negligible under this system. The higher productivity of commercial species could be attributed to the purity of the spawn and the non-mycorrhizal dependence of most commercial mushroom species compared to indigenous species which require symbiotic relationships with specific forest trees for optimal blooms. It is prudent to further evaluate these mushrooms species for conservation and commercialization purposes.**

Key word: Domestication, Indigenous edible mushrooms, Open nursery cultures

INTRODUCTION

Cultivation of mushrooms is a natural process that recycles lignocellulosic organic waste in the environment (Muthangya *et al*., 2013). Mushroom production can commercially be employed to produce wild and domesticated edible mushrooms for food as well as supply useful bioproducts to the pharmaceutical industry. Numerous edible mushroom species including the Paddy straw (*Volvariella* spp), Oyster (*Pleurotus eryngnii*), Button (*Agaricus* spp), *Shiitake* (*Lentinulla* spp), Jews ear (*Auricularia* spp) have been domesticated and commercialized (Taylor, 2019). Out of the 300 edible mushroom species, about, ten (10) are grown commercially. *Agaricus spp* accounts for 97% of the mushrooms produced worldwide (Pilz & Molina, 2002).

Mushroom cultivation is rapidly growing in many parts of Asia, Africa and USA (Manna & Roy, 2014). In 2008, the production of mushrooms was estimated at 3.4 million Tons globally with the largest producers being China and USA at 1.5 and 0.38 million Tons, respectively *(Kumar et al*., 2014). The global demand is occasioned by increased population, and demand by the pharmaceutical and biomedical industries. The current global supply of 3.4M Tons does not even meet 25% of the global demand (Sharma *et al*., 2017). Annually, Kenya produces a paltry 500 Tons of edible mushrooms where 95% of the production accounts for button mushrooms at 476 Tons, while Oyster mushrooms account for about 5% (NFIS, 2015). Kenya is therefore a net importer of mushroom with over 150 Tons required to fix the existing gap in the market demand. There is need therefore to increase production to meet the demand by exporters, pharmaceutical and food industries (Mathew *et al.*, 2009). Commercial mushroom production provides employment opportunities and market for farm-waste that may sustain local livelihoods (Paul, 2020).

Mushroom production is unique because it relies on dead organic matter for nutrients to develop fruiting bodies (Galanda *et al*., 2014). Over the wet seasons, it occurs naturally in the forest when conditions allow. In Kenya, communities living around forests and natural reserves seasonally harvest wild mushrooms for both subsistence and commercial purposes. This has however not been harnessed to its full potential compared to Asia where protection and re-seeding of breeding grounds are managed by local governments. *Matsutake* mushrooms in Asia have thrived well in the market as a result of strict management strategies within the forested regions. In China, the Forest Bureau of Nanhua County, monitors breeding grounds and harvesting patterns to allow for effective development of these wild mushrooms (Yang *et al*., 2009).

The Kenyan coastal line is dotted with over 50 pockets of forests referred to as the Kaya. Kaya are sacred grounds for prayer and rituals for the Mijikenda community. Recently ten (10) of these Kaya forests were recognized as centers for conservation of biodiversity by UNESCO (2008). The blended cultural and ritual values in these communities have conserved the Kaya forests for decades. However, population pressure and mining have increasingly threatened the existence of some of the Kaya forests. Kaya Chivara that sits in Jaribuni region of Kilifi County is an evergreen natural forest rich in biodiversity. Despite the efforts in conservation, Kaya Chivara remains under threat due to encroachment and settlements (Centre, 2008). The forest supports livelihoods, as a source of food, fuelwood and medicine to communities living in its proximity. Mushrooms are an important source of protein that supplement local diets and have seasonally been harvested from these forests. Mushroom "bloom" comes during the wet season (April-June) but with the recent climate changes the harvest seasons have adversely been shortened. Further, mining, lumbering and firewood collection have continuously disrupted the mushroom breeding grounds. Harvests have therefore dwindled and a variety of unique mushroom species that were previously harvested are no longer available.

This project therefore intended to salvage available mushroom species in Kaya Chivara and develop protocols to domesticate them on kitchen gardens to supplement diets in the community. Adoption of this cultivation system shall make unique indigenous mushroom species available to consumers throughout the year. Domestication and re-seeding programs therefore are expected to add value to the conservation efforts of these indigenous edible mushroom species. This study therefore demonstrates domestication

efforts using simple open-nursey system which is simple, cost effective and applicable to low-income producers.

MATERIALS AND METHODS

Study site

Kaya Chivara forest is located at S3 47 55.00 E39 30 52.00 within the Kilifi County of Kenya. Its natural biodiversity has remained intact because it houses a sacred groove managed by the Jibana community. The forest is divided into four sub-sections; South Eastern, South West, North East and South West. The dense South Eastern part of the forest was surveyed for mushrooms in this study based on abundance and established natural breeding grounds.

Sample collection

A group of skilled elders and staff from the National Museums of Kenya (Kilifi) guided the survey and collection of mushrooms using morphological traits based on folklore. A purposive sampling method was adopted where perceived edible mushrooms were collected from the canopies of *Brachystegia speciformis* that housed breeding grounds. Mushrooms with insect or rodent bites, gilled with no spores, those with no latex when pricked and species with local names were considered edible and sampled. Images of sited edible mushrooms were taken in-situ, together with GPS coordinates before harvesting. The local names and related literature on edibility, taste and local recipes were recorded from the elders for further verification. The collected samples were transported in cooler boxes and stored at 4^{o}C for further domestication trials.

Identification of mushroom samples

The color, size and shape of the mushroom caps and stipes, color, texture and pattern of gills were used as markers and matched with existing images and literature to proximate the genus of the mushroom sample using mushroom identification guides by Marklud & Holmberg, (2013) and Jordan, (2016). Collected mushroom images were also run through available digital image search platforms for proximate matching with previously described mushroom species.

Spawn preparation

The collected mushroom samples were washed using sterile distilled water and put into labeled sample jars. The samples were then surface sterilized in 70% ethanol for 10 seconds, then washed three (3) times in series of sterile distilled water (SDW) before air-drying on sterile blotting paper in a lamina flow. The dried mushroom pieces were then plated on acidified Potato Dextrose Agar (PDA) adjusted with 3-5 drops of 3% Acetic acid. The setups were then sealed and incubated at 25^{o}C for 48-72 hours. The developed mycelia were then sub-cultured on water agar (WA) and incubated for 2 days at 25^{o}C. Single hyphae were then cut from the WA plates and cultured on fresh PDA at 25˚C for another 72 hours. The developed pure cultures of each mushroom species were used to inoculate the barley grains. Barley grains (100g) were soaked in 80ml of sterile distilled water (SDW) in a conical flask and autoclaved at 121^{o}C for 20 minutes. The sterile barley grains were then allowed to cool for 1 hour before 3-5 plugs cut from the freshly grown pure mushroom cultures on PDA were introduced. The setup flasks were then sealed and incubated for 10 days at 25^{o}C. Thereafter the flasks were shaken every day to improve colonization until full grain colonization was achieved. After 21 days, the mushroom spawn with fully colonized barley grains were ready for inoculation in open-nursery cultures.

Experimental Design

A Complete Randomized Block Design (CRBD) consisting of 21 plots measuring 1x1m each was adopted. Spawn (100g) from

each of the seven (7) evaluated mushroom species were randomly sown in three (3) replicates randomized within the block.

Preparation of mushroom nursery

A site under a baobab (*Adansonia digitata*) canopy measuring 4m x 10m was prepared and sub-divided into twenty-one (21) plots each measuring 1x1m with 0.5m path in between them (Fig. 1). The soil was excavated from each plot and replaced with 2-3 inches of chopped dried maize stovers. Spawn (100g) prepared from *Hakoranyani*, (*Rusulla sp), Mwasi* (*Rusulla sp)*, *Mbwate (Agaricus sp), Choga nyama* (*Agaricus sp*) *Muhama* (*Agaricus sp)*, *Choga sikiro reruhe* (*Pleurotus sp*), and a commercial mushroom, the *Pleurotus spp* were each sown in three (3) replicates on randomly identified plots within the block. The spawn was introduced directly onto to the maize stovers then covered with a thin (4 cm) layer of soil. Watering (3lts/plot) was then done until the plots were completely wet and regularly maintained throughout the experiment.

Fig 1. Nursery layout three (3) weeks after inoculation

Data collection and Analysis

Mushroom harvests (g) were accumulated per week for each plot across the replicates to get weekly production for each plot. At the end of the four-week harvest season, four (4) entries in grams per species were used to compare performance. The weekly data was analyzed using R-software statistical tool. The data was subjected to analysis of variance (ANOVA) and significant means separated at $\alpha = 0.05$

RESULTS

Indigenous edible mushrooms of Kaya Chivara

Eight (8) indigenous species of mushrooms were collected from Kaya Chivara. These were partially identified as *Rusulla ametica* for *Hakoranyani*, *Pleurotus eryngii* (*Mwasi*), *Rusulla fragrantisma* (*Muhama*), *Agaricus arvensis* (*Mbwate*), *Pleurotus populinus (Choga sikiro reruhe*), *Agaricus bisporus* (*Choga rerema*), *Agaricus spp.* (*Choga nyama*.) and *Agaricus xanthoderma Choga Kadzonzo*. Figure. 2 below describes the morphological characteristics of the study mushroom species as described in our related previous work (Mwafaida *et al*., 2022).

Fig 2: Morphology of indigenous mushroom species collected from Kaya Chivara
1). Hakkoranyani, 2). Mwasi, 3). Muhama, 4). Mbwate, 5). Choga sikiro reruhe, 6). Choga rerema, 7). Choga nyama, 8. Chaga Kadzonzo and 9. Commercial Oyster mushroom for comparison

Productivity of mushrooms under open nursey system

Thirteen (13) days after inoculation, the first harvest was done which lasted for 40 days. Daily harvest entries were made for each plot and cumulative harvest (g) per week for each species were computed across the replications to assess productivity per species per week over the production season. The average cap-weight of a single mushroom ranged from 10-15g for most species. However individual differences per mushroom species were noted.

Commercial mushroom, the *Pleurotus* species had an average yield of 360g per week. This was significantly higher than *Choga sikiro rehure* at 315g, *Muhama* (260g), *Mwasi* (250g), *Hakoranyani* (249g), *Mbwate* (191g) and lastly *Choga nyama* at 120g (P = 0.05). Weekly productivity within the study species did not show any significant variations except for Choga sikiro reruhe at P = 0.05 (Table 1). However, variations in performance was noted across the species when compared to commercial mushroom species (Fig.3). Practically, 12-36 mushroom caps were harvested per week from a 1square metered plot.

Table 1. Productivity (g) of indigenous edible mushroom species under open nursery system

Mushroom spp.	Week/Weight (g)				Mean (g)	*p*-value
	1	2	3	4		
Hakoranyani	320	210	315	150	**249**	0.3708
Mwasi	150	380	250	420	**300**	0.2895
Mbwate	125	270	150	220	**191**	0.6779
Choga nyama	80	180	130	90	**120**	0.9432
Muhama	305	215	385	135	**260**	0.5954
Choga sikiro reruhe	185	260	395	420	**315**	0.0281
Commercial	355	470	410	205	**360**	0.4199

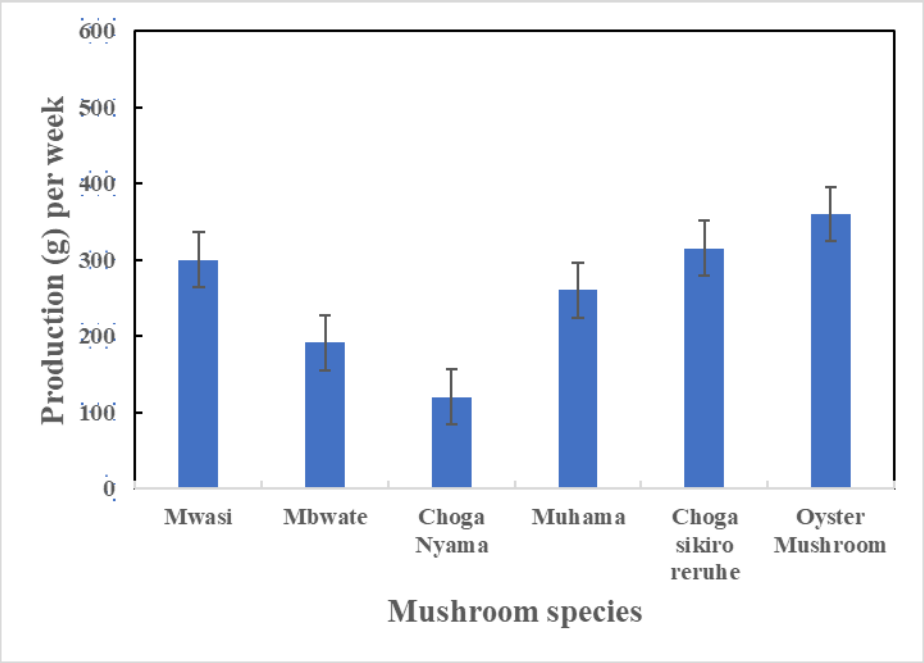

Fig 3: Productivity of selected indigenous edible mushroom species on open-nursey system

DISCUSSION

Identifying edible mushrooms

The Kaya forests are natural habitats for several species of both edible and toxic mushrooms. The local communities have for years interacted with useful mushrooms as a source of food and medicine. Communities have identified useful mushrooms and have coined names to species they have interacted with in reference to their morphologies, tastes and other natural occurring features in the forest. *Hakkoranyani* for example literarily refers to the brightly red-colored rumps of baboons during the mating season; while *Choga sikiro reruhe* literally means the "white-eared mushroom. Interestingly, mushroom harvesting is quite a skillful art and elders appreciate the dangers posed by any slight misjudgments when interacting with new mushroom species. Signs of insect and rodent bites on the mushroom caps are a basic criterion used to determine whether a new mushroom species is edible or not. The mushroom cap is further ripped to probe whether the species releases any latex. Latex producing mushrooms are considered poisonous by the community which is in line with the basic phytochemistry inference for plants and mushrooms (Vijayan & Thampuran, 2004). Species that pass these two tests are further subjected to confirmatory tests that include rubbing the mushroom onto skin. Mushrooms that cause skin irritation are considered poisonous. Lastly, tip-tongue testing is done to probe for any acrid or unpleasant tastes before the species is accepted as edible. This indigenous knowledge is however threatened because the youth are not interested. There is really danger of losing thee skills with the ageing society unless proper documentation is done.

Concept of open nursery system

Our previous undocumented work, noted that spawn contaminated with *Trichoderma species* completely failed to establish cultures on sterile substrates and could not produce any mushroom *blooms* under favorable conditions. However, when the contaminated spawn was disposed, clean and healthy mushroom cultures were observed around the dumpsites. This revelation was used to naturally purify contaminated spawn by allowing the natural microbial competitors to suppress the growth

of *Trichoderma* spp. and other bio-contaminants to allow viable grains spawn to germinate into clean and healthy mushrooms *blooms*. These *blooms* are thereafter aseptically handled to produce fresh and clean mushroom spawns. Soil contains natural competitors that suppress the development of parasitic microbes to mushrooms which allows them to complete their growth cycle and fruit into beautiful mushroom cultures. This is also supported by the natural occurrence of clean mushrooms in the wild and forests canopy where open harvesting has contributed to mushroom production in the world.

Based on this revelation, the open-nursey system was proposed for production of these indigenous edible mushroom species. The Open-Nursery System (ONS) is therefore a trial-production system that intends to produce mushrooms naturally without employing any aseptic protocols that are inaccessible to subsistence producers. The expensive sterile conditions and high-tech equipment is therefore not necessary to achieve production at subsistance level using this protocol. Although this study did not undertake any cost-benefit analysis, ONS is a low-cost production system that can be adopted for kitchen gardens and for re-seeding breeding grounds where communities harvest in-season mushrooms.

Conventional mushroom production today is optimized to utilize domestic water, high electricity demands and other consumables. The highest share of energy consumption is taken by composting at 49% followed by fuel or electricity cost at 45% to maintain production temperatures. Optional use of solar power cuts back on this cost (Robinson *et al*., 2019). The conventional mushroom production systems involve re-use of waste which is usually a plus to environmental conservation. However, the production process require high cost on energy and some financial muscle (Zisopoulos *et al.*, 2016). Besides requiring some financial resources to establish, mushroom production is generally a profitable agricultural activity (Mutema *et al*., 2019). In comparison, the open-nursery system does not require any specialized equipment as long as sterile spawn is provided. The natural shade and moisture in the substrate are adequate for production.

Upscaling ONS for commercial purposes may reduce production cost and ultimately increase availability of mushrooms in the market. Utilizing forest canopy to increase biodiversity through edible mushrooms is a feasible reality where communities are encouraged to conserve the forest while mushroom farming under the canopy. This will give communities a bigger sense of ownership to protect forests and related natural resources in their proximity. Re-seeding is the introduction of sterile spawn into the natural environment where microbial dynamics shield mushroom cultures from *Trichoderma* spp., *Aspergillus* spp. and *Listeria monocytogenes*. These microbes are self-regulating in the soil forming a formidable biocontrol system suitable for open nursery mushroom production (Dygico *et al*., 2020). However, more water is required to maintain the requisite moisture for production. The *blooms* are exposed to high air temperatures in summer which may negatively impact on quality, performance and general productivity. Once a site is seeded, a continuous production of mushrooms *blooms* may be experience for a long season with occasional harvests appearing unexpectedly when the rains come. Meaningful production is however observed in the first four weeks of *blooming*.

Conclusion

Mushrooms just like any other crop can thrive well in natural environment as long as the necessary conditions are provided. Maintaining a moist substrate is however

critical. Over application results in soggy conditions that invite green molds and other fungi which may compete with the mushroom cultures. On average, 300g per week per square meter translates to 3Kg of mushrooms per week on a 10 square-meter kitchen garden. This is enough to supplement protein requirements in a household. Mushrooms make good business. Comparatively, mushrooms fetch twice as much as most meat products (wt/wt) in Kenyan markets today. Having some surplus mushrooms can therefore be good business to these communities.

Recomendations

The open nursery system in mushroom is a low input system that should be recommended for kitchen gardens to supplement local diets on proteins. Provision of quality spawn is however a pre-requisite. Spawn production requires skills, equipment and sterile environment. Pwani University has built capacity to domesticate wild mushroom species and provide spawn for these unique mushroom species. Germplasm of these mushroom species need to be deposited with relevant institutions for conservation. It shall be prudent to re-seed more forest sites to improve seasonal mushroom harvests as a payback plan to communities that willingly shared skills and information on these wild edible mushrooms. Any commercialization efforts on these unique mushroom species need to recognize the contribution of communities in Kaya Chivara where the species rightfully belong.

Acknowledgments

This work was supported by Islamic Development Bank (2020)

References

Centre, U. W. H. (n.d.). *Sacred Mijikenda Kaya Forests*. UNESCO World Heritage Centre. Retrieved December 20, 2020, from https://whc.unesco.org/en/list/1231/

Dygico, L. K., Gahan, C. G. M., Grogan, H., & Burgess, C. M. (2020). The ability of Listeria monocytogenes to form biofilm on surfaces relevant to the mushroom production environment. *International Journal of Food Microbiology*, *317*, 108385. https://doi.org/10.1016/j.ijfoodmicro.2019.108385

Galanda, D., Mátel, L., Strišovská, J., & Dulanská, S. (2014). Mycoremediation: The study of transfer factor for plutonium and americium uptake from the ground. *Journal of Radioanalytical and Nuclear Chemistry*, *299*(3), 1411–1416.

Kemp, M., & Pratt, N. (2019). Uncovering Consumer Demand for Proactive Nutrition Solutions (P17-007-19). *Current Developments in Nutrition*, *3*(Supplement_1), nzz038-P17.

Kumar, S., Chand, G., Srivastava, J. N., & Ahmad, M. S. (2014). Postharvest technology of button mushroom: A socio-economic feasibility. *J Postharvest Technol*, *2*, 136–145.

Manna, S., & Roy, A. (2014). Economic contribution of wild edible mushrooms to a forest fringe ethnic community in some eastern lateritic parts of India. *Journal of Forest Research*, *19*(1), 52–61.

Mathew, G., A.W, M., & M.W, W. (2009, May). *Cultivation and commercialization of edible mushrooms in Kenya: a review of prospects and challenges for smallholder production | International Society for Horticultural Science*. http://www.actahort.org/books/806/806_59.htm

Mutema, M., Basira, K., Savadye, D., & Parawira, W. (2019). Assessment of Oyster Mushroom Production and Profitability in Harare Urban and Periurban Areas (RUWA), Zimbabwe. *Tanzania Journal of Science*, *45*(1), 114–130. https://doi.org/10.4314/tjs.v45i1

Muthangya, M., Mshandete, A. M., Hashim, S. O., Amana, M. J., & Kivaisi, A. K. (2013). Evaluation of enzymatic activity during vegetative growth and fruiting of Pleurotus HK 37 on Agave sisalana saline solid waste. *Journal of Chemical, Biological and Physical Sciences (JCBPS)*, *4*(1), 247.

Mwafaida, J., Okoko, M., & Kahindi, J. H. (2022). The occurrence of indigenous edible mushrooms in Kaya Chivara forest in Kilifi,

Kenya. *African Journal of Horticultural Science*, *19*.

NFIS, N. (2015, November 30). Mushroom Farming in Kenya: How You Can Make Your First Ksh1,000,000 In Just 2 Months. *Kuza Blog*. http://www.kuzabiashara.co.ke/blog/mushroom-millions/

Paul, K. (2020, February). *Small-scale farmers who are making over Sh0.5m a year from mushroom farming*. Farmbiz Africa. https://farmbizafrica.com/profit-boosters/2025-small-scale-farmers-who-are-making-over-sh0-5m-a-year-from-mushroom-farming

Pilz, D., & Molina, R. (2002). Commercial harvests of edible mushrooms from the forests of the Pacific Northwest United States: Issues, management, and monitoring for sustainability. *Forest Ecology and Management*, *155*(1–3), 3–16.

Robinson, B., Winans, K., Kendall, A., Dlott, J., & Dlott, F. (2019). A life cycle assessment of Agaricus bisporus mushroom production in the USA. *The International Journal of Life Cycle Assessment*, *24*(3), 456–467.

Sharma, V. P., Annepu, S. K., Gautam, Y., Singh, M., & Kamal, S. (2017). Status of mushroom production in India. *Mushroom Research*, *26*(2), 111–120.

Taylor, T. (2019). *Twelve edible mushrooms of the United States* [Article]. Wikisource.

Vijayan, K. K., & Thampuran, R. A. (2004). 11 Pharmacology and Toxicology of Cinnamon and Cassia. *Cinnamon and Cassia: The Genus Cinnamomum*, *259*.

Yang, X., Wilkes, A., Yang, Y., Xu, J., Geslani, C. S., Yang, X., Gao, F., Yang, J., & Robinson, B. (2009). Common and privatized: Conditions for wise management of matsutake mushrooms in Northwest Yunnan province, China. *Ecology and Society*, *14*(2).

Zisopoulos, F. K., Becerra Ramírez, H. A., van der Goot, A. J., & Boom, R. M. (2016). A resource efficiency assessment of the industrial mushroom production chain: The influence of data variability. *Journal of Cleaner Production*, *126*, 394–408. https://doi.org/10.1016/j.jclepro.2016.03.066

Blank Page