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Successful domestication of *Lentinus sajor-caju* from an indigenous forest in Tanzania.

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ABSTRACT

Objective: Indigenous forests in Tanzania provide a good environment for saprophytic wild edible mushrooms (SWEM) to grow. In rural areas, mushroom eaters rely on seasonal collection of wild edible mushrooms during the rain seasons, which make them unreliable source of nutrients during dry seasons. The study aims to identify and domesticate SWEM so that it is available throughout the year.

Methodology and results: Eight types of SWEM were identified, collected; their germplasm isolated and experimented for domestication their biological efficiency. Three species out of eight were successfully isolated and included *Pluteus umbrosus, Lentinus sajor-caju* and *Panus conchatus*. Of the three isolates, only one species *L. sajor-caju* was successfully domesticated and formed fruit bodies at 18% biological efficiency. Furthermore, results on vegetative growth on mixed substrates of dried banana (70%) and wood shred (30%) administered at different inoculation rates of 1%, 3% and 5% in glass columns revealed different colonization rates with the highest record of 24.94±0.38 mm/day at 3% spawn inoculation with *P. conchatus.*

Conclusion and application of results: The successful domestication of *L. sajor-caju* shows the potential of SWEM being cultivated and is a stepping-stone towards more domestication of indigenous mushroom species. More studies on optimizing its growth parameter are recommended for growing and introduction to mushroom growing industry.

Key words- Colonization, Domestication, Indigenous forests, *Lentinus sajor-caju*, SWEM

INTRODUCTION

Wild edible mushrooms cultivation is a biotechnological process for lignocellulosic organic waste recycling (Muthangya *et al*., 2013). The production of mushrooms is regarded as the second most important commercial microbial technology next to yeast (Xia *et al*., 2016). The world production of mushrooms was around 3.4 million tons in 2008, the largest producers being China, with 1.5 million tons, and the USA with 0.38 million tonnes (Mishra, 2012, Kumar *et al*., 2014). The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Pleurotus* species, *Lentinula edodes*, and *Flammulina velutipes* (Valverde *et al*., 2015). More than 25 species out of the 2000 mushrooms that are said to be main edible species are cultivated commercially, and only ten of those are on an industrial scale (Reis *et al*., 2012, Hussein *et al*., 2015, Rezaeian *et al*., 2016, Juma *et al*., 2016). Over the past few decades, interest in commercial harvesting of Wild Edible Mushrooms has been increasing considerably in many parts of the world (Tibuhwa, 2013, Manna , 2014). Cultivating

mushroom is quite dissimilar with growing green plants since mushrooms are fungi do not contain chlorophyll and therefore obtain food from decaying organic matter (Daba *et al*., 2008). They absorb nutrients from the substrate they grow and incorporate those nutrients into their fruit bodies (Galanda *et al*., 2014). Traditionally wild edible mushrooms have been obtained through collection in the forest specifically during rain seasons. This makes them seasonal and not available throughout the year. Climate change has also contributed towards decreases of mushrooms in the forests apart from the growing human population, pollution, human occupational activities including firewood gathering, bush burning, agriculture methods and logging) and urbanization (Okigbo , Nwatu, 2015). As a result, this limits the location of mushrooms deeper into forests, hence demands extra hunting efforts and more risk including exposure to wild animals (Osarenkhoe *et al*., 2014). Currently there is a growing interest in cultivation of mushrooms to supplement, or replace the wild harvest (Mishra, 2012). Mushroom collection, local utilization and preservation have driven the priority for their domestication (cultivation) in the course of time. In some countries of western Africa, portable logs previously colonized by mushrooms are relocated from the wild to home gardens where they are nurtured until the appearance of the next flush (Osarenkhoe *et al*., 2014). Mushroom cultivation process involves providing medium and the right environment for the fungi (mushroom) to expand their mycelia to the point the mycelia mass will transform into new fruiting bodies (Mensah, 2015). In Tanzania, domestication of the saprophytic edible mushrooms has recently gained popularity (Raymond *et al*., 2013) since its introduction by the ministry of agriculture and cooperative in 1993. Tanzania has a very large availability of various types of raw substrate material such as sugarcane bagasse, wheat straw, paddy straw, chicken manure, de-oiled cakes banana peels and many other agricultural industrial wastes. Domestication of wild edible mushrooms can provide year-round source of nitrogen, and increase household incomes, specifically for women and children who are main collector and growers (Djikeng, 2013, Hasan *et al*., 2015). Very few studies have been done in the country to domesticate indigenous mushroom. They include those by Mshandete and Cuff (2008) who domesticated four species of SWEM namely *Coprinus cinereus*, *Plerotus flabellatus, Volvariella volvaceae and by* Magingo *et al*. (2004) who domesticated *Oudemensiella tanzanica* nom. pro. This study presents one more successfully domesticated SWEM, *Lentinus sajor-caju* from a Tanzanian indigenous forest.

MATERIAL AND METHODS

Sample collection: The study was conducted within natural forests in Tanga region, and Kazimzumbwi forest near Kisarawe town in the Coast region (Figure 1). Topography of these natural forests provides favourable conditions for the mushrooms to grow. The study targeted natural forests and sites were selected on basis of close proximity to the University due to budget constraint. In selecting the study sites, an experienced mycologist was engaged and suggested the natural forests where saprophytic mushrooms were most likely to be found. The study samples were collected during the rainy season from April to May and November to December 2012. Mushroom samples were collected as per Hussein *et al*. (2014). The collected mushrooms were *Polyporus tenuiculus*, *Lentinus sajor-caju* W (Wild), *Lentinus squarrosulus, Macrolepiota procera, Panus conchatus,* and *Auricularia auricular-judae*. All collected samples were subjected to domestication trials.

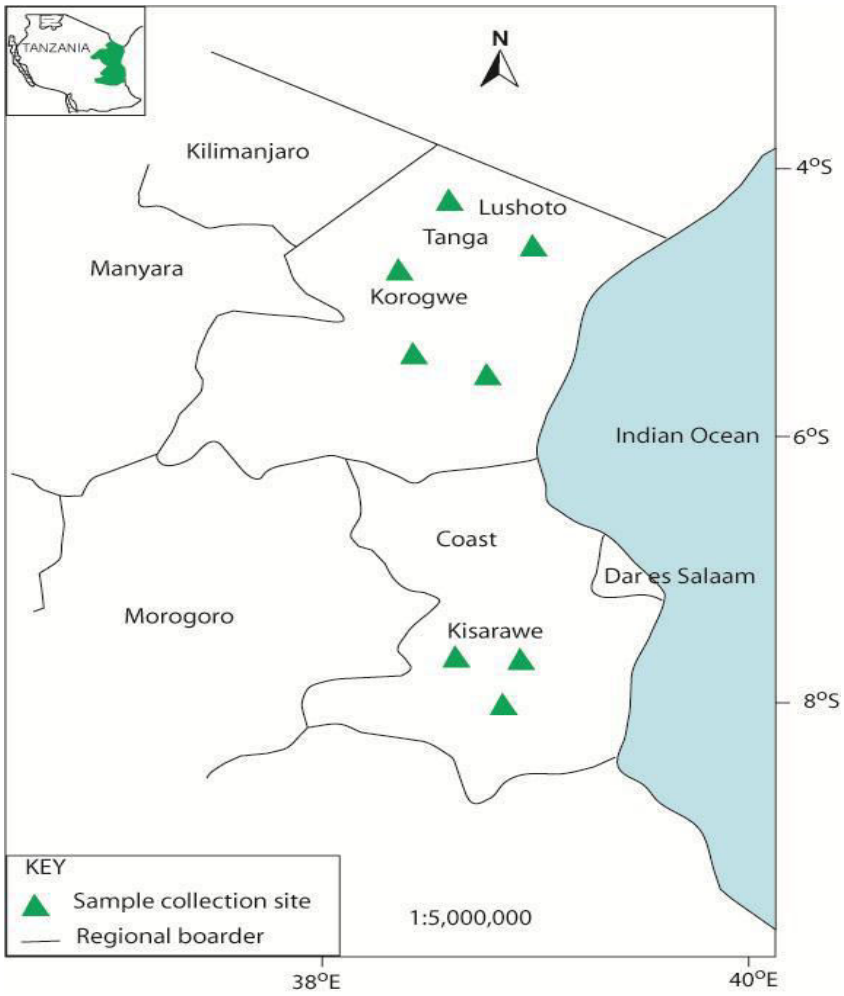

Figure 1: The map of Tanzania showing sampling sites.

Tissue culture: Culture for domestication of SWEM was prepared as described by Tibuhwa (2012). It involved cleaning of collected fresh fruit bodies from the field by thoroughly pre-washing in water, and then wiped clean with a damp paper towel to remove dirty and damaged external tissues. They were then swabbed with ethanol (70% (v/v) to remove any contaminant on the surface. Small inner part tissue were aseptically taken from a young and healthy fruit body and transferred to the petri dish containing potato dextrose agar (PDA) (OXOID Ltd Basingstoke Hampshire, England) and malt extract agar (MEA) (OXOID Ltd Basingstoke Hampshire, England). Sub culturing was performed to obtain pure cultures and the inoculated petri dishes were incubated upside down at 28°C in the dark for 4 days for *L. sajor-caju* and *P. conchatus* and for 7 days for *P. umbrosus*. Pure cultures obtained were preserved on MEA (OXOID Ltd Basingstoke Hampshire, England) at 4°C by subculturing onto fresh media after every one month (Mshandete, 2011) for short term preservation. Long-term storage of the culture was done in liquid nitrogen (LN2) at -196°C in a LN2 unit (Thermolyne (Bio-Cane TM 20, CAN and CANE SYSTEM, USA).

Spawn preparation: Spawn was prepared as per Mshandete (2011) on intact sorghum. The grains were washed thoroughly, and 3 kilogram of the grains was soaked in 6 liters of water for 20 min then paraboiled for 15 minutes. The grains were left for 10 minutes in hot water to allow them to absorb moisture. They were then decanted over a sieve to remove excess water and allowed to cool to ambient temperature. Thereafter, they were mixed with 1% (w/w) $CaSO_4$ and 2% (w/w) $CaCO_3$ to prevent sticking and for adjusting the pH, respectively. One hundred gram wet weight grains were put in 500 ml wide mouth bottles which were later on covered with aluminium foil. Bottles were sterilized at 121°C and 1

atmosphere for 20 minutes. Thereafter the grains were aseptically inoculated with four 1 cm^2 pieces of mycelia taken from 7 days old cultures of *Pluteus umbrosus* (ID 32) (Plate 1a), and 4 days old for *Lentinus sajor-caju* (ID 36) and *Panus conchatus* (ID 44) (Plate 1d). The inoculated grains were incubated in darkness at 28 ± 2 ºC until they were fully colonized by mycelia (Plate 2b).

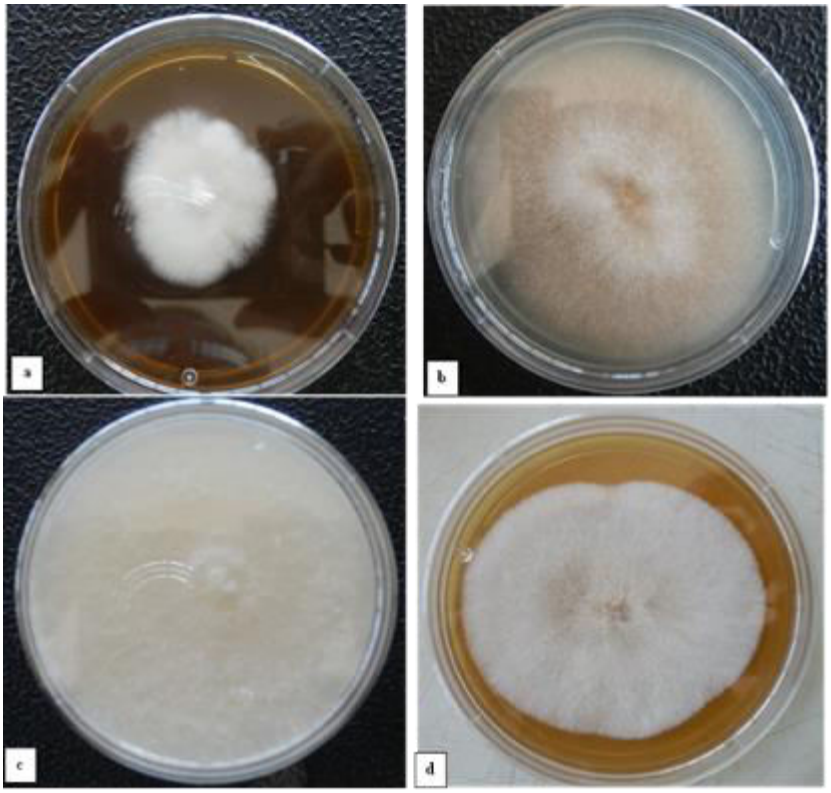

Plate 1: Tissue culture of studied mushroom species (a) *P. umborosus* young and b after 7 days of colonization (c) fully colonized plate of *L. sajor-caju* and (d) *P. conchatus*.

Substrate preparation: Substrate preparation was done according to Onyango *et al*. (2011). All fresh substrates (banana leaves, wood shreds) were sun dried for seven days before any degradation had occurred. Banana leaves were chopped into 3-7 cm long bits (Plate 2 a) followed by soaking in tap water overnight (relative hours 12 hours) and for 4 days for dried banana leaves and wood shred, respectively for moisture absorption. Excess water was allowed to drain until no water was dripping from the substrates. A palm squeeze test was then performed to ensure the right amount of moisture in the substrates. With no water dripping between palm fingers confirmed, that moisture in the substrate was correct to sustain mushroom cultivation. The substrate used was a mixture of 70% banana leaves and 30% wood shreds. Five hundred grams 500 grams wet weight) of each substrate (which corresponded to 150 g dry weight of substrate) was packed into transparent polypropylene bags each bag was kept open on both ends but tied loosely by a sisal rope. The bags were autoclaved at 121ºC for 3 hours (Koninklijke AD Linden JR.BN-Zwijinderect, Holland). The substrates were allowed to cool for 12 hours before inoculation with spawn.

Plate 2: (a) Substrate preparation by chopping into small size (3-7 cm long) (b)Sorghum grain fully colonized by mycelia in spawn (c-d) Vegetative growth of mushrooms under domestication trials 3% and 5% in plates c and d respectively (e) Early stage mycelia colonization on

Vegetative growth measurement: Two hundred 200g (w/w) of prepared substrate as explained above was packed into punched glass column (with volume 500 mL, height 27.4 cm, inner diameter 4.7 cm, outer diameter 5.5 cm, 20 holes each 9 mm diameter for aeration were made each 5 cm apart). Each 200 g (w/w) was equivalent to 95 g dry weight of substrate, which was a mixture of banana leaves 70 % and wood shred 30 %. The columns were covered by transparent sol tape to prevent aeration. These substrates were inoculated with spawn at rates of 1%, 3% and 5% (w/w) of the substrate by layer spawning method (Alananbeh *et al*., 2014). Each spawning rate was done in triplicate and glass columns not inoculated with spawn (only with 200 g moistened substrate) were included as control (i.e. three for each spawn rate). Both inoculated and controls were incubated on disinfected (10% bleach) wooden shelves in the darkness in the mushroom house to allow mycelia to colonize the substrates. Mycelium colonization was closely monitored and length of mycelia growth was recorded after every two days by using a ruler and expressed as mm/day (Plate 2 c and d). On the other hand, the volume occupied by mycelia on the substrate expressed as cm^3 was determined using the formula 22/7 or 3.14 x r^2 (r=inner diameter/2 cm i.e. 2.35 cm) x height (27.4 cm) (of glass column). The density (ρ) of mycelia was obtained by mass (g) of the spawn loaded for each spawn rate divided by volume (V cm^3) of the mycelia obtained) and was expressed as gram per cubic centimetre (g/cm^3) (Table 1). Photographs were taken using a digital camera (Samsung HD DV 300F).

Inoculation and spawn running: Inoculation and spawn running was done as per Mshandete (2011) where the plastic bags containing the substrates (500 g w/w) were inoculated by 3% of spawn by layer spawning method. After inoculation, the bags were placed horizontally on disinfected bench (by 70% ethanol) in a spawning running room. The room had a concrete floor pre-disinfected with 70% ethanol. The windows and the doorframes were covered with wire gauze to block insects and rodents. Shelves were covered with disinfected black plastic paper to create darkness and to limit fresh air circulation. The spawning room was kept humid by pouring 15 liters tap water on the floor per day. Vegetative development was followed by direct observation of the inoculated substrates until the substrates were fully colonized by mycelia for *L. sajor-caju* (ID 36) and *P. conchatus* (ID 44) mushroom species. Contaminants of green colour (genus *Trichoderma*) were also observed and noted (Raymond *et al*., 2012). During spawn running and fructification

humidity and temperature was recorded using a weather forecast clock (which simultaneously measures temperature and humidity) (Max-min Thermo hygro, MEGA) (Plate 3a). The temperature and humidity of the room was 28 ± 1ºC and 79 ± 2%, respectively.

Fructification and Harvesting: The fructification was done as per Onyango *et al*. (2011) with some modification as done by Mshandete (2011). Sealed substrate bags were soaked in cold water for 15 minutes, and then inverted to remove excess water for another 15 minutes. This was done to lower substrate temperature. The small holes were made on the bags with a sterile blade to increase air exchange. The environmental conditions: moisture, air exchange, temperature and light in mushroom house were altered to meet requirement for fructification. Black plastic paper was removed from the shelves to allow ventilation and more light. This was done to lower carbon dioxide concentration in the cropping room as well as to initiate formation of pinheads (Mshandete, 2011). Air temperature was lowered to 28 ± 2°C and relative humidity in the room was increased to 90-95% by covering the floor of the culture room with thin mattress and pouring 20 liters of tap water per day. Furthermore, the bags were sprayed with tap water twice a day using a hand sprayer to maintain a moisturized environment. Fruit bodies that developed were picked by hand from the substrate then weighed the same day and some were preserved for further analyses. Mushroom yield (MY) was calculated according to Mshandete (2011) where MY= (Weight of fresh mushrooms harvested (g) per fresh substrate weight (kg)). In addition, the biological efficiency (BE) was determined as BE = (Weight of fresh mushrooms harvested (g) /dry substrate weight (g) x100.

RESULTS AND DISCUSSION

In this study, eight SWEM were collected and the germplasm isolation was successful for the three species out of 8. The successful isolates included *Pluteus umbrosus* (ID 32), *Lentinus sajor-caju* (ID 36) and *Panus conchatus* (ID 44). The cultures of these species were preserved for short term in 4ºC and for long term in liquid nitrogen LN_2 unit (Thermolyne (Bio-Cane TM 20, CAN and CANE SYSTEM, USA). The spawn was prepared followed by inoculation and colonization as well as cultivation trials for all the three species.

Tissue culture Spawn characteristics of newly domesticated wild edible mushrooms: Mycelia were observed to grow similarly well on both media, PDA and MEA with variation in colonization rate among the isolates For *P. umbrosus* it took 7 days to colonize the whole plate while *L. sajor-caju* and *P. conchatus* took only 4 days. The mycelium of *P. umbrosus* was slow growing, whitish, soft and hairy at the beginning and later it developed some pinkish colour (Plate 1a and b). On the other hand, mycelia for *L. sajor-caju* were fast growing, milky in colour; lathery and hard/tough (Plate 1c). The spawn colonized the sorghum within 10 days for *L. sajor-caju* and *P. conchatus* while *P. umborosus* was a slow grower and took 17 days (Plate 2 b). The spawn of *L. sajor-caju* was white, leathery and soft at the beginning but it became tough (hard to separate) after about a month. On the other hand, *P. conchatus* was white and soft but after about one month the spawn became soft and began to pull away slightly from the walls of their glass containers. The hardness of mycelia was increasing as it become old (Plate 1 c). The toughness of mycelia could be due to the species possessing either trimiticor dimitic hyphae as it has been reported by Stamets (2000). Mycelia of *P. conchatus* was white, soft, and hairy (Plate 1 d), but about one month the spawn became soft, lathery and began to pull away slightly from the walls of the glass containers. Similar characteristics were reported by Hibbett *et al*.(1993) and thought it could be due to moisture loss.

Vegetative growth of domesticated wild edible mushrooms on substrates in glass column: The highest rate of mycelia colonization after 6 days (early colonization) was observed in *P. conchatus* at 10.07±0.06 mm/day, 14.97±0.21 mm/day, and 15.50±0.08 mm/day on 1%, 3% and 5% respectively, followed by *L. sajor-caju* at 7.70±0.13 mm/day on 1%, 10.53±0.06 mm/day on 3% and 10.93±0.07 mm/day on 5% of spawning rate. The lowest mycelia colonization rate was on *P. umborosus* where 4.87±0.07 mm/day, 4.90± 0.09 mm/day and 5.33±0.06 mm/day on 1%, 3% and 5% were obtained, respectively (Table 1, Plate 2 c, d). A similar trend was observed even after 24 days with *P. conchatus* being able to fully colonize the column in 19 days only for 3% and 5% (w/w) spawn rates. However, for *L. sajor-caju,* the column was fully colonized after 24 days for 3% and 5% (w/w) spawn rates while *P. umborosus* failed to colonize the substrate and growth stopped in a few days (Table 1 and Figure 3). The rate of mycelia colonization in this study revealed that the higher the spawn rates the faster the mycelia colonization rate. This was so evident with the trend of 5% being fastest followed by 3% while 1% of spawn inoculation had the least colonization rate.

Table 1:Mycelia colonization rate, volume and density of domesticated wild edible mushrooms on 70% banana leaves and 30% shred wood substrate (n=3), C=controls.

Colonization stage	Species	Mycelia colonization rate (mm/day)				Mycelia volume (cm^3)				Mycelia density (g/ cm^3)			
		C	1%	3%	5%	C	1%	3%	5%	Co	1%	3%	5%
Early colonization	*P. umbrosus*	0	4.87±0.07	4.90± 0.09	5.33±0.06	0	84.39±1.00	84.97±1.73	92.48±2.65	0	0.0237±0.06	0.0706±0.03	0.1081±0.02
	L. sajor-caju	0	7.70±0.13	10.53±0.06	10.93±0.07	0	133.52±3.00	182.65±4.36	189.59±1.00	0	0.0150±0.02	0.0329±0.01	0.0527±0.06
	P. conchatus	0	10.07±0.06	14.97±0.21	15.50±0.08	0	174.56±6.09	259.53±4.00	268.78±4.59	0	0.0115±0.01	0.0231±0.01	0.0372±0.01
Full colonization	*P. umbrosus*	0	5.50±0.10	6.33± 0.17	6.33± 0.25	0	95.37±3.00	109.82±3.61	109.82±2.65	0	0.0210±0.07	0.0182±0.09	0.0182±0.06
	L. sajor-caju	0	16.67±0.29	25.87±0.42	26.00±0.10	0	289.01±5.30	448.54±2.65	450.86±3.00	0	0.0069±0.13	0.0045±0.06	0.0044±0.07
	P. conchatus	0	17.27±0.59	26.00±0.38	26.07±0.44	0	299.42±2.65	450.86±6.94	452.01±2.65	0	0.0067±0.06	0.0044±0.21	0.0044±0.08

Based on these observations this study suggests 3% spawn rate to be used taking into consideration of the anticipated cost implications of the spawn especially for large-scale production. On a mixed substrate of 70% banana leaves and 30% shred wood *L. sajor-caju* and *P. conchatus* were observed to have higher mycelia colonization rates compared to *P. umborosus* (Plate 2e). It took about 24-28 days for *L. sajor-caju* and *P. conchatus* to fully colonize the substrate.

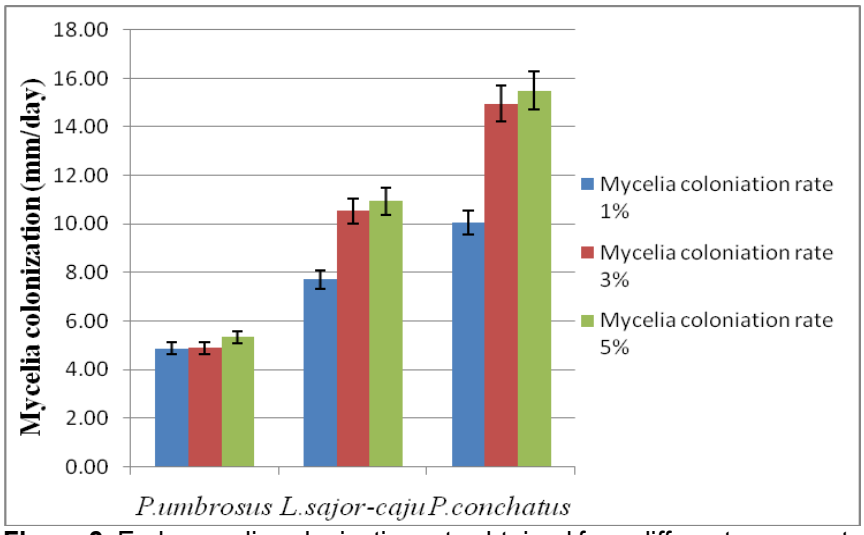

Figure 2: Early mycelia colonization rate obtained from different spawn rate among studied mushrooms after 6 days.

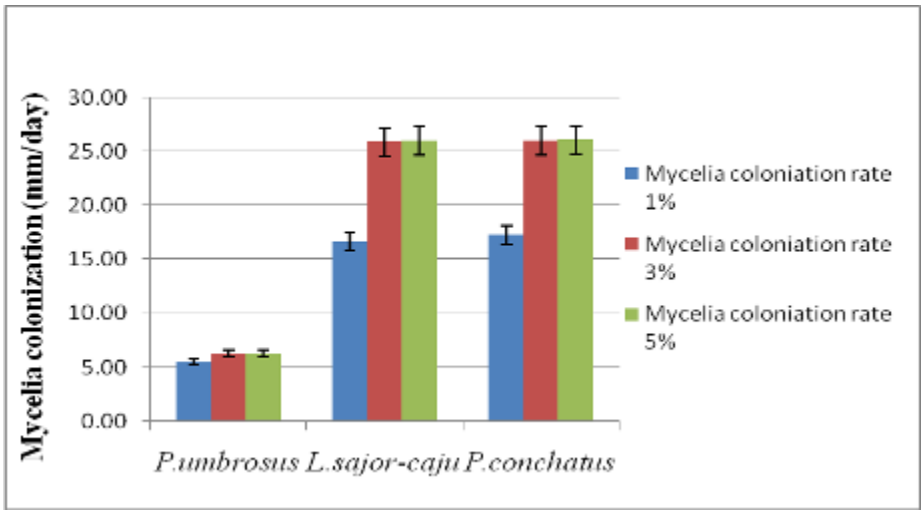

Figure 3: Full mycelia colonization rate obtained from different spawn rate among studied mushrooms after 24 days.

Fructification and harvesting of newly domesticated wild edible mushrooms: Out of the three species that were under domestication trials, only *L. sajor-caju* fructified. It took about 49-52 days for a small fruit body of *L. sajor-caju* to appear which took about 1-2 days to develop fully for harvesting (Plate 3 a and b). The fruit bodies were harvested in two flushes with the last flush having the lower yield compared to the first because the amount of nutrients available in the substrate diminished after each flush. This is in agreement with the study of Raymond *et al*. (2012) who reported a decrease in the number of fruit bodies from one flush to another. The calculated mushroom yield of *L. sajor-caju* was 52 g fresh mushrooms/kg of wet weight substrate at a biological efficiency of 18%. Biological efficiency was calculated to determine how efficient the mushrooms utilized nutrients

present in the substrates, which for this study it was very low. This could be due to the reason that no supplements were added to the substrate during the cultivation trials. Use of supplements in mushroom cultivation has been proven to increase biological efficiency and yield during mushroom cultivation since they increase nutrient contents (carbohydrates and nitrogen) to the substrate(Yang *et al*., 2013, Arce-Cervantes *et al*., 2015, M Hasan *et al*., 2015). Low biological efficiency in non-supplemented substrates have been reported by Raymond *et al*. (2013) and Pardo-Giménez *et al*. (2015). Nevertheless, this was a domestication experiments thus more studies on optimizing its growth parameter is recommended for increasing the biological efficiency and yields at large.

Plate 3: Showing (a) Young fruit and (b) mature fruit bodies of *L. sajor-caju*.

In domestication of *P. conchatus* the substrate was fully colonized but no fruit body appeared even after inducing fructification by a cold shock. Also for *P. umbrosus* the mycelia growth was very poor and could not fully colonize the substrate. There could be some factors that hindered the fructification. Temperature is one factor that could have contributed to the failure of fruit formation in *P. conchatus*. Temperature is important in fructification of mushrooms. This study was conducted in Dar es Salaam, Tanzania where the temperature is above 30^{o}C for most time of the year. The higher temperature compared to the place where the sample was collected could contribute to hinder fructification. As reported in (Gwanama *et al*., 2011) cultivation of button mushrooms became difficult since it requires low temperature and high selective and complex substrate resulted from composting. The substrate also plays an important role in mushroom cultivation. Some species of mushroom require specific micro-environment including complex nutrients for the mycelia growth and fructification (Petre and Teodorescu, 2012). Taking in consideration that some species of mushroom require composted substrate to grow (Chioza , Ohga, 2014). Possibly the substrate used for cultivation of this species did not have suitable nutrients to support fructification. Also adaptation to the environment for the domesticated species could be the reason for failure to fructify. Since the mushroom was collected from highland forests (Lushoto) and brought to be domesticated in low land region (Dar es Salaam) they face difficulty in adoption. A previous study reported failure of *L. edodes* isolate to adapt to tropical regions and form primordial (Marino *et al*., 2003). Nevertheless Hibbett *et al*. (1993) reported successful cultivation of *P. conchatus* on blume sawdust and rice bran, thus proving the possibility of growing of this mushroom species. More studies are needed to understand the optimization conditions (substrate and environmental condition optimization) required for successful cultivation of indigenous species.

Domestication challenges: During the domestication trials, the main challenge encountered that might have contributed to the unsuccessful domestication of the other species was temperature. Mushrooms are strongly affected by temperature and humidity. This study was conducted at Dar es Salaam where temperature ranges

28-33 ^{0}C in most time of the year. The studied mushrooms were collected from the forests where the temperature is low 19-21 ^{0}C. Therefore, high temperature of Dar es Salaam could be the contributing factor for unsuccessfully fructification of other studied mushroom species. However, the temperature in mushroom house was lowered by allow more air to flow and cold-shock of colonized substrate bags as suggested by (Onyango *et al*., 2011).

CONCLUSION

Saprophytic Wild Edible Mushroom (SWEM) has potential of being domesticated. Under optimal conditions, they grow fast with requirements of small area for cultivation. Mushroom cultivation not only that it will generate income to the people practicing it but also it will help to provide balanced diet, boost immunity, possible alternative to fight chronic diseases and will also help recycling of these wastes and ensuring pollution control. More studies are needed to increase varieties of wild mushroom to be cultivated and optimize their growing conditions for optimum yields.

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