PRODUCTION OF VIABLE, PRODUCTIVE AND PURE SPAWN IN OYSTER MUSHROOM (*Pleurotus ostreatus*)

L.Pathmashini[1], V.Arulnandhy[1], R.S.W. Wijeratnam[2]
[1]Department of Agricultural Biology, Faculty of Agriculture, Eastern University, Sri Lanka
[2]Food Technology Division, Industrial Technology Institute (ITI), Colombo – 07, Sri Lanka

ABSTRACT

An investigation was carried out to produce oyster (*Pleurotus ostreatus*) mushroom spawn on different grain substrates. Locally available kurakkan (*Eleusine coracana*), maize (Whole and broken) (*Zea mays*), sorghum (*Sorghum bicolor*), bajra (*Pennisetum typhoides*) and paddy (*Oryza sativa*) were tested for spawn production. A newly developed methodology was tested to produce the spawns, in which $CuSO_4$ of 0.5% was used to treat all types of grains. Out of the grain substrates tested whole maize and bajra failed to develop spawns. Broken maize (*Zea mays*) showed the highest density with mean score of mycelia density 4. Kurakkan (whole grain) expressed an acceptable density with mean score of mycelia density 3 while paddy and sorghum showed the low densities with mean score of mycelia density 2. The maximum points of attachments were found in broken maize followed by kurakkan, sorghum and paddy respectively. Broken maize showed very rapid colonization in about 11 days of incubation. Sorghum and Paddy took 16, 17 days of incubation respectively and kurakkan showed colonization in 14 days.

Key words: *Eleusine coracan*, *Oryza sativa*, *Pennisetum typhoides*, *Pleurotus ostreatus*, *Sorghum bicolor*, Spawn, *Zea mays*

INTRODUCTION

World Bank (2008) reported increasing food prices as a cause of food scarcity around the world. Diversifying to food sources is essential to meet the food requirements of an ever increasing population. In this respect mushrooms are a prominent and circumscribed group of edible fungi and have the ability to bioconvert into highly palatable food (Shah *et al*, 2005). Mushrooms are the fruiting bodies of filamentous and fleshy fungi (Chang, 1981). They are not only a nutritious food but also a valued delicacy. This product is gradually gaining popularity in Sri Lanka. Mushroom production as a source of income has good potential due to

the availability of suitable substrates such as post- harvest agricultural materials like straw, dried leaves, waste from food industries, etc. A spawn or mushroom seed comprises mycelium of the mushroom and a supporting medium which provides nutrition to the fungus for its early growth (Bahl, 1998). Therefore a pure culture of a desired mycelium is added to the grains substrate. The procedure is called spawning and the pure culture is called spawn. Spawn production is a critical process in mushroom production (Lelley, 1988). The production of viable productive and pure spawn is the key operation in mushroom cultivation.

The objectives of this study were to the production of viable productive and pure spawn, evaluate selected grain media for spawn production, and develop a suitable protocol with the most suitable media for spawn production.

MATERIALS AND METHODS

Location
Experiment was carried out in Microbiology laboratory, Industrial Technology Institute (ITI), Colombo.

Source of Mushroom
Pure culture of *Pleurotus ostreatus* mushroom maintained on Potato Dextrose Agar was obtained from mushroom tissue.

Types of grain substrates
Five types of locally available grains were tested.
Kurakkan (*Eleusine coracana)*
Maize (*Zea mays*) – whole and broken
Sorghum (*Sorghum bicolor*)
Paddy (*Oryza sativa*)
Bajra (*Pennisetum typhoides*)

Spawn production technology
In this study 0.5% $CuSO_4$ was used as treatments for different kinds of grains. Untreated samples of these grain types were used as controls. Each treatment was replicated thrice in a CRD experiment as design.

Methodology for Treated Grain Preparation
The cleaned grains were soaked in 0.5% $CuSO_4$ for 10 minutes and the soaked grains were thoroughly washed and soaked in tap water for 2 hours. After that the soaked grains were drained, excess water was

removed and the additives were added; which included rice bran at the rate of 10%, chalk ($CaCO_3$) at the rate of 2%, and epsom ($MgSO_4$) at the rate of 0.2% on dry weight basis of the grains and the additives were thoroughly and evenly mixed well with the grains.

Then the grain media were packed in polypropylene bags of 200 gauges, with dimensions of 37.5 cm length, and 17.5 cm breadth. About 200 grams of the medium was packed in each bag. Then the bags were sealed at the neck region by putting cotton plugs into the conduit/ poly vinyl chloride pipe rings, and covered with a piece of paper by tying a rubber band around the neck. The bags were sterilized in autoclave at 121°C, 15 psi, for 30 minutes and the sterilized bags were allowed to cool for 24 hours.

Methodology for Untreated Grain Preparation*

The cleaned grains were thoroughly washed and soaked directly in tap water for 2 hours without soaking in 0.5% $CuSO_4$ and the rest of the procedure are same as with treated grains

The treatments were

T1 - Untreated sorghum grains
T2 - Untreated kurakkan grains
T3 - Untreated broken maize
T4 - Untreated paddy grains
T5 - Treated Sorghum grains
T6 - Treated kurakkan grains
T7 - Treated broken maize
T8 - Treated paddy grains

*Bajra and whole maize were assigned in this manner as treatments but they are omitted in the discussion part as there was no appreciable colonization of mycelium.

Inoculation of Culture into the Grain Substrates

The agar carrying the inoculum piece was transferred immediately and aseptically in to the grain bags and the mouth of the bags sealed immediately using sterilized cotton plugs. The inoculated bags were kept in incubator at ambient conditions until the mycelia fully colonize the bags.

RESULTS AND DISCUSSION

Out of the media tested whole Maize kernels and Bajra (*Pennisetum typhoides)* seeds were found unsuitable to the given methodology of

spawn production. Whole Maize showed colonization, but at a very slow rate i.e. approximately 25 days to colonize completely in the bag. The mycelia appeared dried and dead on Bajra (*Pennisetum typhoides*) seeds within 5 days. The mycelium was unable to colonize on the bajra grains and found as coagulated. Therefore these two media were not considered for further discussion.

In this study overnight soaking and cooking were not applied. Instead a new methodology was adopted, which is the treatment of grains with 0.5% $CuSO_4$ soaked for 10 minutes. It is economical as well as, energy and time saving, and assumed to be most reliable technology. To overcome the problem in whole maize, the maize kernels were ground slightly to break the grain in order to reduce size. The broken maize yielded highest density of mycelium with mean score of mycelia density 4. (Table2). The reduction in size increased the points of attachments of inoculum and yielded maximum density of inoculum (Table 1). Next to the broken maize, kurakkan (whole grain) expressed an acceptable density with mean score of mycelia density 3 while paddy and sorghum showed the low densities with mean score of mycelia density 2 (Plates 1- 4).

Table1. Colony morphology of *Pleurotus ostreatus* on Grain Media

Media	Color	Texture	Max.Days Colonize	Points. attchmts*	Growth Period
Sorghum	White	Cottony	16	++	21
Kurakkan	White	Cottony	14	+++	17
Broken maize	White	Cottony	11	++++	15
Paddy	White	Cottony	17	+	21

* + = Low. ++ = Medium. +++ = High. ++++ = Very High.

Meanwhile maximum points of attachments were found in broken maize followed by kurakkan, sorghum and paddy. It was found when the size of the grains increases the points of attachment decreased and caused reduction in density. This may be the reason for the variation in the density of inoculum (Table 1).

Broken maize showed very rapid colonization in approximately 11 days of incubation. Paddy and sorghum took 17, 16 days of incubation respectively (Table 1). The seed coat hardness and the large size of thegrains are the causal factors which determine colonization. Kurakkan showed colonization in 14 days because of its small size and soft texture. It was noted that, the points of attachment were low in paddy because of very rough seed coat. Points of attachment depend on seed coat hardness and seed size.

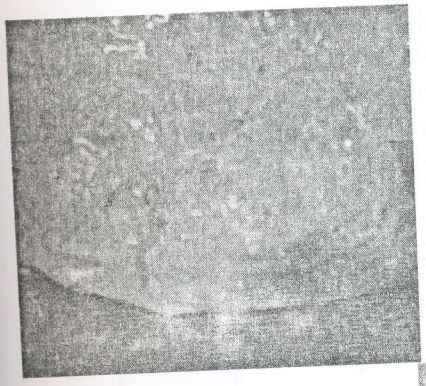

Plate 1. Pleurotus ostreatus grown on broken Maize

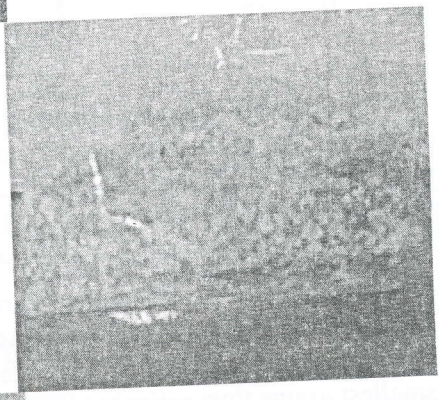

Plate 2. Pleurotus ostreatus grown on Sorghum

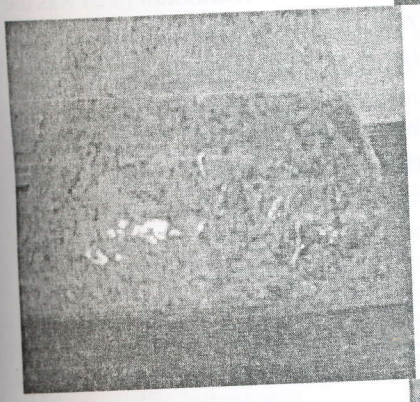

Plate 3. Pleurotus ostreatus grown on Paddy

Plate 4. Pleurotus ostreatus grown on Kurakkan

The density observed in untreated grains was found to be less than in treated grains (Table 2). It is possible that $CuSO_4$ increased the metabolic activity in *Pleurotus ostreatus* as copper is known to be a constituent of a number of enzyme systems (Eswaran & Ramabadran, 2000).

Table 2. Contamination and mycelial density of treated and untreated grains

Treatment	No. Contaminated Bags	Specific ratings of mycelia density
T1	nil	1
T2	3	2
T3	2	3
T4	1	1
T5	nil	2
T6	nil	3
T7	nil	4
T8	nil	2

1 = Very low density 2 = Low density 3 = Medium density 4 = High density

The contamination was completely arrested with the treatment of $CuSO_4$ (T5-T8). $CuSO_4$ acted as a contact fungicide and prevented the contamination mainly caused by green moulds. Kurakkan (T2) was the smallest grains thus actively utilized by the micro organisms for their metabolism and it showed the higher percentage of contamination.

The loss in the weight of grain media was noticed but not remarkable over time during the incubation period. This loss in kurakkan grain (T2) was greater compared to other grains and it may be due to the efficient utilization of kurakkan grain substrates by the *Pleurotus ostreatus* (Figures 1-2).

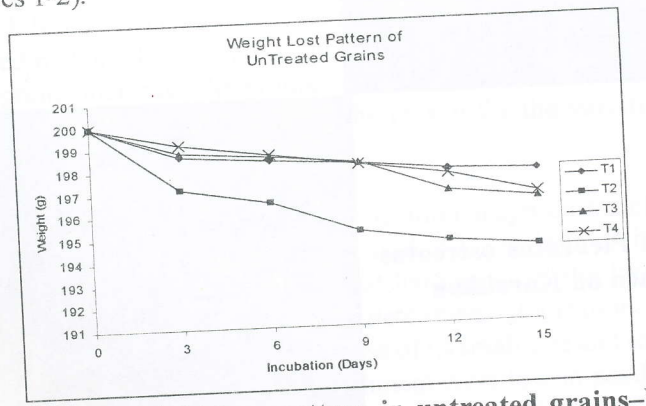

Figure 1: Weight loss pattern in untreated grains–Weight plotted against time (in days)

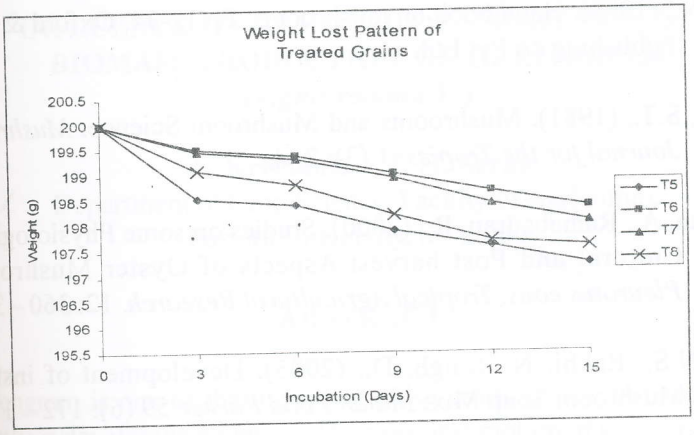

Figure 2: Weight loss pattern in treated grains-Weight plotted against time (in days)

(T1;untreated sorghum, T2;untreated kurakkan, T3; untreated maize, T4; untreated paddy, T5; treated sorghum, T6;treated kurakkan, T7; treated maize, T8; treated paddy)

CONCLUSIONS

Soaking grains with 0.5% copper sulphate ($CuSO_4$) for 10 minutes completely arrested the contamination and enhanced the development of mycelium of oyster mushroom favorably. In this study broken maize treated with 0.5% copper sulphate ($CuSO_4$) has observed to be the most suitable medium for spawn production in oyster mushroom. However as far as the whole grains are concerned kurakkan (*Eleusine coracana*) treated with 0.5% copper sulphate ($CuSO_4$) was found to be the best grain medium for the production of oyster (*Pleurotus ostreatus*) mushroom grain spawns, compared to maize (*Zea mays*), sorghum (*Sorghum bicolor*), and paddy (*Oryza sativa*). These findings will contribute to economic spawn production with, energy and time saving benefits to the industry.

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