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Domestication of Seven Tanzanian Indigenous Saprophytic Edible Mushrooms

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Abstract

Seven Tanzanian indigenous edible mushroom species were subjected to domestication trials using dried sugarcane tops as a substrate. Out of seven experimented species only two were successfully domesticated; Amylosporus sp. IJ-2014 and Polyporales sp. 'Kusaghizi'. Generally spawn running period ranged from 2.5 to 7 weeks although the primordia formation and fructification differed markedly among the species. Amylosporus sp. IJ-2014 showed a short period as its premordia formed in 31±2 days while fructification commenced in 43±2 days since substrate spawning. In Polyporales sp. 'Kusaghizi' primordia formed in 67±2 days whereas fruitbodies developed in 105±2 days since substrate spawning. The higher mushroom yield was observed in Polyporales sp. 'Kusaghizi' with 23.29 g/ kg wet substrate compared to Amylosporus sp. IJ-2014 with 15.50 g/ kg wet substrate. The biological efficiency mean values were 4.62% and 3.16% for Polyporales sp. 'Kusaghizi' and Amylosporus sp. IJ-2014, respectively. Further researches have to be done on the two species to establish optimum requirements for their cultivation hence escalating the yield. For the rest species which did not form fruitbodies, further work should be done especially by deploying strain specific-breeding techniques.

Keywords: Domestication, sugarcane tops, *Amylosporus sp. IJ-2014*, *Polyporales sp. 'Kusaghizi'*.

Introduction

For a long time people have been eating edible mushrooms for nourishment, medicine and enjoyment. Some mushrooms are intentionally cultivated but most of them are gathered from the wild during rainy season[1-3]. Mushrooms are wealthy in protein, minerals, vitamins, fibres and essential amino acids making them good supplement to cereals[1,3]. Some mushrooms have medicinal values as they contain bioactive substances with immunomodulating effects[4,5]. In rural and urban areas edible mushrooms are used as a source of income[1,3].

In Tanzania, mushroom cultivation was introduced in the country by the Ministry of Agricuulture and Coopoeratives in 1993. Since then, exotic mushroom strains and very few indigenous wild mushrooms have been cultivated. Indigenous mushrooms species which were successfully domesticated and hence cultivated are *Coprinus cinereus*, *Pleurotus flabellatus*, *Volvariella volvacea* and *Oudemansiella tanzanica*[6,7]. Nevertheless, up to this moment the indigenous mushroom strains are not performing well in mushroom cultivation industry of Tanzania. Several reasons are associated with poor performances of local strains. *Coprinus cinereus* is short lived due to the cup autolysis at maturity. *Oudemansiella tanzanica* has a small fruitbody and strange taste not liked by most of consumers. This causes mushroom cultivation industry in Tanzania to be dominated by exotic species which sometimes do not cope well with the weather prevailing in our tropic climate. This work is therefore aimed at domesticating more Tanzanian indigenous edible mushroom species and introducing them to mushroom farmers for adoption.

Material and Methods

Sample Collection and Identification: The study was carried out in selected natural forests of Tanga and Pwani regions as well as some planted trees of University of Dar es Salaam, Mwalimu JK Nyerere Mlimani Campus, in Dar es Salaam region. Among the forest reserves surveyed are Lutindi, Shume-Magamba, Kieti and Kazimzumbwi. Lutindi and Shume-Magamba forest reserves are located in Tanga region at 4° 52′ 0″ S, 38° 37′ 60″ E and 4° 40′ 0″ S, 38° 15′ 0″ E, respectively. Kazimzumbwi forest reserve is located in Pwani region at 6° 58′ 0″ S, 39° 2′ 60″ E. The studied sites are shown on figure-1. Field trips were conducted during rainy seasons (March-May and September-November in 2011/2012). Morphological and molecular markers (nrDNA ITS and LSU) were employed in identification of the collected mushroom. Dietary, culinary, therapeutic and other ethnomycological exploitation of wild mushrooms was investigated by face-to face interviews for over 150 local people. The detailed mushroom identification and ethnomycological utilization will be reported separately elsewhere. The studied mushroom samples are presented in table-1.

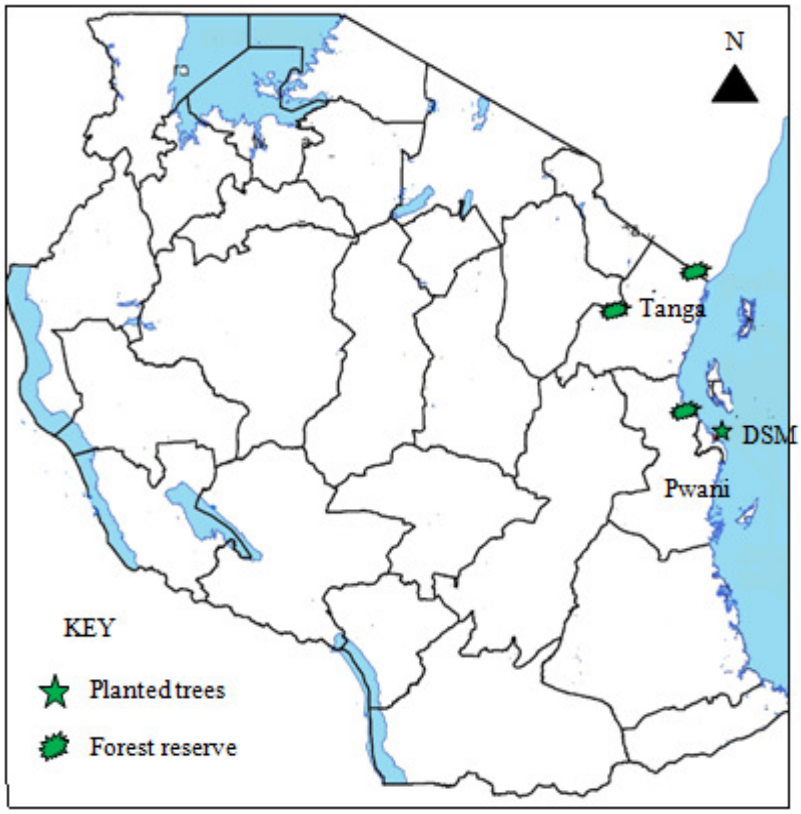

Figure-1
A map of Tanzania showing studied sites

Table-1
Studied mushroom samples

Sample No.	Sample ID	Sample name
1	29	*Amylosporus sp. IJ-2014*
2	34	*Polyporus tenuiculus*
3	35	*Pleurotus cystidiosus*
4	38	*Laetiporus sp. IJ-2014*
5	40	*Polyporales sp. 'Kusaghizi'*
6	46	*Auricularia polytricha*
7	50	*Lentinus sajor-caju*

Germplasm Isolation, Spawn and Substrate Preparation: Germplasms of seven species of wild edible mushrooms were isolated according to Stamets and Dhouib et al. with few modifications as detailed by Mshandete and cuff[6-9]. Mushroom tissues were aseptically cultured on potato dextrose agar (PDA) (OXOID Ltd Basingstoke Hampshire, England). Tissue culture was done in laminar flow hood or in artificial inoculating box in case of distant sampling sites. The inoculated agar plates were incubated upside down at 25±2°C in the dark and monitored daily for mycelia growth. Mycelia obtained from tissue culture were used to develop mushroom spawns using sorghum grains according to Mshandete and cuff[6]. The substrate used for mushroom cultivation was dried sugarcane tops from mushroom substrate stocks deposited at the Department of Molecular Biology and Biotechnology, University of Dar es Salaam. They were chopped into approximately 5 cm pieces and then soaked in water for 24 hours according to Onyango et al.[10]. The soaked substrates were drained on inclined woven wire sieves and then divided into 500g lots. Each lot was packed into separate transparent polypropylene bags (Simba Plastics, Dar es Salaam). Each bag was kept open at both ends and those ends were tied loosely with sisal rope before being sterilized in autoclave at 121°C, 1 atm for 2 hours (Koninklijke AD Linden JR BN-Zwijinderect, Holland) and finally cooled at room temperature overnight.

Inoculation of Substrates: The substrates were inoculated by adding spawn at a rate of 5% (wet weight spawn/ wet weight substrate). Surface spawning method was adopted for all mushroom species. The sisal ropes were removed before spawning and then replaced after spawning. During each experiment, three prepared bags/ trays of substrates were not inoculated and hence used as controls.

Spawn-running: Mycelia Colonizing the Substrate: Subsequent to inoculation the bags/ trays were placed on wooden shelves disinfected with 70% ethanol in a spawn-running room of the mushroom house at the Department of Molecular Biology and Biotechnology, University of Dar es Salaam. The room was previously cleaned with water and disinfected 70% ethanol. The wooden shelves were covered with black cotton curtains to create darkness and to limit fresh air circulation around inoculated bags/ trays as recommended by Staments and Chilton[11]. The spawning room was kept humid by pouring over 20 litres of tap water on the floor on a daily basis and the darkness was maintained in the room. The inoculated substrates were observed daily for vegetative development until the substrates were completely colonized by mycelia of mushrooms. Contaminants were also monitored and contaminated bags were discarded. The number of spawn running days for mycelia of each mushroom species was recorded. During spawn running and fructification humidity and temperature were monitored using weather forecast clock (Bright Weather Care, Scholer Quartz, Swiss). During spawn running the temperature and relative humidity in the room were 27.5±1.5°C and 70±4%, respectively.

Fructification: When mycelia of the wild edible mushrooms fully colonized substrates, the bags and trays were transferred to a fruiting room with the same dimensions and shelves as that of the spawning room. The fruitbody formation was triggered by shifting the environmental variables namely moisture, air exchange, temperature and light in the cropping room[8]. For increasing light intensity and air circulation, window and shelves were left open and the sisal ropes were loosen on bags. In addition to that, begs were perforated with sterile iron nails. These processes accelerated primordia initiation, introduced sufficient fresh air which at the same time lowered carbondioxide concentration in the fruiting/cropping room. Relative humidity was increased to 86±4% and temperature lowered to 25±2°C by pouring over 25 litres of cold water and ice blocks per day on the floor and on the walls, respectively. Occasionally the moisture of the bags and trays was maintained with the use of moist sprayers.

Harvesting and Crop Yield: Fruit primordia were allowed to grow to the recommended harvesting stage and were picked. The substrate adhering to the stipe was taken away and the mushrooms in their entirety were weighed the same day. Dates of each harvest were also recorded. Six aspects of crop yield were evaluated according to Morais et al. and Tisdale et al.[12,13]. These aspects are mushroom size (MS), biological efficiency (BE), biological yield (BY), mushroom yield (MY), flush number and fresh weight. Biological efficiency values were calculated according to Staments[8]. On the other hand, mushroom yield values were calculated as previously reported by Morais et al.[12]. Biological yield was calculated according to Amin et al.[14]. The average weight of individual mushrooms was determined as quotient of the total fresh weight mushrooms harvested by their total numbers according to Phillipoussis et al.[15]. The average mushroom size was calculated as total fresh weight of mushrooms harvested divided by total number of mushrooms. The following formulae were used in determination of BE, MY and BY:

$$BE = \frac{\text{Weight of fresh mushroom harvested (g)}}{\text{Dry substrate weight (Kg)}} \times 100 \quad (1)$$

$$MY = \frac{\text{Weight of fresh mushroom harvested (g)}}{\text{Fresh substrate weight (Kg)}} \quad (2)$$

$$BY = \frac{\text{Weight of fresh mushroom harvested (g)}}{\text{Dry substrate weight (Kg)}} \quad (3)$$

Data analysis: Data on spawn-running, primordia formation, fruitbody formation, biological efficiency, biological yield, mushroom size and mushroom yield were subjected to analyses of variance (One-way ANOVA) and significance was accepted at 0.05 probability level. Post hoc tests were made under SNK-Dunkan/ LSD Waller.

Results and Discussion

Mushroom Germplasm Isolation: Mycelia of *Laetiporus sp. IJ-2014* were white, cottony, lightly attached on the surface of the medium and its growth was in concentric manner. For *Lentinus sajor-caju*, initially the growth of mycelia boundaries was asymmetrical, but the overall growth was in concentric manner. The colour of mycelia was initially white, it changed with maturity to light brown and then yellowish brown with scattered whitish patches. Likewise initially the mycelial mat was slim, smooth and concentric but with development towards maturity, the mycelial mat became thick and rough. Finally it appeared leathery with regular margins and concentric pattern of growth. Mycelia of this mushroom were contaminated with other fungi as shown on figure-2a. They were not involved in next steps of mushroom cultivation.

Mycelial growth of *Auricularia polytricha* was longitudinally linear, thickening with age to form concentric, uneven dense cottony white mycelial mat, which became spotted with brown discolourations at maturity.

For *Pleurotus cystidiosus*, following inoculation of the fresh tissues on PDA medium, irregular mycelia growth started on the entire inoculated tissues after four days. At first, a large amount of hyaline, aerial mycelia appeared which in due course become whitish. Coremia formation started in the form of small swellings on the entire tissues. Tiny watery droplets having blackish colour appeared on the white stalk which, after three days of swellings appearance, terminated into distinct capitate structures called toxocysts as shown on figure-2b, which is characteristic feature of *Pleurotus cystidiosus*. Mycelial growth was irregular and cottony. After a long run of serial subculturing, the mycelia were free from toxocysts. The bottom of mycelial mat characterized with concentric circles of pale-to-

deep brown colouration with few broken radial lines on the agar medium.

For *Polyporales sp. 'Kusaghizi'* the mycelia were characterized by irregular, cottony appearance and were lightly attached to the solid growth medium. With age, the mycelial mat was thin and formed some fine concentric circles of pale brown to lightly yellow as appreciated on figure-4a and b.

Amylosporus sp. IJ-2014 displayed mycelia with unique characteristic features as well. The mycelia were typically uneven, vividly cottony and strongly attached on the surface of PDA medium. Initially the mycelia were white and soft but with age they acquired brown or brick red colouration, stiff and rigid texture. Moreover, the mycelia appeared to have ability of extending on and tightly cling to the PDA-free walls of the agar plate. With age, the mycelial mat detached itself from the surface of the plate as a crust. The colour of mycelia may be appreciated on figure-5b.

Mushroom spawns: With the exception of *Lentinus sajor-caju*, spawns of all mushroom samples were successfully obtained as shown on figure-3, 4c and 5a and b. The coremia developed in spawn of *Pleurotus cystidiosus* which appeared like black contaminants as evidenced on figure-3d and e.

Spawn-running, Primordia Formation and Fruitbody Formation: The results for spawn-running, primordia formation and fruitbody formation are shown on table-2 as well as figure-4 and 5.

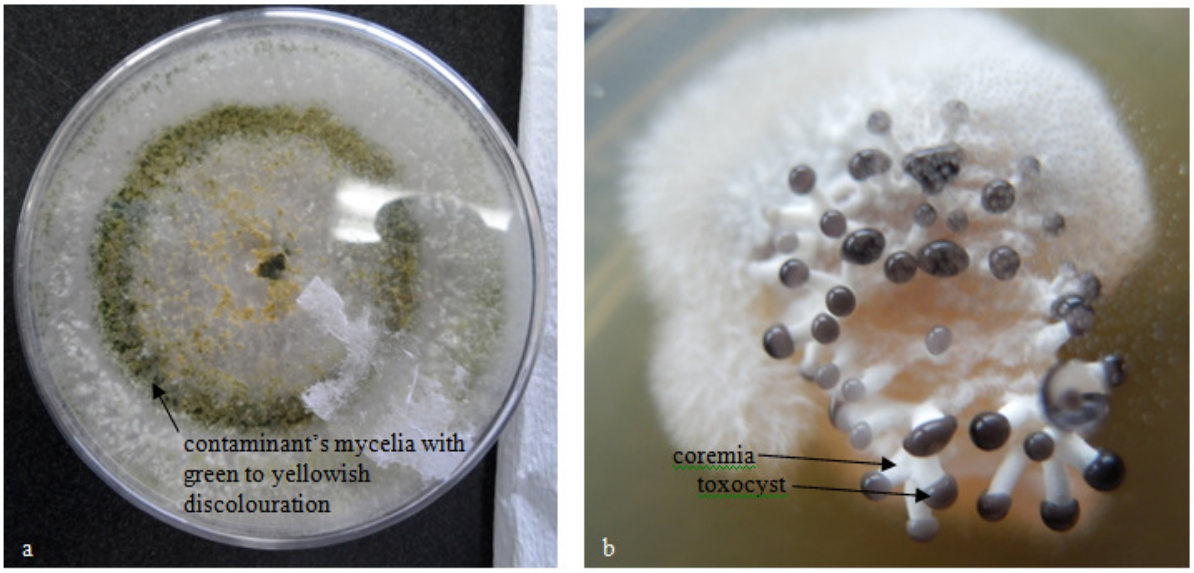

Figure-2
Mushroom mycelial cultures on PDA plates a: Contaminants on *Lentinus sajor-caju*. b: *Pleurotus cystidiosus* with toxocysts formed on the coremia

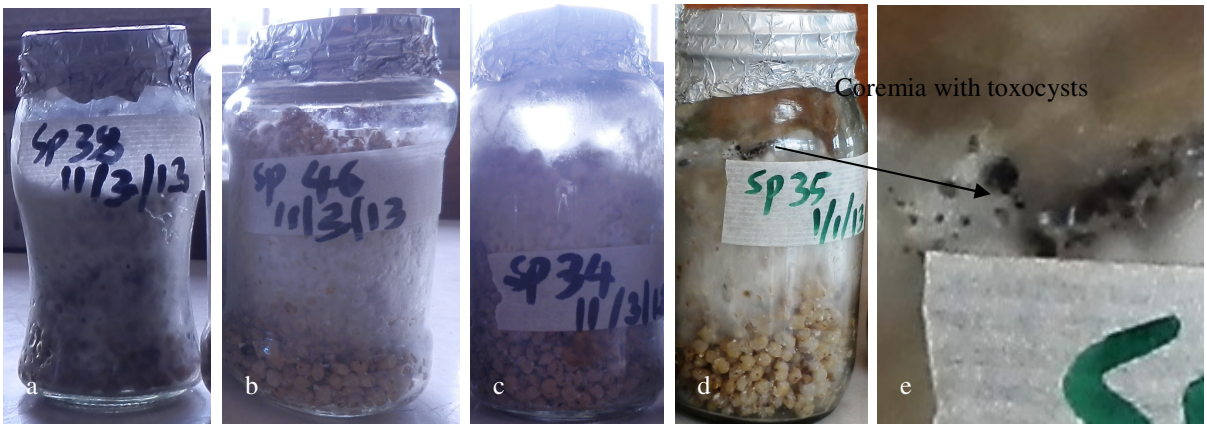

Figure-3
Mushroom spawns a: *Laetiporus sp. IJ-2014* b: *Auricularia polytricha* c: *Polyporus tenuiculus* d, e: *Pleurotus cystidiosus* with coremia development

Table-2
Days for spawn-running, primordia formation and fruitbody formation

Mushroom sample	Spawn-running	Primordia formation	Fruitbody formation
Amylosporus sp. IJ-2014	23±2[b]	31±2	43±2
Polyporales sp. 'Kusaghizi'	48±2[d]	67±2	105±2
Polyporus tenuiculus	20±1[a]	nil	nil
Pleurotus cystidiosus	27±2[c]	nil	nil
Laetiporus sp. IJ-2014	24±1[b]	nil	nil
Auricularia polytricha	27±2[c]	nil	nil

Numbers with different letters on the same column are statistically different at $p < 0.05$.

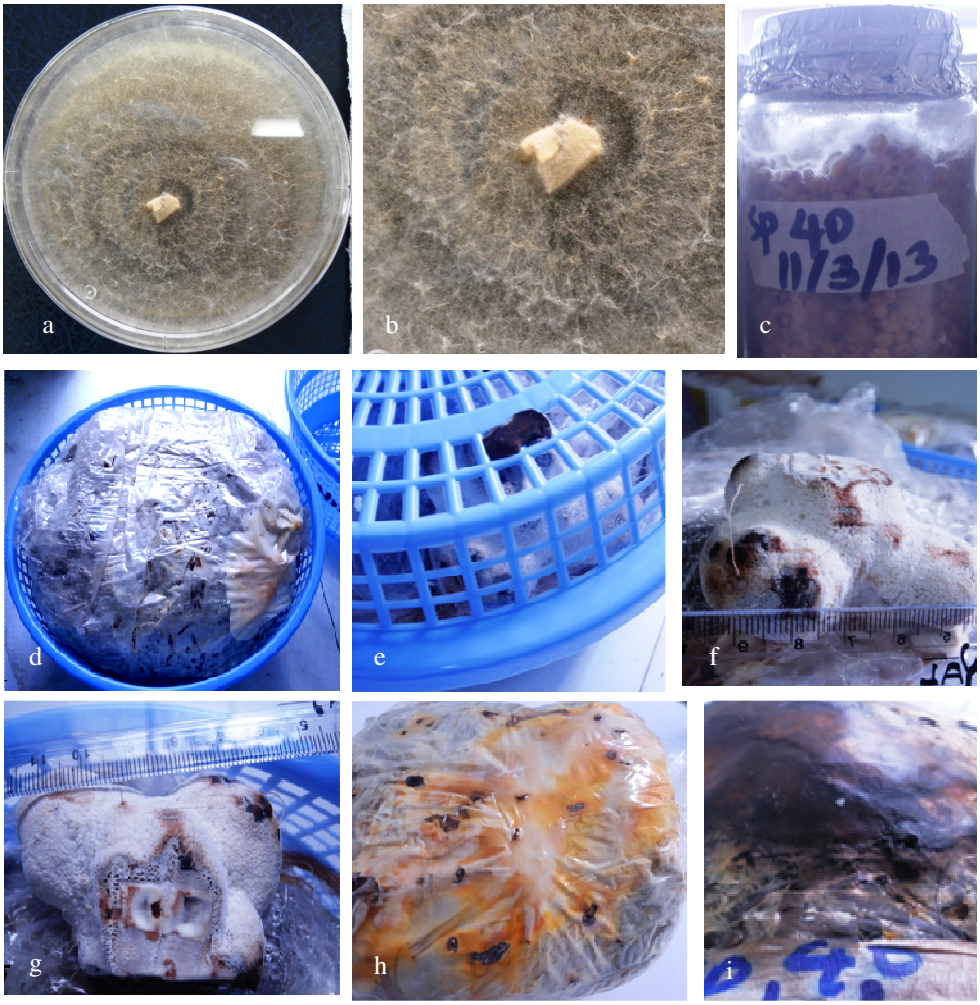

Figure-4
Stages of domestication of *Polyporales sp. 'Kusaghizi'*. a-b: Mycelia on PDA plate. c: Spawn. d: Colonized substrate with pale yellow primordia formed in dark. e: Fruitbody developed in dark within container and its tissues engulf part of the lid. f: Fruit body after opening container. g: Fruitbody displaying its part that contacted lid. h: Colonized substrate with deep yellow primordia formed in bright light. i: Mushroom mycelia and premordia have been overrun with fungi from genera *Penicillium* and *Trichoderma*

This is the first report on spawn-running, pin head formation and fruitbody formation for the six indigenous edible mushroom species cultivated on dried sugarcane tops in Tanzania. It is the first cultivation trials for *Amylosporus sp. IJ-2014* and *Polyporales sp. 'Kusaghizi'* in Tanzania. The three phases which are important in cultivation of any mushroom differed among the six local edible mushroom samples investigated. This is due to variation in the genotypes of mushrooms cultivated since other determinant factors such as the type of substrate, substrate formulation, spawn type, the level of spawning rate and prevailing mushroom growing conditions were the same for all six local edible mushroom species[6,15,16]. In this study, spawn running took 2.5 to 7 weeks after inoculation of substrate depending on the mushroom species. *Polyporus tenuiculus* recorded the shortest colonization period of 20±1 days whereas *Polyporales sp. 'Kusaghizi'* recorded the longest colonization period of 48±2 days. These results concur with that of Atikpo et al. who reported that the genotype of the mushroom species/ strains determine their mycelia colonization on different substrates[17]. In this study, *Pleurotus cystidiosus* observed to have 27±2 days for complete colonization of substrate. This finding is within the range of 12-41 days that has been reported as spawn-running period for various *Pleurotus* species cultivated on composted or non-composted substrates[13,18,19]. Mycelia of *Laetiporus sp. IJ-2014* fully colonized the substrate in 24±1 days. This study finding is contrary to Pleszczyn´ska et al. who reported that *Laetiporus sulphurius* mycelia colonized the substrate completely after 4 weeks of incubation[20]. This disagreement may be due to the difference in genetic nature of the two distinct species and variation of substrates used in the two studies as well as environmental factors. Likewise, in this study the mycelia of *Polyporus tenuiculus* colonized substrate entirely after 20±1 days of incubation as opposed to the study of Omarin et al. which established a total of 60 days of incubation at 25°C needed to produce solid block for *Polyporus tenuiculus* cultivated on saw dust and wheat straw[21]. The difference in the two findings might arise from the strains of the species cultivated, varieties of substrate used as well as environmental influences during cultivation. Interestingly, the spawn-running period for *Auricularia polytricha* was observed to be 27±2 days, coinciding with the finding of Ahila devi et al. who reported a range of 21.3-46.3 days as spawn-running period for *Auricularia polytricha* cultivated on saw dust and wheat bran substrates[22]. The spawn running period of *Amylosporus sp. IJ-2014* was observed to be a 23±2 days which does not differ significantly from *Laetiporus sp. IJ-2014* among the studied taxa.

Primordia formation is the second stage of mycelia growth during cultivation of mushrooms. Among edible mushrooms investigated in this study, only *Amylosporus sp. IJ-2014* and *Polyporales sp. 'Kusaghizi'* managed to form primordia which later developed into mushroom fruitbodies as shown on figure-4 and 5. Other mushroom species did not form primordia and fruitbodies irrespective of various techniques employed in fructification induction.

As shown in table-2, the time elapsed for primordia formation starting from the day of spawning the substrates differed among the mushrooms investigated. *Amylosporus sp. IJ-2014* recorded the earliest formation which was 31±2 days whereas *Polyporales sp. 'Kusaghizi'* recorded the last formation which was 67±2 days. This variation might arise from inherent properties of the two distinct mushroom species and the fructification strategies employed to them.

Among several methods of fruitbody induction tested, cooling the colonized substrate bags at 2-4°C for 24 hours and subjecting the colonized substrate bags in the dark until fructification were observed to be effective for *Amylosporus sp. IJ-2014* and *Polyporales sp. 'Kusaghizi',* respectively. Other methods such as injection of a portion of cold water into colonized substrate bags, exposure of the fully colonized substrate to atmospheric air and perforating the completely colonized substrate bags using 70% ethanol-swabbed rod proved to be useless due to infection of the substrate surfaces with fungi from the genera *Penicillium* and *Trichoderma*. Within 4 to 5 days, the mycelia of these fungi overran completely the exposed mushroom mycelia or primordia formed as indicated on figure-4i. Fruitbodies of *Amylosporus sp. IJ-2014* and *Polyporales sp. 'Kusaghizi'* were observed 43±2 days and 105±2 days, respectively, after spawning substrates. The growth stages of *Polyporales sp. 'Kusaghizi'* and *Amylosporus sp. IJ-2014* are illustrated on figure-4 and figure-5, respectively.

Mushroom Yield, Mushroom Size, Biological Yield and Biological Efficiency: The crop was harvested in the first flush as no higher flushes obtained. The crop yield parameters are presented in table-3.

Analysis of mushroom yield revealed that *Polyporales sp. 'Kusaghizi'* had 23.29 g / kg fresh substrate and such mushroom was superior to *Amylosporus sp. IJ-2014* which had 15.50 g / kg fresh substrate. This demonstrated that mushroom yield is related to the mushroom species cultivated. The mycelia of the two species have different colonizing potentials for the substrate on which they were grown, and this corresponds to the yield obtained. With this observation in mind, different researchers had reported different mushroom yields for different mushroom species and substrates[6]. The mushroom yield values are within the range of 12.03-184.64 g / kg fresh substrate that has been reported from other mushroom species, Oyster mushroom (*Pleurotus HK-37*), cultivated on solid sisal waste fractions supplemented with cow dung manure[23].

The mean values for biological efficiency of *Polyporales sp. 'Kusaghizi'* and *Amylosporus sp. IJ-2014* were 3.16% and 4.62%, respectively. The low biological efficiency values were due to inadequate mushroom yield which obtained only in first flushes. Variation in the biological efficiency of the two species revealed their varying potentials in conversion of dry substrate material into their biomass of which *Polyporales sp. 'Kusaghizi'* was superior to *Amylosporus sp. IJ-2014*. Researchers have been reporting the effect of substrates and mushroom species on

biological efficiency[6,23]. The biological efficiency values are within the range of 1.02-11.06% reported for *Coprinus comatus* cultivated on *Pleurotus* mushroom spent substrates though they are distinct species and were grown on different substrates[24].

Table-3
Biological efficiency (BE), mushroom yield (MY), biological yield (BY) and mushroom size (MS) of indigenous edible mushrooms

Mushroom sample	BE (%)	MY (g/kg wet substrate)	BY (g/ kg dry substrate)	MS
Amylosporus sp. IJ-2014	3.16	15.50	31.63	3.02
Polyporales sp. 'Kusaghizi'	4.62	23.29	46.22	3.81

The mean values for biological yield of *Polyporales sp. 'Kusaghizi'* and *Amylosporus sp. IJ-2014* were 46.22 and 31.63 g / kg dry substrate, respectively. This suggests that *Polyporales sp. 'Kusaghizi'* had a higher potential of converting dry substrate weight into its biomass compared to *Amylosporus sp. IJ-2014*.

The average values for mushroom size of *Polyporales sp. 'Kusaghizi'* and *Amylosporus sp. IJ-2014* were 3.81 and 3.02, respectively. This variation results from inherent properties of the two mushroom species since other determinant factors such as substrate type, level of spawning substrate and environmental conditions were similar[6].

Conclusion

Among seven indigenous edible mushroom species subjected to domestication trials, only two species were successfully domesticated which are *Polyporales sp. 'Kusaghizi'* and *Amylosporus sp. IJ-2014*. Both species did not grow to appreciable size of their respective wild forms and thus displayed low yield. This suggests that optimum requirements for their growth had not been attained. Further researches have to be undertaken on these two mushroom species for establishing their optimum growth requirements. For the rest that did not form fruitbodies, further work should be done especially by deploying breeding techniques.

Acknowledgement

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List of abbreviations: DSM - Dar es Salaam; BE - Biological efficiency; BY - Biological yield; MS - Mushroom size; MY - Mushroom yield.

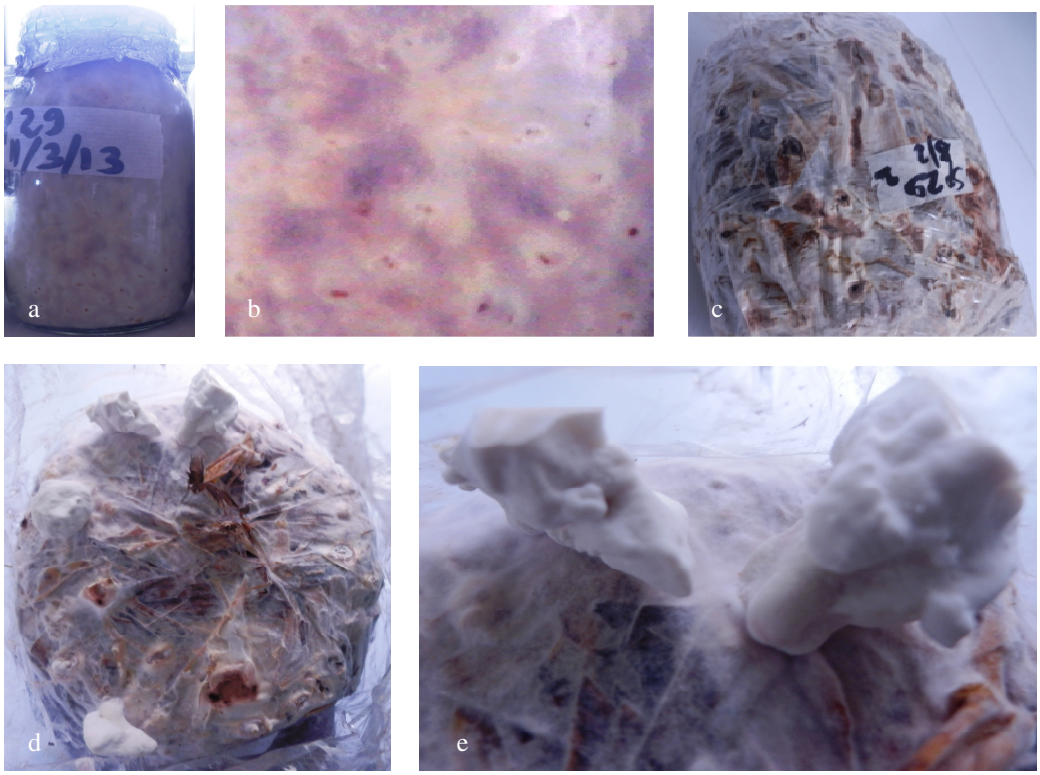

Figure-5
Stages of domestication of *Amylosporus sp. IJ-2014* a: Spawn. b: Spawn's mycelia colour d: Fruitbodies developing from primordia. e: Fruitbodies

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