INFLUENCE OF SUBSTRATE PASTEURIZATION METHODS ON THE YIELD OF OYSTER MUSHROOM (*Pleurotus species*)

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To evaluate the influence of pasteurization methods on cotton waste substrate on yield of oyster mushroom (*Pleurotus* spp. Cotton waste subjected to different methods of pasteurization, namely pasteurization with steam, hot-water treatment and chemical sterilization with formalin, which were compared with control (without pasteurization). Three species of *Pleurotus* i.e. *Pleurotus florida, Pleurotus pulmonarius* and *Pleurotus ostreatus* were selected. Steam pasteurization produced the best results as for as the performances of individual species are concerned, *Pleurotus pulmonarius* completed the mycelial growth in the shortest time. Formalin treatment behaved poorly as the different *Pleurotus* spp, took maximum time to complete mycelial growth. Steam pasteurization technique produced more yield, whereas *Pleurotus florida* behaved better in all the treatments than other species. Substrate was analyzed chemically for N: P: K to determine their contents at different stages. N: P: K contents were increased after the completion of mycelial growth in all the treatments, but were decreased after fructification as the fruiting bodies consumed nutrients for their growth.
Keywords: *Pleurotus* spp, mycelial growth, yield, mushroom

INTRODUCTION

Mushroom is one of the man's earliest foods which have come to be recognized as highly nutritive food, low in calories, but rich in proteins and certain vitamins. A distinctive feature of mushroom protein is that it comprises of all the essential amino acids and has highly digestible value.

The vitamins of mushrooms are not destroyed by cooking, drying and freezing (Nair, 1982). Mushroom has been used as a food and medicine by different civilizations since immemorial time, due to its delicious taste and dietetic qualities. But technology for artificial cultivation of mushroom is recent innovation especially of oyster mushroom. Mushrooms are also known for their medicinal properties, these are low in calories and ideal food for diabetic and heart patient. Mushroom has qualities like lowering the blood cholesterol level, warding against cancer and invigorating hair growth. Tewari (1986) reported that the fresh mushroom contains about 85-90% moisture, 3% protein, 4% carbohydrates, 0.3-0.4% fats and 1% minerals and vitamins.

The world production of oyster mushroom is estimated to be 875,000 tons in 1997 (Chang, 1999). China was responsible for 87% of world supply, oyster mushroom is the easiest to produce and least expensive to grow. For small scale cultivation with limited budget, oyster mushroom is the clear choice for gaining entry into the mushroom industry. Different *Pleurotus* species grow on a wide array of forest, agricultural and industrial materials. The cultivated species of *Pleurotus* are *P. ostreatus, P. pulmonarius, P. systetiosus, P. eryngii, P. djmor, P. citrinopileatus* and *Pleurotus Pulmonarius*.

The cultivation of mushroom as a source of fungi growing has been suggested one of the ways to meeting the demand of growing population of Pakistan. Further, mushroom can be grown on almost all ligno-cellulosic agricultural and industrial wastes which are in abundance in Pakistan. Mushroom cultivation can be adopted as cottage industry. Being a protected crop, it does not compete for area with any other crop because it is usually produced indoor and thus is safe from vagaries of nature. Its commercial success will help in saving foreign exchange, now being used for importing mushrooms. It has also tremendous export potential as a foreign exchange earning commodity.

Successful cultivation of mushroom often requires pasteurization of the substrate, prior to inoculation with spawn (Chang and Hayes, 1978). Oyster mushroom growers in Pakistan, usually use different methods of substrate pasteurization which results in large variation in their mushroom production. Therefore, the present research was undertaken to find out the most appropriate method of pasteurization which will be helpful for further improvement in yield.

MATERIALS AND METHOD

Cotton waste which is considered successful substrate for the cultivation of *Pleurotus* spp, and for being abundantly available in industrial area was used as experimental substrate for growing oyster mushroom.

For present studies, three strains of oyster mushroom viz., *Pleurotus florida, Pleurotus pulmonarius* and *Pleurotus ostreatus* were collected from the "Culture Bank" of Department of Horticulture, University of Agriculture, Faisalabad. These selected strains were

multiplied on Malt Extract Agar **(**Malt Extract 20 g, Dextrose 20 g, Agar 20 g, Peptone 1 g, Distilled water 1000 ml).The medium was sterilized in autoclave at 15 PSI at (121°C) for 15 minutes and then poured in clean test tubes. Streptopenicilline was added in the sterilized medium at the rate of 1 g/liter. Test tubes were incubated at 25°C until the completion of mycelial growth. Spawn was prepared on wheat grains as described by Pal and Thopa (1979).By- product of textile industry (cotton waste) 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%. This material was filled in polypropylene bags of 5 x 7" size. Total weight of substrate filled in each bag was 270 g and mouth was plugged with cotton wool.

Pasteurization technique

i. Control (without pasteurization)
ii. Hot-water treatment with boiling water for 30 minutes.
iii. Pasteurization through steam at 80°C for one hour.
iv. Chemical sterilization with formalin, in which half liter of formalin was diluted with 10 liters of water and were used for one cubic meter of substrate.

Spawning was done at the rate of 5% of the net weight of the substrate. After the completion of spawn running, the mouth of the bags were cut with the help of scissors or blades. These bags were placed in shelves of growing room for cropping. Efforts were made to maintain temperature, relative humidity and aeration at desired level.

The appropriate statistical method was used for the analysis of data. LSD test at 5 % probability was applied to compare the differences among the treatments (Steel and Torrie, 1984).

RESULTS AND DISCUSSION

Chemical analysis of the substrate for N:P:K

Cotton waste substrate was chemically analyzed before treatments, after treatments, on the completion of mycelial growth and after cropping for nitrogen, phosphorus and potassium.

Nitrogen percentage in different treatments

Data on nitrogen percentage of different treatments of substrate are given in Table 1. Analysis of variance shows that there was no significant change in nitrogen percentage before and after treatments. However, nitrogen percentage increased after the completion of mycelial growth, which decreased after the appearance of fruiting bodies. Maximum nitrogen contents (0.56 %) were found in all treatments after the completion of mycelial growth. Whereas, minimum nitrogen contents (0.28%) were observed in substrate before and after treatments. However, nitrogen percentages (contents), after the cropping period were decreased in all treatments and species. These findings have shown that fruiting bodies consumed nitrogen for their growth which resulted in deserved nitrogen contents after the cropping period. Considering the interaction of treatments and species, there was no significant variation in nitrogen percentage. The mystery behind in increased nitrogen percentage after the completion of mycelial growth was the nitrogen fixing ability of *Pleurotus* species. Nitrogen contents was consumed by the fruiting bodies and decreased accordingly in treatments and specie. These results are in close conformity of the findings of Rangaswamy *et al.* (1975), Shetty and Krishnamoorthy (1980), Zafar *et al.* (1981), Khan *et al.* (1982) and Ahmad (1990).

Table 1. Percentage of Nitrogen, phosphorus, potassium (N:P:K)

Substrate	*P. florida*			*P. palmonarius*			*P. ostreatus*		
	N	P	K	N	P	K	N	P	K
Substrate without treatments	0.28	0.16	1.096	0.28	0.16	1.096	0.28	0.16	1.096
Substrate after treatments	0.28	0.16	0.52	0.28	0.16	0.52	0.28	0.16	0.52
Substrates after mycelial growth	0.56	0.64	0.80	0.56	0.64	0.80	0.56	0.64	0.80
Substrates after cropping in control (No treatment)	0.42	0.48	0.84	0.50	0.56	0.96	0.46	0.50	0.90
Substrates after cropping in hot-water treatment	0.40	0.44	0.67	0.48	0.50	0.75	0.44	0.46	0.70
Substrates after cropping in steam pasteurization	0.36	0.40	0.54	0.42	0.46	0.60	0.39	0.43	0.57
Substrate after cropping in formalin treatment	0.38	0.42	0.65	0.45	0.48	0.71	0.42	0.44	0.68

Phosphorus percentage in different treatments

Data regarding phosphorus percentage in different treatments are given in Table 1. Analysis of variance shows that phosphorus percentage was not changed in substrate before and after treatments. Interaction between treatments and species showed no change in phosphorus content. However, phosphorus contents increased in different treatments after the completion of mycelial growth, which decreased after the cropping period, as consumed by the fruiting bodies. Maximum phosphorus contents (0.64%) were observed in substrate after the completion of mycelial growth, while minimum (0.16%) were determined before and after treatments. Increase in phosphorus contents, after the completion of mycelial growth, might be due to acidity produced through CO_2 released through respiration of *Pleurotus* species which resulted in the formation of H_2CO_3. It on hydrolysis gave the hydrogen ions resulting in decreased substrate pH. Therefore, the availability of phosphorus may be due to acidic pH which was maintained by adding $CaCO_3$ in the substrate. The results are in line with Galdwell (1986).

Potassium percentage in different treatments

Data pertaining to potassium percentage of different treatments are shown in Table 1 results show that there was a significant change in potassium percentage before and after treatments of substrate. Potassium contents decreased after pasteurization, which were increased on the completion of mycelial growth. Maximum contents (1.096%) were observed in substrate, before subjecting to different treatments. Minimum (0.52%) were determined in substrate after the pasteurization. However, potassium contents increased after the completion of mycelial growth, which again reduced after the cropping period, as it was consumed by the fruiting bodies. Whereas interaction of treatments and species remained non-significant.

Days taken to complete mycelial growth

The variation in the duration of mycelial growth in different treatments was observed. Similarly different species also behaved differently (Table 2). Pasteurization selectively kills temperature sensitive micro-organisms. The population left intact presents little competition to the mushroom mycelia for initial period giving ample opportunity for the mushroom mycelium to colonize (Staments, 1993). Treatment with formalin produced poor results as all the species of Pleurotus took more time for the completion of mycelial growth. Chemical sterilization is usually recommended for the substrates which are incorporated with any carbon source and no such source was added in the present study. The duration for the completion of mycelial growth is also affected by the growth behaviour of different species/strains substrates and treatments. Studies conducted by Tan (1981) revealed that cotton waste was the best substrate for the cultivation of Pleurotus ostreatus and related species. The results showed that spawn-running took two to three weeks. Similarly, Leong (1980) confirmed that a fast growing strain of Pleurotus florida took 9-12 days for spawn-running on cotton waste when held at 20 to 30°C.

Table 2. Number of days taken to complete mycelial growth of *Pleurotus spp*. in different treatments.

Species	Mycelial growth (100 percentage) in days			
	Control (with out treatment)	Hot water treatment	Steam pasteurization	Formalin treatment
P. florida	20	16	13	25
P. pulmonarius	18	14	12	24
P. ostreatus	15	12	11	23

Total weight of fruiting bodies (gm)

Data regarding total weight of fruiting bodies (gm) are given in Table 3. Analysis of variance shows that interaction of treatments and species varied

Table 3. Total weight of fruiting bodies (gm).

Species	Substrates			
	Control (No treatment)	Hot-water treatment	Steam pasteurization	Formalin treatment
P. florida	14	16	20	17
P. pulmonarius	10	12	15	13
P. ostreatus	12	14	18	15

significantly. Steam pasteurization produced the best results, followed by formalin, hot-water and control (without pasteurization) treatments. In steam pasteurization, Pleurotus florida produced maximum weight (20 gm) whereas, Pleurotus ostreatus and Pleurotus pulmonarius produced 18 and 15 gm weight respectively. In formalin treatment, Pleurotus florida produced 17 gm weight. Whereas, Pleurotus ostreatus and Pleurotus pulmonarius produced 15 and 13 gm weight respectively. In hot water treatment, Pleurotus florida produced 16 gm weight. Whereas, Pleurotus ostreatus and Pleurotus pulmonarius 14 and 12 gm weight respectively. Pleurotus florida produced 14 gm total weight in control (without treatments), while Pleurotus ostreaus and Pleurotus pulmonarius produced 12 and 10 gm weight respectively. Interaction of treatments and species shows that steam pasteurization produced the maximum total weight for different species of *Pleurotus*. As far as species are concerned, *Pleurotus flordia* yielded more weight as compared to the other species.

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