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Shiitake, a new mushroom variety proposed in Mauritius

P Huzar Futty Beejan*
AREU
Email: areu@intnet.mu

R D Nowbuth
AREU
Email: areu@intnet.mu

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Abstract

Shiitake (*Lentinula edodes*) is an edible mushroom introduced in Mauritius in order to diversify the mushroom varieties cultivated locally. Till recently, the oyster mushroom (*Pleurotus* sp) was the only species cultivated in Mauritius. Shiitake is already present on the local market, imported in a major part in the dried form. As such, the Shiitake mushroom being proposed is readily acceptable among local consumers. With the expected increase in tourism inflow into the island, the demand for this specialty mushroom (both in the fresh and processed forms) is expected to increase. A potential was found for its cultivation locally using the bag method. The substrate being used actually is maize grains. The appropriate cultural practices needed to be fine tuned in order to initiate fruit formation in this temperate species of mushroom. Research work is ongoing in order to identify other potential substrates such as sawdust for its cultivation. Several growers have started Shiitake production on a small scale from bags purchased at the Mushroom Unit of the Agricultural Research and Extension Unit (AREU) and few growers have started producing their own Shiitake substrate bags. The scopes and limitations of the cultivation of the proposed mushroom will have to be thoroughly taken into consideration in order to further promote the sustainable cultivation of Shiitake in Mauritius.

Keywords: Shiitake, maize grain, *Lentinula edodes,* cultural practice, Mauritius, edible fungi

* to whom correspondence should be addressed

1.0 INTRODUCTION

Shiitake (*Lentinula edodes*) is a vastly popular edible mushroom and was previously known as *Lentinus edodes* (Chen, 2001). The name Shiitake is a synergy of the Japanese words "Shii" referring to the shiia tree on which it occurs naturally and "take" meaning mushroom. In China, where it originates, Shiitake is known as 'xiang-gu', in USA as the 'black forest mushroom' and as the 'lentin' in France. It is also obtained in two other forms arising as a result of cold temperatures during mushroom development notably the 'dong-gu' or winter shiitake and the 'hua-gu', the flower shiitake. The latter is distinguished by the flower-like pattern on its cap surface and is most highly prized on the world market (Chen, 2001).

In nature, Shiitake is saprophytic and grows on dying or dead broadleaf trees in temperate conditions. It is indigenous to the Far East where it can be seen in the wild in China, Japan and Korea (Chen, 2005). There are several records of Shiitake collections from the wild. However, Shiitake cultivation is reported to have been started by Wu Sang Kang in 1100 A.D (Luo, 2004). Since then, its cultivation has grown to become a worldwide phenomenon with an estimated fresh weight production of 2 million tonnes in 2002 (Luo, 2004). According to Chang and Miles (2004), China is the leading Shiitake producer, followed by Japan and other countries such as Taiwan, USA. Shiitake is currently the world's second most commonly cultivated mushroom after the button mushroom and ranks first amongst the specialty mushrooms (Kwon and Hobbs, 2005). The popularity of Shiitake can be attributed to its excellent culinary characteristics, flavour as well as its medicinal and nutritional properties.

Shiitake is considered as a gourmet mushroom with excellent flavour and is used in several dishes ranging from stir fries to elaborate dishes. It is a good source of minerals, essential amino acids, dietary fibre and vitamin B and D. The protein content of dried Shiitake is comparable to chicken and meat but with lower fat content (Kwon and Hobbs, 2005). Its beneficial medicinal properties have been valued for thousands of years in Chinese traditional medicine. Modern science has gone a long way to take stock of the anti-tumour, anti-viral, anti-bacterial, hepatoprotective and cardiovascular effects of Shiitake (Kwon and Hobbs, 2005).

Mushrooms are highly valued on the local market as well as in the tourism sector. The demand is expected to rise with the increase in tourist inflow. Imports of various mushrooms, both in fresh/chilled and processed forms, have reached around MUR 41 million annually. Shiitake is brought in from countries such as China and Thailand. It is available in Mauritius in dried forms as well as canned. The local production of mushrooms is estimated at

65 to 70 tonnes annually and is insufficient to cater for the local demand. Oyster mushroom (*Pleurotus* sp) was, till recently, the only species cultivated locally. In order to propose diverse varieties for local cultivation, the Agricultural Research and Extension Unit had started research on new mushroom species (Huzar Futty, 2003). The Shiitake, with its above mentioned benefits and its acceptability on the local market due to its popularity in local cuisine, was a primary option.

Shiitake strains were introduced from overseas laboratories. Parameters such as growth rate, mycelial maturation, substrate selectivity and fruit quality and yield are strain related (Chen, 2001). Strains of *L. edodes* can also be distinguished by their preferences for fruiting in colder or warmer temperatures (Stamets and Chilton, 1983). Accordingly the introduced strains were evaluated in order to identify a strain better adapted to our local warmer conditions. Contrarily to oyster mushrooms being cultivated locally, Shiitake is a temperate mushroom for which a whole package of cultural practices had to be determined in order to establish local cultivation.

Shiitake can be cultivated by 2 methods: bag method and log method. The latter is the traditional method of cultivating shiitake mushrooms. This method consists of drilling holes into cut logs of suitable species and filling in with spawn. After incubation, whilst temperature, humidity and ventilation are maintained at an optimal level, logs are induced to fruit. This method is, however, too lengthy and yields relatively less than the bag method.

Even though sawdust supplemented by bran and gypsum etc is generally favoured for bag method of cultivation, use of other materials e.g. wheat straw and other additives are also suitable for Shiitake cultivation. At AREU, the substrate used for evaluation of bag method of cultivation was maize grains with additives of lime and sugarcane bagasse.

The purpose of this study was to establish adequate cultivation practices for *L. edodes* in order to diversify the varieties cultivated locally so as to provide a mushroom with culinary, nutritive and health benefits to the market.

2.0 MATERIALS AND METHODS

Fifteen strains of *L. edodes* are being maintained in the Culture Collection at the Mushroom Unit of AREU. Complete randomised design was adopted for the majority of trials. These strains were evaluated in the laboratory for their rates of mycelial growth at different temperatures (15^0, 20^0, 25^0, 30^0C) in order to identify a strain better adapted to our local conditions. The strain

introduced from Taiwan with Culture Code number CC 111 was found to be more promising and was used for all further trials.

The Shiitake strain was subcultured onto Potato Dextrose agar petridishes. Plugs of actively growing mycelia were aseptically excised from petridishes and used to inoculate mother spawn flasks. Standard millet based substrate was used for mother spawn production. After inoculation, the flasks were placed in incubators set at 25 ± 1^0 C. At the end of the incubation period, colonised mother spawn flasks were used to inoculate the substrate.

The bag method was adopted for local Shiitake cultivation. Broadly speaking, the bag method consists of preparing a substrate with appropriate nutritional requirements for shiitake development, placing them in plastic bags, sterilising the substrate followed by inoculation with desired strain and, after incubation, fructification can be promoted. Several raw materials were evaluated for possible use as substrates among which were maize cobs, maize leaves/sheaths, maize grains, 6 formulations of sawdust substrate, both composted and uncomposted bagasse substrate and bagasse/chicken manure compost. The moisture content of all substrates was adjusted such that a relative humidity of around 60 % was achieved. The substrates were then assessed for mycelial run, contamination rates and yield. The maize grain substrate was found acceptable for shiitake substrate preparation. The latter was prepared by boiling maize seeds for 20 minutes, draining followed by addition of sugarcane bagasse and lime. After thorough mixing, the substrate was filled into heat resistant plastic bags and weighed. Shiitake bags of 0.75 kg were produced and stoppered with cotton plug. Production of larger bags was refrained from in order to avoid too lengthy substrate colonisation times and increased risks of contamination. In order to assess the need for compaction, maize seed substrate was filled into bags and was either not compacted (control) or manually compacted with 0, 10, 20, 30% starch addition.

In order to determine the optimal heat treatment regime for Shiitake, prepared bags were subjected to either pasteurisation (3-4 hours at 80^0C) or sterilisation in an autoclave at 121^0C for varying time spans. The cooled Shiitake bags were transferred to a clean and disinfected inoculation area or inoculation hood. The bags were aseptically inoculated with colonised mother spawn prepared previously. Localised spawning of Shiitake bags was carried out. After inoculation, the bags were incubated.

The time taken for incubation and the stages that can be observed during the incubation phase of Shiitake were noted. The light requirement during incubation of shiitake bags was determined by allowing 20 replicated bags

to colonise under 3 luminosity regimes, that is, in complete darkness, in 24 hour artificial light, in semidarkness.

After incubation, the Shiitake bags were transferred to the growing house which is preferably a well sheltered, cool, humid and ventilated area. Fructification in Shiitake was induced by subjecting the bags to a temperature shock. The effect of temperature shock on fruit initiation was observed either by immersing the bags in cold water for 4 hours, spraying the bags with water or placing the bags for 4 hours in a cooled area (12-16^{0}C).

After initiation of fructification, bags were placed on racks or inverted on a layer of 1-2 inch rocksand and lightly sprayed with water 1-2 times daily. Shiitake fruiting bodies' yield was determined. Preliminary work on Shiitake postharvest was run.

3.0 RESULTS

Following the strain selection carried out in the laboratory, the strain CC111 was used to produce fruiting bodies. Strain CC 111 was observed to yield mushrooms with a circular convex fleshy cap, light to dark brown in colour and 3 to 8 cm in diameter. The cap becomes almost plane at maturity and its surface has white hairy flecks (veil remnants), especially along the margin. Cap margin is in-rolled in early stages. Gills are white and crowded. The mushroom stipe is centrally attached, tough with scattered fibrillose partial veil remnants (Fig 1).

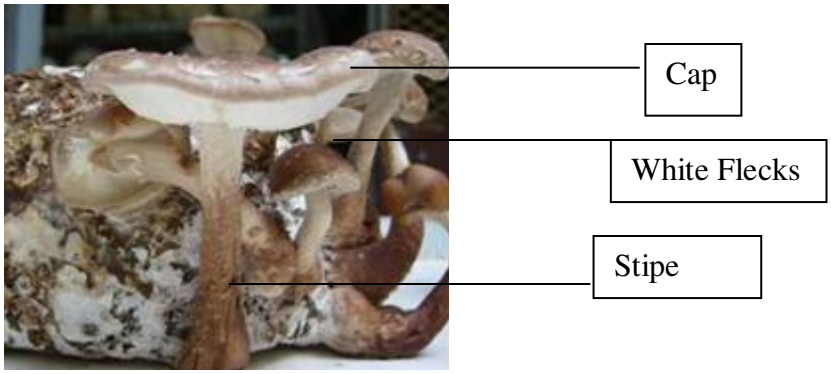

Figure 1: Shiitake mushroom

For local cultivation, a maize seed based substrate was found acceptable for Shiitake production. It was important to ensure that the substrate ingredients used were of good quality, well dried and free from pests and diseases. Mycelial growth was observed to be very slow and uneven in bagasse which was followed by growth inhibition. Similarly, growth of mycelium was found unsuitable in chicken manure/bagasse compost. It was noted that

Shiitake bags had to be well compacted manually during substrate filling of bags. Addition of starch did not result in significantly different results in terms of mycelial growth and yield for Shiitake bags of 750g capacity. Batches of 35 bags were prepared for each experimental treatment. Starch was found to promote compaction in the event that manual compaction is inadequate e.g. in bags of greater than 0.9 kg substrate capacity. Table 1 shows the formulation for substrate for Shiitake bag.

Table 1: Substrate Formulation for Shiitake Cultivation

(After promising results obtained, all subsequent bags were prepared using this formulation)

Item	Quantity
Maize seeds	100 kg
Sugarcane bagasse	5 kg
Lime	1 kg
Water	60 % R_H*

(*Water added till substrate attains relative humidity of 60%)

The trial on heat treatment of prepared maize seed substrate bags indicated that pasteurisation was not suitable (100% contamination rate). Instead, sterilisation of bags in an autoclave at 121^0C for 55-65 minutes is recommended. Contamination rate after sterilisation of bags was around 15 – 30%.

Determination of the optimal light regime during incubation indicated that all 3 treatments enabled mycelial development. In all cases, 2-3 harvests were recorded per bag. The rate of mycelium growth was faster in bags incubated in total darkness. Though comparable numbers of fruiting bodies were recorded in both diffuse and full light incubation, higher yield of 86.1g in terms of fruiting bodies' weight per bag of 750 g capacity was noted in bags in semi-darkness as compared to those in light regime (68.9 g).

Incubation of bags was carried out in at ambient temperature in a darkened room as there is no light required during this vegetative phase. The time taken for incubation was observed to vary between 3 to 5 months and was broadly divided into 5 stages (Table 2).

Table 2: Stages during Shiitake Spawn Run

Stage 1	Colonisation (Mycelial growth)
Stage 2	Mycelial coat formation
Stage 3	Blistering stage
Stage 4	Browning stage
Stage 5	Bark formation

During stage 1, the substrate was colonised by vigorous mycelium growth. The white mycelia of shiitake completely covered the substrate and the bag became white in colour. At the end of colonisation during stage 2, a thicker white coat developed at the outer periphery of the bag block (Figure 2).

Figure 2: Different stages of mycelial colonisation in Shiitake bags

The blistering stage was recognised by the appearance of popcorn-like bumps or 'blisters' on the surface of the bag within the plastic. These knot-like blisters are in fact formation of clumps of mycelia. The time of appearance of the blisters vary with respect to the inoculation time and temperature during incubation. Shiitake primordia are produced at the apex of some blisters (Chen, 2001). A majority of blisters are abortive and do not give rise to primordia. Stage 4 is distinguished by the browning of the substrate surface (Fig 3).

Figure 3: Browning stage in Shiitake bags

For the fructification phase, ambient natural light was required as opposed to the incubation phase. Simple racks were built for the indoors stacking of the mushroom bags (Fig 4a). For lower humidity areas, it was found that stacking the Shiitake bags on a 1-2 inch layer of moist rocksand improved fruit formation (Fig 4b).

Figure 4a: Simple racks for stacking Shiitake bags

Figure 4b: Perforated rack with rocksand layer

All 3 methods of inducing fructification using cold shock were found to trigger fruiting bodies' formation. Subjecting the Shiitake bags to a cold shock by soaking them in water at 12 - 16 ^{0}C for 4 hours was determined to promote optimal initiation of fruiting (Fig 5). The other 2 methods of initiation do not constantly trigger subsequent mushroom fruiting bodies' formation unlike with soaking.

Figure 5: Cold water immersion of Shiitake bags with completely removed plastic

After immersion, bags were removed and placed on the prepared racks. The covering plastic of Shiitake bags can be either partially or completely removed from the Shiitake bags. In the cases of partial plastic removal, bags were, after immersion, inverted on the racks for 2-3 days to promote drainage of superfluous humidity after which they were placed upright. Shiitake pinheads started developing 2-7 days after water soaking (Fig 6a and 6b). Bags, more aptly referred to as 'blocks' during the fruiting stage, were very lightly sprayed with water during this period. If required, the pinheads were gently covered with a transparent plastic for humidity retention.

Figure 6a: Shiitake pinhead formation

Figure 6b: Pinhead elongation

Pinheads of Shiitake reach maturity in 5-8 days (Fig 7). Spraying of substrate was avoided during the 12 hours preceding harvest. Harvest stage can be distinguished when the mushroom cap is approaching plane and the cap margins are still tightly in-rolled.

Shiitake was picked manually by firmly holding the base of the mushroom stalk and twisting it off the substrate blocks (Fig 8). The stalks were then trimmed, if needed. Right after harvesting, any remnants of mushroom stalk were trimmed off the substrate blocks to avoid contamination.

After harvest, the blocks were not watered for 2-4 weeks to reduce humidity. At the end of this period, the blocks were again triggered for another flush of fruiting by immersion in cold water for the time span previously mentioned.

Figure 7: Shiitake mushrooms at harvest stage

Figure 8: Twist to harvest

Normally, 2 to 4 harvests are obtained per bag. The total yield of fresh shiitake mushrooms harvested from each bag of 0.75 kg capacity was around 100 g.

After harvest, fresh Shiitake have a relatively short shelf life. Fresh Shiitake are packed in Styrofoam punnets and covered with cling film before being stored at 2-5^{0}C. Cold stored under such conditions, the mushroom shelf life is around 10 days. For long term storage, mushroom caps were either sun dried or dried in a ventilated oven at 40-50^{0}C, cooled and packed in polyethylene bags. Dried Shiitake were thus preserved for 6-15 months. Also, dried stipes was blended into powder. The latter showed a potential for use as flavouring.

4.0 DISCUSSIONS

An adequate substrate formulation was determined for Shiitake cultivation. The non-composted materials used in the formulation allow rapid preparation times as described by Stamets for *Pleurotus* etc (1983). Some of the other raw materials assessed were not found suitable. This may be due to inadequate C:N ratio as in the case of composted chicken manure/bagasse substrate, slow mycelial colonisation and contamination rates. Preliminary evaluations of sawdust formulations were likewise found not promising even though it is commonly used as substrate. This may be due to unsuitable wood genera or possibly inhibitory substances within the sawdust. Compaction was found to improve mycelium colonisation. Compaction is especially important in the case of Shiitake in order to recreate the compactness encountered in wood logs where the mushroom naturally occurs as well as to promote mycelium development and minimise possibility of occurrence of contaminants. The substrate was compressed such that it forms a 'block' during bag production. This is in line with the observation of Kyung Wha Choi (2000) who recommends 'putting the substrate in the shape of a block, column or log'. After substrate preparation, the bags were sterilised in an autoclave. High pressure steam

sterilisation is recommended for creating a selective substrate for Shiitake cultivation. Pasteurisation, as in the case of oyster mushroom cultivation, was not found adequate for Shiitake cultivation as previously reported by Kwon (2001). It was noted that the sterilisation time must be adjusted based on the nature and quantity of substrate packed, the plastic type/ grade used and the bag size produced. For example, bags of around 2 kg must be sterilised for around 2 hrs at 121^0C (Chen, 2001).

The determination of optimal light regime for Shiitake indicated that no light is required for incubation, but diffuse light was required for fructification phase. The incubation phase of Shiitake bags, which is also known as the 'spawn run' period, is when mushroom mycelium growth occurs so as to permeate the bag (colonisation), followed by a period of maturation. Browning, also called 'light culturing' (Choi, 2000), is the formation of a brown membrane and, occasionally, exudates on the substrate surface. This is observed at the last stage of maturation. The plastic may be partially or completely removed off the Shiitake bags as reported by Chen (2001) to promote pinhead formation. When the bags were exposed to air, it was noted that the exposed substrate surface forms a dark brown drier protective and hardened layer that functions like a tree bark. The inner substrate softens and becomes moist such that inside moisture content reaches a maximum of around 80 %, ideal for onset of fructification. The drawback of this softening was that some bags crumbled during immersions. After maturation, fructification was initiated in bags. Several methods have been reported to be successful in inducing shiitake mushroom fruit formation. These methods include physical or electric shocks, abrupt changes in temperature or oxygen and carbon dioxide gas concentrations and high humidity (Lee, 1998). The temperature shock was selected to trigger fruiting as reported in several studies, for example the study of Mata and Savoie (1998). After fruiting bodies' initiation and harvest, bags were allowed to have a dormancy period before initiation of subsequent flushes. Harvested Shiitake can be stored in cold temperatures in order to extend the shelf-life. This is in line with the report of Fan *et al* (2005) regarding storage of Shiitake at 1-4^0C which extends shelf life to about 2 weeks. It was further reported that, with refrigeration of the mushrooms at temperatures below 10^0C, keeping time of Shiitake is up to 7 days as obtained locally. For long term preservation, freezing, drying, canning, pickling and powder or tea preparation (Fan *et al*, 2005) can be adopted.

4.1 Constraints

There are some factors that have to be taken into consideration during local Shiitake cultivation.

a) Shiitake being a temperate mushroom therefore, for low investment cultivation, bag production and fructification have to be earmarked for during the cooler months of the year.
b) The long duration of spawn run results in a higher susceptibility of Shiitake bags to contamination. The types of contamination noted during spawn run are invasions by various competitor moulds such as *Trichoderma* , *Aspergillus* etc. Susceptibility to contamination is also noted after water soaking and during fructification. This may be avoided by not immersing bags with signs of contaminants with uncontaminated ones and also by preventing excessive humidity build up during fruit formation.
c) The chosen strain should show good mycelial vigour, be a pure culture and show consistent mushroom strain characteristics. Ensuring good laboratory practices and monitoring strain purity whilst avoiding excessive transfers help maintain required quality. Visual and olfactory screening of mother spawn to detect good quality mother spawn is carried out.
d) In addition to avoiding contaminations during cultivation, good hygienic conditions have to be maintained in the growing house. Rats are a common pest encountered in growing houses, which can be controlled using repellents and rodenticides. Mushroom flies are controlled by using insect proof netting on all openings. Less frequently, slugs have also been seen on the Shiitake fruiting bodies to which they are attracted. They can be controlled by manual removal as they appear or by using slug bait.
e) Bad timing of plastic bag removal from Shiitake bags at end of incubation may result in improper development of 'bark' stage (Chen, 2001). This combined with the softened core of bags following fungal metabolism may result in the crumbling of blocks during immersions and loss of flushes.
f) People working indoors in the growing houses and who are prone to spore allergy risk an immune reaction. Protective masks must be worn to minimise this risk.

5.0 STATUS OF SHIITAKE CULTIVATION LOCALLY

With training from AREU, shiitake cultivation has already been started on a small scale by a few mushroom growers. After the official launching of Shiitake to the mushroom planting community in 2007, there have been several more growers purchasing Shiitake bags from AREU and those having started to prepare their own Shiitake bags.

6.0 FUTURE WORK

The future research at AREU is geared towards improving yield as well as the actual cultural practices package developed for the cultivation of Shiitake locally. These include:

a) Research into identification of other substrates e.g. sawdust, agrowastes, grasses etc for the cultivation of Shiitake bags.
b) Determining the optimal bag sizes for the different substrates.
c) Determining the optimal sterilisation regimes for bags.
d) Identifying ways to diminish strain attenuation.

7.0 CONCLUSION

Shiitake mushroom is well appreciated globally as well as locally for its numerous benefits. Its cultivation is a promising agribusiness. Research work done so far at the AREU has identified a set of cultural practices for successful Shiitake cultivation locally. Substrate formulation, bag preparation, incubation and fructification steps have been investigated in order to diversify the range of mushrooms cultivated locally. Future work will be aimed at improving the existing substrate, cultural practices and Shiitake yield so as to ultimately cut down on imports.

8.0 ACKNOWLEDGEMENT

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