



IMPACT OF VARIOUS STERILIZATION METHODS USING DIFFERENT SUBSTRATES FOR YIELD IMPROVEMENT OF *PLEUROTUS SPP.*

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ABSTRACT

Different sterilization methods viz., Lab autoclave, Country style autoclave (2hr), Country style autoclave (1hr), Hot water treatment (1/2hr) and Ordinary water (1/2 hr) were investigated. Oyster mushroom was cultivated on saw dust, wheat straw, and rice husk with different treatments which included, wheat straw 50% + saw dust 50%, saw dust 100%, wheat straw 50% + rice husk 50% and rice husk 100%. Among the sterilization methods, the significantly effective method was lab autoclave followed by others. It was observed that the *Pleurotus ostreatus* (P-19) gave the maximum yield in the first flush followed by second, third and fourth flush and lab autoclave was recommended one of the best methods for the yield improvement of *Pleurotus spp.*

Key words: *Pleurotus ostreatus* (P-19), sterilization methods, agricultural wastes, yield.

INTRODUCTION

Pleurotus ostreatus (Jacq.Fr.) commonly known as Oyster mushroom is cultivated worldwide, especially in southeast Asia, India, Europe and Africa. The genus is characterized by its high protein content 30-40% on dry weight basis (Sharma & Madan 1993). Mushrooms are liked all over the world due to their taste, flavor and health properties and as a balanced diet. Most of them are edible which are sufficient for human consumption. Various agricultural wastes are being used as substrates for cultivation of oyster mushrooms. Some of these wastes include banana leaves, mango fruits, wheat straw and rice straw (Thomas *et al.*, 1998). It is also considered the best substrate in terms of yield and high protein content. In Europe, wheat straw is used while in southeast Asian countries sawdusts is more common. The majority of these substrates can be used as animal feed. Pakistan's economy is mainly dependent on agriculture. Food production in large quantity is a challenge but safe disposal of crop residues is a great problem. Edible fungi are natural recyclers which convert lignocelluloses wastes into protein rich health food. It is not only low in calories but also provides sufficient amount of digestible protein containing most of indispensable amino acids. The agriculture shares about 21% of total GDP and contributes substantially to Pakistan's export. It also contributes to growth as a supplier of raw material to industry as well as market for industrial products. It employs 44.3 percent of country's work force and 66 percent of country's population in rural areas is directly or indirectly linked with agriculture for their livelihood

(Anonymous, 2007). Its present production is approximately 1.5 million tons in the world. Every year 90 tons of mushrooms are exported to Europe from Pakistan (Shah *et al.*, 2004). Oyster mushroom can be cultivated on any type of ligno and cellulosic materials like (saw dust, wheat straw and rice husk). The Oyster mushrooms can be cultivated on a wide range of cellulosic materials (Ficior *et al.*, 2006). Different sterilization methods can be used for cultivation of oyster mushroom production and its yield improvement (Khan, 2009). Using such appropriate methods, spawning will assure better resistance against any disturbance of competitive micro-organisms. Apahidean, (2006) and Oei, (1996) determined that sterilization of substrates is much more appropriate method for effective and smooth cultivation of mushrooms to remove the existence of a number of microorganisms. Malnutrition is a problem in developing third world countries like Pakistan. Mushrooms with their flavour, texture, nutritional value and high productivity per unit area have been identified as an excellent food source (Eswaran and Ramabadrana, 2000). Oyster mushroom can help in solving the problems of malnutrition and disease. The present study aimed to investigate the sterilization methods using different substrates for their effective utilization by cultivation of oyster mushroom.

MATERIALS AND METHODS

Selection of *Pleurotus* strain: *Pleurotus ostreatus* (P-19) was obtained from the Department of Plant Pathology, University of Agriculture, Faisalabad,

Pakistan. The selected strain was multiplied on malt extract agar (MEA) having the following constituents of 20g/L, dextrose 20g/L, agar medium 20g/L, peptone 1g/L.

Preparation of cellulosic and lignocellulosic substrates. Wheat straw was taken from the livestock farm whereas rice husk was collected from field area near the Department of Plant Pathology, University of Agriculture, Faisalabad and saw dust of Kikar wood was collected from Raza-abad near university gate. These substrates were mixed with water thoroughly and stocked on a cemented floor, so as to remove the excess water from the substrates to get desired moisture (70%). Lime was mixed at the rate of 5% with different proportions of substrates and treatments with four replications were made by the following substrates which includes Saw dust, Wheat straw, Rice husk and these were fermented for 5 days by covering with polythene sheets before filling the bags, 1000 gm of each moist substrate was filled in polypropylene bags and their mouths were plugged by inserting water absorbing cotton with the help of plastic rings of 2.5 × 1 cm size made from PVC pipe. The only one local strain (*Pleurotus ostreatus* P-19) was used for its effective cultivation to screen out the various cellulosic and lignocellulosic substrates.

Using different sterilization methods for increasing yield: Five different sterilization methods were used for sterilization of the substrates.

(i) Lab autoclave: By this method the substrates were filled in the polypropylene bags and the polypropylene bags filled with moist substrate were autoclaved for one hour at 121 °C and 151 psi.

(ii) Country style autoclave. By this method after wetting the substrates were filled in the polypropylene bags. Then the bags were put in the country style autoclave for one hour.

(iii) Country style autoclave. By this method after wetting the substrates were filled in the polypropylene bags. The polypropylene bags filled with moist substrate were put in the country style autoclave for two hour.

(iv) Hot water treatment. By this method cellulosic and lignocellulosic substrate of sufficient material of bags (8/10) sizes were put in boiling water for ½ hour. This material was then filled in bags after cooling and drained off excessive water still the desired percentage of moisture was obtained.

(v) Ordinary water/control. By this method the substrates were treated in simple water.

Spawning of bags: The spawning was done at Seven percent of dry weight of the substrate in each bag of 56 gm used for all treatments. These bags were

incubated for spawn running under complete darkness at controlled temperature of 25 °C in spawning room. Temperature was maintained with the help of electric heater. Data was recorded of 100% (full growth) of spawn completion in days of substrate bags.

i. Temperature: Mushroom cultivation has two important phases spawn running and fructification, while temperature and humidity are two vital factors involved at both phases. So temperature was controlled by electric heater for 3-4 hours during day time and for whole night.

ii. Humidity: The humidity of the growing room was maintained between 80-90% by sprinkling on floor and moisture requirements of the bags were accomplished by sprinkling water on them thrice a day using sprinkler.

iii. Ventilation: The exhaust fan was used which was operated 3-4 times for air flush to fulfill oxygen requirements during the fructification of mushroom.

iv. Layout: The experiment was laid out in a complete randomized design (CRD). The data was analyzed statistically.

Recording of Data.

i) Days for completion of spawn running: Time was recorded in days for the completion of 25%, 50%, 75% and 100% growth of mycelium on each substrate in polypropylene bags.

ii) Days for the appearances of pinhead: The data was recorded in days taken for appearance of primordial formation in substrates.

iii) Maturation of fruiting bodies: Time period was recorded in days from pinheads to maturation of fruiting bodies in all treatments.

(iv) Yield: The data was recorded for the harvesting of mushroom in four flushes. The first and respective harvesting was done at maturity and the yield of different flushes of fruiting bodies were noted.

(v) Temperature and humidity of growing room: Temperature of growing room was recorded three times with dry and wet bulb thermometer, while relative humidity was recorded with the help of hygrometer.

RESULTS AND DISCUSSION

Spawn running: The analysis of variance for the spawn running of local Oyster mushroom *Pleurotus ostreatus* (P-19) showed in Table (1), that minimum mean comparison of days were required (15.75) when saw dust was used alone upto 100%. However, it required (17.50) days for spawn running when substrates combination of saw dust 50% + wheat straw 50% was used. The number of days increased to the time of (21.00) days when rice husk 50% +

wheat straw 50% was used. Maximum (23.75) numbers of days were needed when substrate combination of Rice husk 100% was used. Shah *et al.* (2004) reported that spawn running took 2 weeks for its completion on saw dust. Khan, (2009) reported that *Pleurotus Ostreatus* (P-19) took 24-25 days for completion of 100% spawn running on wheat straw.

Pinhead formation: The analysis of variance of the pinhead formation of local Oyster mushroom *Pleurotus ostreatus* (P-19) showed in Table (1) that minimum mean comparisons of days were required (22.75) when saw dust was used alone up to 100%. However, it required (25.50) days for spawn running when substrate combination of saw dust 50% + wheat straw 50% was used. The number of days increased to the time of (28.50) days when rice husk 50% + wheat straw 50% was used. Maximum (33.75) numbers of days were needed when substrate combination of Rice husk 100% was used. Sopit (2006) studied that pinhead formation took 40 days for the appearance, when Oyster mushroom was cultivated on sawdust, coconut husk. This difference in pinhead formation may be due to different substrates and strains of Oyster mushrooms. The

pinhead initiation takes 17-29 days and total number of pinheads was the highest (48.8) in the bag size (8x12") as compared to bag size (6x12") which gave minimum number of pinheads (25.4) reported by Khan *et al.* (2004).

Fruiting bodies formation: The analysis of variance of the fruiting bodies formation of local Oyster mushroom *Pleurotus ostreatus* (P-19) showed in Table (1) that minimum mean comparison of days were required (29.50) when saw dust was used alone up to 100%. However, it required (25.00) days for spawn running when substrate combination of saw dust 50% + wheat straw 50% was used. The number of days increased to 33.50 days when rice husk 50% + wheat straw 50% was used. Maximum (35.50) numbers of days were needed when substrate combination of Rice husk 100% was used. The 24 days for pinheads formation was observed on sawdust medium and the days for pinhead formation, days for flush (fruiting bodies) formation recorded in this study were longer than previous findings reported by Shah *et al.* (2004). The spawn running took three weeks and fruiting body appeared after 2-3 days observed by Tan (1981).

Table 1: Days for completion of spawn running, pinhead formation and fruiting body formation of different phases of mushroom production on different substrates

Substrates	Spawn running	Pinhead formation	Fruiting body formation
	(Days)		
Saw dust 100%	15.75 C	22.75 C	29.50 D
S.D 50% + W.S 50%	17.50 B	25.50 B	25.00 C
Rice husk 100%	23.75 A	33.75 A	35.50 B
R.H 50%+ W.S 50%	21.00 A	28.50 A	33.50 A
LSD at P= 0.05	1.0320	1.1455	1.0420

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

Different Sterilization Methods used for yield production of Oyster mushroom: The yield performance of each sterilization method is showed in Table (2). There is a significant difference among yield of different sterilization methods. The results revealed that lab autoclave (1hr) proved one of the best sterilization method which gave 1180g yield in flush first as appeared most efficient method for obtaining yield of *Pleurotus ostreatus* (P-19) and statistically close to country style autoclave(2 hr) which gave 1030.4 g in flush first and 750.55, 580.30, 430.25 g respectively in second third and fourth flush. After words, the method country style autoclave (1 hr) gave 680.4g in flush first and 470.42g, 370.45g, 245.40g respectively in other

flushes. Similarly, Hot water treatment for (½ hrs) gave 510.10g yield in first flush and 480.35, 375.33, 246.19 in other flushes. Khan *et al.* (2002) studied that the effect of different sterilization methods on the production of oyster mushroom (*Pleurotus ostreatus*) on different substrate. However, sterilization of substrates is not an easy job for the cultivation of mushroom. In order to work out an easy handy procedure for substrate (rice straw, wheat straw and cotton wastes) sterilization, different sterilization techniques (hot water treatment, country-style autoclave, laboratory autoclave in that all methods the lab autoclave method gave good result as compared to other sterilization methods. Khan (2009) reported that lab autoclave was most effective

method with respect to yield of Oyster mushroom. The pathogen population but also thermophytic fungi helps in biodegradation and pathogenic fungi and bacteria deteriorate the substrate resultingly in a very poor yield. Hafeez (2010) found the cultivation of three exotic oyster mushrooms i.e *P. (florida)*

ostreatus (WC-536), *P. (sajor-caju)* *pulmonarius*(WC-537) cultivation and *P. ostreatus*(WC-522) on different cellulosic substrates and sterilization of mushroom bags was done through lab autoclave. Finally, lab autoclave was found one of the best sterilization method among others.

Table:2 Evaluation of various sterilization methods on yield of mushroom production.

Sr. No	Sterilization methods	Ist Flush	2nd Flush	3rd Flush	4th Flush
		(yield in grams)			
1	Country style autoclave (1 hr)	680.4 c	470.42d	370.45d	245.40d
2	Lab auto clave (1 hr)	1180.3 a	845.38a	540.53b	279.39b
3	Country style autoclave (2 hr)	1030.4 b	750.55b	580.30a	430.25a
4	Hot water treatment for (½ hrs)	510.10 d	480.35c	375.33c	246.19c
5	Ordinary water or control for(½ hr)	490.33 e	310.34e	280.25e	197.29e
LSD at P= 0.05		0.7202	0.8219	0.7011	0.5876

Means sharing similar letter in a row or in a column are statistically non-significant (P>0.05).

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