EFFECT OF PASTEURIZATION TECHNIQUES ON MYCELIAL GROWTH OF OYSTER MUSHROOM, *PLEUROTUS* SPP.

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ABSTRACT

These studies were conducted at the Institute of Horticultural Sciences, University of Agriculture, Faisalabad during 2002. Different techniques of pasteurization including control, hot water treatment, steam pasteurization and chemical sterilization with formalin were applied to cotton waste to evaluate optimum method for best mycelial growth of three species of oyster mushroom. Results showed that steam pasteurization gave maximum mycelial growth which completed in shortest period of time. Formalin treatment behaved poorly as the species took maximum time to complete their mycelial growth.

KEYWORDS: *Pleurotus;* growing media; mycelium; Pakistan.

INTRODUCTION

Mushroom is fungi and lacks chlorophyll. It cannot produce its own food and depends entirely on organic materials for nutrition.The nutrients are provided by the medium called compost. Mushroom belongs to the class Basidiomycetes and order Agaricales in fungal classification. Agaricale order is composed of fungi, forming fleshy usually umbrella like bodies. The term mushroom refers to fruiting body.

Mushroom is popular for its nutrients and can make a very important contribution to human food. The protein contents of mushroom are equal to corn, milk and legumes and higher than other root and leafy vegetables and fruits. Low in cholesterol and calories, mushrooms are referred to as slimming foods (5).

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Mushrooms can be grown on almost all lignocellulosic material which is in abundance in Pakistan. Mushroom cultivation can be adopted as cottage industry. Being a protected crop, it does not compete for an area with another crop because it is usually produced indoor and thus remains safe from vagaries of weather, etc. Its commercial success will help save foreign exchange.

The oyster mushroom, which belongs to genus *Pleurotus*, grows wild under conditions on logs and stumps of trees in forests of North West Frontier Province and Azad Kashmir and other plantations in the plains of Punjab and Sindh during monsoon (2). The oyster mushroom refers to "Dhingri" in local vernacular. Oyster mushrooms are easier to produce and least expensive. For small scale cultivation with limited budget, oyster mushroom is the clear choice for entering into mushroom industry. Different species of *Pleurotus* grow on wide array of forest, agricultural and industrial waste materials. The cultivated species of *Pleurotus* are *P. ostreatus*, *P. pulmonarius*, *P. systetiosus*, *P. eryngii*, *P. djmor* and *P. citronopileatus*. Successful cultivation of mushroom often requires sterilization of the substrate, prior to inoculation with spawn (3). Oyster mushroom growers in Pakistan usually use different methods of substrate pasteurization which result in large variation in their mushroom production.

Present studies were undertaken to find out the most appropriate method of pasteurization which will further improve the yield and production.

MATERIALS AND METHODS

The studies were conducted in glass house, at the Institute of Horticultural Sciences, University of Agriculture, Faisalabad during 2002. Layout system of the experiment was completely randomized design with four replications. Three strains of *Pleurotus* namely *P. florida, P. pulmonarius* and *P. ostreatus* were selected and collected from the "Culture Bank" of the Institute whereas strain AS-64 was isolated from Faisalabad. These strains were multiplied on malt extract agar (MEA) and potato dextrose agar (PDA) media, the composition of which is given on next page.

Malt extract agar (MEA)

Malt extract	=	20g
Dextrose	=	20 g
Agar	=	20 g
Peptone	=	1 g
Distilled water	=	To make one litre (1000 ml)

Potato dextrose agar (PDA)

Potato starch	=	20 g
Dextrose	=	20 g
Agar	=	20 g
Distilled water	=	To make one litre (1000 ml)

The medium was sterilized in autoclave at 15 PSI and 121°C for 15 minutes, poured into clean test tubes and inoculated with the pure culture of two strains. The test tubes were incubated at 25°C until the completion of mycelial growth.

Spawn preparation

Spawn was prepared on sorghum grains as described by Pal and Thupa (4). The sorghum grains were boiled for 30 minutes and mixed with 20 percent calcium carbonate and 4 percent calcium sulphate to avoid culming of grains. These grains were sterilized at 121°C for one hour. The spawn was ready after about 30 days incubation at 28 $\pm$ 2°C during which the mycelia fully covered the grains.

Preparation of substrate

Byproduct of textile industry i.e. cotton waste was used in the experiment. The substrate was soaked in water for 72 hours to moisten. The substrate was spread on the inclined cemented floor to remove excessive water from the substrate to 70 percent. This material was filled in polypropylene bags of 5 x 7 sizes. Total weight of substrate filled in each bag was 270 g and mouth was plugged with cotton wool.

Pasteurization techniques

Substrate filled bags were subjected to following different pasteurization techniques.

1. Control (without pasteurization)
2. Hot water treatment with boiling water for 30 minutes.
3. Chemical sterilization with formalin, in which half litre of formalin was diluted with 10 litres of water and was used for a cubic meter of substrate.
4. Pasteurization through steam at 80°C for one hour by using steam drum in such a way that plastic bags filled with substrate were kept in the steam drum filled with 4-5 inches bottom layer of water and heated at 80°C for one hour.

Spawning

Spawning was done @ 5 percent of the net weight of substrate. The inoculated bags were kept at 25°C in complete darkness for spawn running until substrate became white due to impregnation by fungal mycelium.

Observations on the completion of mycelial growth were recorded by recording numbers of days taken to complete mycelial growth. The mycelial growth was calculated in such a way that spawn grains were kept in the bottom of test tube (9 inches long) and substrate was filled in test tubes. The length of test tube was equally divided in to four parts. The percentage of growth of mycelia was measured along the length of test tube by taking each part as 25 percent of total growth. In this way number of days taken to complete 25, 50, 75 and 100 percent mycelia growth were counted.

RESULTS AND DISCUSSION

It is concluded from results that steam pasteurization gave better performance than all other methods (Table 1), followed by hot water treatment. In steam pasteurization technique, *Pleurotus ostreatus* completed mycelial growth rapidly in all stages of mycelial growth (25, 50, 75 and 100%) in 2, 7, 9 and 11 days, respectively, whereas *Pleurotus pulmonarius* and *Pleurotus florida* took 3, 8, 9 and 12 and 4, 9, 10 and 13 days, respectively.

Table 1. Number of days taken to complete mycelial growth of *Pleurotus* spp.

	Mycelial growth(%)			
Species	25	50	75	100
P. florida	4	9	10	13
P.pulmonarius	3	8	9	12
P. ostreatus	2	7	9	11

Data regarding mycelial growth in hot water treatment (Table 2) showed that *Pleurotus ostreatus* completed mycelial growth in minimum number of days, followed by *Pleurotus pulmonarius* and *Pleurotus florida.*

Table 2. Number of days taken to complete mycelial growth of *Pleurotus* spp. in hot water treatment.

	Mycelial growth(%)			
Species	25	50	75	100
P. florida	5	12	14	16
P.pulmonarius	4	10	12	14
P. ostreatus	3	8	10	12

The results from control treatment (Table 3) showed that *Pleurotus ostreatus* took less time to complete mycelial growth in all four stages as compared to all other species followed by *Pleurotus pulmonarius* and *Pleurotus florida.*

Table 3. Number of days taken to complete mycelial growth of *Pleurotus* spp. in control (without treatment)

	Mycelial growth(%)			
Species	25	50	75	100
P. florida	7	14	17	20
P.pulmonarius	5	13	15	18
P. ostreatus	4	10	13	15

In formalin treatment (Table 4) *Pleurotus ostreatus* took less time to complete mycelial growth in all four stages as compared to *Pleurotus pulmonarius* and *Pleurotus florida.*

Table 4. Number of days taken to complete mycelial growth of *Pleurotus* spp. in formalin treatment.

	Mycelial growth(%)			
Species	25	50	75	100
P. florida	8	15	20	25
P.pulmonaria	9	14	21	24
P. ostreatus	8	14	18	23

In case of complete mycelial growth (100%) in all treatments, steam pasteurization technique proved better followed by hot water treatment (Table 5).

Table 5. Number of days taken to complete mycelial growth of *Pleurotus* spp. different treatments

Species	Mycelial growth(%)			
	Control (without treatment)	Hot water treatment	Steam pasteurization	Formalin treatment
P. florida	20	16	13	25
P.pulmonaria	18	14	12	24
P. ostreatus	15	12	11	23

Variation in duration of mycelial growth in different treatments was observed. Different species behaved differently. Pasteurization selectively kills temperature sensitive micro organisms. The population left intact offers little competition to mushroom mycelia for initial period giving opportunity for the mushroom mycelium to colonize (6). Formalin treatment gave poor results as all the species took more time for completion of mycelial growth. Chemical sterilization is usually recommended for the substrate which is incorporated with any carbon source and no such source was added in the present study.

The duration for completion of mycelial growth was also affected by the growth behaviour of different species/strains substrate and treatments.

Earlier study (7) revealed that cotton waste was the best substrate for cultivation of *Pleurotus ostreatus* and other related species. The results also showed that spawn running took two to three weeks. Similarly, Leong (3) confirmed that fast growing strain of *Pleurotus florida* took 9-12 days for spawn running on cotton waste when held at 20 to 30°C.

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