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Assessment of Potential for Domestication of *Termitomyces microcarpus*: An Indigenous Edible and Medicinal Mushroom from the Lake Victoria Basin

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Abstract: Most of the mushrooms, which are used as food by communities bordering Lake Victoria wetland areas, have neither been documented nor studied. These indigenous mushrooms are used solely as products of the wild. While the cultivation of mushrooms for food is a lucrative economic activity even in some developed countries, in East Africa, this has not yet been fully exploited. In the studies reported here, local people around the Lake Victoria basin participated in ranking mushroom species according to their nutritional, medicinal and toxicological significance. *Termitomyces microcarpus* was the highest ranked edible mushroom in the region. The *T. microcarpus* mushroom caps at umbrella stage were excised and inverted over dry sterile filter paper in a sterile petri-dish and incubated. The caps were then removed leaving pink "spore prints". Potato Dextrose Agar (PDA) medium was prepared by dissolving 39g of the powder in 1L of distilled water and autoclaved at 121°C for 20 min. On cooling the media was poured in petri-dishes and left to solidify. Using a flame sterilized inoculation wire loop, spores were transferred from the "spore print" and S-streaked on the fresh PDA medium. The inoculated plates were incubated at ambient temperature (25°C) in the dark for 10 days. Three discrete pure colonies were separately subcultured onto fresh PDA medium and incubated under the previous conditions for 60 days. These constituted the three monospore cultures; S_1 S_2 and S_3 which were used as starter cultures for further studies. The growth of the monospore starter cultures was monitored and a record taken of their colony diameter once a week during the 60 day incubation period. Using the liquid culture technique, the grain mother spawn for monospore starter culture S_3 had fully colonized compared to that for monospore starter cultures S_1 and S_2 which attained full colonization at 75 and 90 days, respectively. However, there were differences in the intensity of colonization with grain mother spawn of monospore starter culture S_3 giving a much more intense mycelial growth as compared to grain mother spawn of monospore starter culture S_1, which gave a moderate mycelial growth. Mycelial colonization for grain mother spawn of monospore starter culture S_2 was a bit scanty. The grain mother spawn prepared using the agar culture plug technique were very slow. After four months mycelial colonization was at most 25% of the total volume of millet substrate of grain mother spawn for monospore starter culture S_1, but not more than 10% for the grain mother spawn of monospore starter cultures S_2 and S_3. Further studies are needed to initiate fruiting body formation which has not been possible under the present test conditions. This will require a better understanding of the relationship between the fungus and the termite and the ecological relationships therein.

Key words: Potential for domestication, indigenous, edible and medicinal much room, Lake Victoria Basin, PDA

INTRODUCTION

Mushrooms have been prized and relished as a delicacy for hundreds of years because of their subtle flavor (Priestly, 1984), nice aroma and physical taste appeal (Bhatti *et al*., 1989). Varying opinions have also been expressed regarding the true nutritive value of edible mushrooms. In some countries mushrooms were considered luxurious food and a delicacy for the rich because of their unique flavor and exotic taste (Priestly, 1984).

In East Africa mushrooms are highly treasured in the rural village communities. They start growing soon after the first rains and become very handy and tasty vegetables long before the agricultural crops that have been planted are ready for harvesting. But the natives

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mainly eat them as an addition to the menu because of their being in plenty and inexpensive. The knowledge ofthe nutritive value and the ecology of the indigenous mushrooms, however, is rather scanty (Engola *et al*., 2007). The fast growing mushrooms have received a remarkable amount of interest in recent decades with the realization that they are a good source of delicious food with high nutritional attributes and some have medicinal values as well. Today, they are a potential protein source especially in developing countries where animal protein is scarce and expensive (Hand and Hayes, 1981).

Most of the mushrooms, which are used as food by communities bordering Lake Victoria wetland areas, have neither been documented nor studied. These indigenous mushrooms are used solely as products of the wild. While the cultivation of mushrooms for food is a lucrative economic activity even in some developed countries, in East Africa, this has not yet been fully exploited. Few investigations have also been done on the nutritional value and the possibility of deriving novel antibiotics from mushrooms in East Africa (Opige *et al*., 2006a) Furthermore, the extent of land reclamation and deforestation in the region implies that some of the mushrooms may be disappearing due to the destruction of their habitats. The problems of minimal documentation, diminishing supplies, unsustainable, unregulated and indiscriminate use of the wetlands, provides an opportunity for investigations on ways to improve their conservation and utilization for contribution to rural well being and livelihood in the region. It is the rural people and those who live in the areas bordering wetlands that have the most to lose if this priceless indigenous resource disappears. It is also the same people who have the most to gain if this knowledge is documented and programmes established to domesticate and conserve mushrooms, as well as creating new markets for the products from them (Opige *et al*., 2006b).

Termitomyces microcarpus is by far the most popular of the wild edible mushrooms that grows in the Lake Victoria Wetland region. It is praised for its good flavour and is used to treat some ailments. It is a small white-cream mushroom that grows in symbiotic association with the cubitermes termite species in cropland, grassland, woodland and forests. It is wide spread in tropical Africa and in Southern Asia. It is found throughout the wetter parts of East Africa and is harvested from the wild mainly during the rainy season. It grows on the fungus comb which has been shade out from the termitarium of the associate termite. The cap is 5-25 mm across, generally convex, white -cream, the edge is entire, lobed or splitting. The gills are free, thin and white turning pale pink at maturity. The stalk is slender (20-40 mm long and 3 mm wide) smooth, solid with fibrous tissue and a bulbous base. Its "spore print" is pale pink.

Termitomyces microcarpus is one of the most versatile mushrooms in the Lake Victoria basin. In all our previous studies, it was ranked highest by the communities from the medicinal and nutrional viewpoint (Opige *et al*., 2006c). Its main medicinal benefit seems to be in the treatment of measles in children. Here we now report preliminary attempts at domesticating this extremely interesting indigenous mushroom.

MATERIALS AND METHODS

Study sites: This mushroom project covered 3 regions: Uganda (Rakai and Bugiri); Tanzania (Musoma and Mwanza districts) and Kenya (Busia and Siaya districts). These areas are selected on the basis of their differing cultural practices, socio-economic structure and location within the Lake Victoria basin. Selection of wetlands was based on geographical factors and wetland type. Preliminary studies and opinions of experienced local personnel were considered in selecting suitable sites for mushroom collection. Local people participated in ranking mushroom species according to their nutritional, medicinal and toxicological significance. The focus group findings reported here was collected from Mayuge district in Uganda.

Field data collection and analysis

Reconnaissance surveys: Reconnaissance surveys were conducted in the three regions, with the purpose of getting acquainted with the study areas, selecting the sites, discussing the proposal with district and village leaders and building teams for the field investigations. Criteria for selection of villages for study were, among others, vegetation cover, demonstration of mushroom knowledge by community, environmental awareness and accessibility by road and water, ease of follow-up and response of the village authorities to the work. Furthermore, gender balance in gathering information was considered since men and women can value mushrooms differently.

Participatory rapid appraisals: Participatory Rapid Appraisal (PRA) was used as the main method for gathering information from the communities. Standard PRA techniques, including semi-structured interviews, pair wise ranking and focus group discussions were used to aid in capturing indigenous knowledge and uses of the mushrooms. The community perceptions on the priority mushrooms for cultivation were also captured.

Domestication trials on *Termitomyces microcarpus*

Production of monospore starter culture: Fungus comb freshly exposed by termites was collected from the surface of the termitarium of the associate termite and cultured on 3 layers of sterile moist blotter papers in a petri-dish covered with a beaker. The set up was incubated at room temperature overnight. The following morning the mushroom caps at umbrella stage were excised and inverted over dry sterile filter paper in a sterile petri-dish and incubated as above. The caps were then removed leaving pink "spore prints". Potato Dextrose Agar (PDA) medium was prepared by dissolving 39 g of the powder in 1 L of distilled water and autoclaved at 121°C and 15 kg cm^{-2} for 20 min. On cooling the media was poured in petri-dishes and left to solidify.

Using a flame sterilized inoculation wire loop, spores were transferred from the "spore print" and S-streaked on the fresh PDA medium. The inoculated plates were incubated at ambient temperature (25°C) in the dark for 10 days. Three discrete pure colonies were separately subculture onto fresh PDA medium and incubated under the previous conditions for 60 days. These constituted the three monospore cultures; S_1 S_2 and S_3 which were used as starter cultures for further studies. The growth of the monospore starter cultures was monitored and record taken of their colony diameter once a week during the 60-day incubation period.

Preparation of mother grain culture: Good, relatively clean millet grains were bought from St. Balikuddembe (Owino) market. They were then further thoroughly cleaned by winnowing and washing in plenty of water to float off the husks and diseased grains and to remove dust. The grains were then soaked in tap water for 12 h to imbibe, then boiled in a sauce-pan over a hot plate for 20 min and drained of excess water immediately. The final moisture content measured using the oven method was 50%. The grains were then mixed with calcium carbonate and gypsum each 1% w w^{-1} to stabilize pH and to improve on texture. The gains were then packed in clean flat 150 mL flat bottles 75g each. The bottles were firmly plugged with non-absorbent cotton wool and autoclaved at 121°C and 15 kg cm^{-2} for 1h.

Inoculation: Two approaches were used, namely; agar culture plug and liquid inoculation method.

Preparation of inocula for liquid inoculation method: Three stainless steel blenders with metallic cover, each containing 100 mL of water were autoclaved at 121 °C and 15 kg cm^{-2} for 1 h. After cooling to room temperature, one plate for each of the three pure monospore starter cultures was aseptically cut out (mycelium + medium) and separately mixed in the sterile water in the blenders at low speed for 5 sec. The resultant solutions (inocula) were transferred into separate sterile 250 mL conical flasks and covered with cotton wool and aluminum foil on top. They were then incubated on a shaker running a low speed at room temperature for 24 h.

Inoculation of grains: With the aid of a sterile syringe, 5 ml of inoculum were aseptically introduced in each bottle containing sterile grains. The bottles were shaken to evenly wet the grains with the liquid inoculum. A separate syringe was used for each monospore culture and nine bottles were inoculated for each culture using this technique. For the agar culture plug method, a culture plug (medium + mycelium) of 1×1 cm in size was cut using a sterile scapel blade and introduced into the grains. The starter cultures were also subcultured on agar slants to create stock cultures for future use.

RESULTS AND DISCUSSION

Pra and survey findings: Of all the conditions reported by the respondents the arrival of rain is the most commonly listed variable (70%) while the season of white ants was also a known connection. This would suggest that the indigenous knowledge points to the fact that most of the mushrooms in the area are of the *Termitomyces* sp. (Table 1).

It is clear from these finings that the community perception in the area is that there is need for rain and white ants for the mushrooms (Table 2). The most highly ranked mushroom was *T. microcarpus* for edible and second ranked for medicinal (Table 3 and 4).

Performance of monospore cultures in millet grains

Liquid inoculation method: By the 5-7th day after inoculation, the culture was seen to have started `leaping' (establishing) onto the millet grains. At 30 days after inoculation the culture was seen to have established all over the grains mass was appearing as a white mycelium on the surface of the grains. At the end of the second month post-oculation the spawn was ready for inoculation into test growing substrates (cotton seed hulls and weathered saw-dust).

Agar culture plug method: The cultures did not exhibit and observable growth even at the 10th day from inoculation. At 30 days from inoculation the cultures had colonized small patches just around the point were the agar culture plug was introduced.

Table 1: Perceptions of general growth requirements of mushrooms

General requirements for growth	% Respondents
Sunshine	10
Good soil	10
Rain	30
White ants	5
Decayed matters	25
Banana peelings	5
Millet	5
Sorghum	5
Cool places	15
Dry wood	5
Cow dung	10
Ant hills	10
Hard soil	5
Omwisiga	5
Darkness	5

Table 2: Community perceptions of requirements for mushroom growth

Growth requirements	% Respondents
Termites	5
Rain	70
Logs of wood	5
Forests	5
Cow dung	10
Anthills	15
Hard soil	5
Good soil	10
Passion fruit plants	5
Emponzira	5
Sunshine	10
Land free from cultivation	5
Rotten wood	5
Banana peelings	5
Millet	5
Sorghum	5
Rotten rubbish	5
Burning of bush	5
Season of white ants	10
Omwisiga	5
Darkness	5

Table 3: Edible mushrooms recommended for domestication

Good mushrooms to be planted in homes	% Respondents
Bunabugogo (*NI)	5
Bwidhanamadhi (*T. eurhyeusus*)	55
Butyabule (NI)	15
Bwisonkere (*T.microcarpus*)	60
Bitutwe (NI)	5
Gudu (*Termitomyces titanicus*)	5
Tandabira (NI)	5
Nabusa (NI)	15
Emponzira NI	25
Kinula (*Termitomyces* spp)	40
Obubaala *Termitomyces* spp	20
Mugulunundo (NI)	5
Bubwikampuli (NI)	5
Amelele (NI)	5

* NI = Not yet botanically indentified

Table 4: Priority for domestication of medicinal mushrooms

Medicinal mushroom	% Respondents
Obwisonkere (*T.microcarpus*)	35
Nabusa (NI)	5
Emponzira (NI)	10
Obubaala (NI)	10
Obwidhanamadhi (*T.eurhyseus*)	55
Tandabira (NI)	5

Growth of *Termitomyces microcarpus* after 60 days: After 60 days from inoculation with liquid culture technique, the grain mother spawn for monospore starter culture S_3 had fully established as compared to that for monospore starter cultures S_1 and S_2 which attained full colonization at 75 and 90 days, respectively. However, there were differences in the intensity of colonization with grain mother spawn of monospore starter culture S_3 giving a much more intense mycelial growth as compared to grain mother spawn of monospore starter culture S_1, which gave a moderate mycelial growth. Mycelial colonization for grain mother spawn of monospore starter culture S_2 was rather scanty (Table 5 and 6).

The grain mother spawn prepared using the agar culture plug technique grew very slowly. After four months mycelial colonization was at most 25% of the total volume of millet substrate of grain mother spawn for monospore starter culture S_1, but not more than 10% for the grain mother spawn of monospore starter cultures S_2 and S_3.

Table 5: Growth (Colony diameter in mm) of the three monospore starter cultures over a period of 2 months on PDA medium

	Colony diameter (mm)		
Age of culture (Days)	S_1	S_2	S_3
5	12	11	12
9	16	15	15
13	19	20	19
19	22	28	25
23	25	31	29
28	27	32	32
35	30	33	34
40	31	33	35
50	35	33	38
60	40	33	39

Key: S_1=Monospore culture 1, S_2 = Monospore culture 2, S_3 =Monospore culture 2

Table 6: Colonization period (in days) and intensity of mycelial growth for the *Termitomyces microcarpus* grain mother spawn

Culture	Colonization period (Days)	Mycelial growth
S_1		
R_1	75	Moderate
R_2	75	Moderate
R_3	75	Moderate
R_4	75	Moderate
R_5	75	Moderate
S_2		
R_1	90	Scanty
R_2	90	Scanty
R_3	90	Scanty
R_4	90	Scanty
R_5	90	Scanty
S_3		
R_1	60	Intense
R_2	60	Intense
R_3	60	Intense
R_4	60	Intense
R_5	60	Intense

Key: S_1, S_2 and S_3 = Monospore starter cultures used to prepare the grain mother spawn. R_1, R_2, R_3, R_4 and R_5 = Are replicates of the grain mother spawn for the respective monospore starter cultures

Initiation of mushroom formation: The 3 grain mother spawn were tested for mushroom initiation by incubating about 10 g of the grain mother spawn on 3 layers of sterile moist blotter papers in a pair of petri dishes. Even after two months no mushroom premordia had developed and some even got contaminated in the process. No fruiting body formation was elicited under this test conditions.

The persistent difficulties in domesticating the termitomyces are a pointer to the fact that the information on the ecology and growth requirements, especially the relationship between the termite and the mushroom, is not complete. The difficulties in fruiting still present a problem. It should, however, still be possible to make use of the spawn for most of what is needed, the flavor and the medicinal potential.

The other problem is the market potential. Because of the small size of the mushroom, it is probably not easily accepted by western markets. But the flavour would be good for the soup in high flying hotels in the region. It is also possible to genetically establish the source of the flavour and then use it in *Pleurotus* sp., a much bigger species that is more readiliy accepted by the other markets. But then there would be a problem faced by all GMO. These issues all need to be addressed in further studies.

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