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Sarita
Research Scholar, Department of Plant Pathology, RCA, MPUAT, Udaipur, Rajasthan, India

SS Sharma
Professor, Department of Plant Pathology, RCA, MPUAT, Udaipur, Rajasthan, India

Somdutt
Senior Research Fellow, SKRAU, Bikaner, Rajasthan, India

Chirag Gautam
Assistant Professor, Agriculture University, Kota, Rajasthan, India

Roop Singh
Ph.D., Scholar, Department of Plant Pathology, RCA, MPUAT, Udaipur, Rajasthan, India

Corresponding Author:
Sarita
Research Scholar, Department of Plant Pathology, RCA, MPUAT, Udaipur, Rajasthan, India

Optimization of spawn rate for Oyster Mushroom cultivation in southern Rajasthan

Sarita, SS Sharma, Somdutt, Chirag Gautam and Roop Singh

Abstract
The present experiment was carried out to find out the optimum spawn rate to get maximum yield and biological efficiency. Two strains (PL-19-05 and PL-19-06) of oyster mushroom were cultivated by using wheat straw as substrate and wheat grain spawn at different rates (3%, 4%, 5% and 6%). Among four spawn rates early spawn run and pin head initiation were observed at 6% spawn rate for both strains, followed by five percent, four percent and three percent spawn rate. Similarly, yield and biological efficiency were also higher at six percent spawn rate for both strains, followed by five percent, four percent and three percent Spawn rate. Based on the results six percent spawn rate would be suggested as optimum spawn rate for sub-humid conditions of Udaipur region to get higher yield.

Keywords: Oyster mushroom, spawn rate, yield and biological efficiency.

Introduction
Mushrooms are becoming a popular food among masses, because of their health benefits as per scientific findings available in authentic journals and literature. Mushroom comes under higher fungi with a unique fruit body which can be either hypogeous (underground) or epigeous (overhead the ground) and mushroom size is large enough to see with the naked eye and also pick by the hand. The oyster mushroom has two distinct part, a fleshy pileus and stalk or stipe (Chang and Miles, 1992) [4]. Fruiting bodies of mushroom are fleshy spore bearing structure and contain several spores that have similar function to seeds of the higher plants for propagation.

Mushrooms, also called 'boneless vegetarian meat' or 'white vegetables' (Thakur, 2014) [12] contains high nutritional value and good source of protein, vitamins (B1, B2, B12 and C), carbohydrates, minerals but low in fat (Subramamiam and Shanmugasundaram, 2015) [11]. Oyster mushroom also has good medicinal values and prevents or reduces some serious diseases like; cancer (Jedinak and Sliva, 2008) [7], high blood presser, diabetes (Agarwal *et al*., 2010) [1], averts fungal, viral and bacterial growth and balances the blood sugar level.

The process of photosynthesis is believed to produce approximately 200 tons of organic matter every year (Zhang, 2008) [15]. The majority of this organic matter, as well as numerous agro-industrial wastes, generate a considerable volume of solid waste, by-products and residues, all of which pollute the environment (Lal, 2005) [9]. Innovative technologies and disposal strategies are being developed around the world to convert these lignocellulosic wastes into more valuable resources (Chang, 2006) [3]. Among various techniques mushroom cultivation is one of the most efficient environmentally friendly techniques for the recycling of lignocellulosic organic waste into food, feed and fertilizer (Mandeel *et al*., 2005) [10]. Agricultural by-products such as rice and wheat straws are produced in large amounts. Mushroom cultivation, on the other hand, can recycle this waste into protein-rich foods.

Globally most cultivated mushroom is *Agaricus bisporus* followed by *Pleurotus sp* (commonly known as oyster mushroom). In India mushroom cultivation began in the year 1962 in Solan district of Himachal Pradesh and holds 4th position in mushroom production. Oyster mushroom belongs to Phylum–*Basidiomycota*, Class–*Agaricomycetes*, Family–*Pleurotaceae*. The important and commercially growing spp. of oyster mushroom are *Pleurotus sajor-caju*, *P. florida*, *P. ostreatus*, *P. eryngii*, *P. eous*, *P. comucopiae*, *P. fossulatus*, *P. platypus*, *P. flabellatus*, *P. columbinus*, *P. membranaceus* and *P. tuberreginum*. Different species of *Pleurotus* grow well in wide range of temperatures, hence they are well suited to year-round cultivation in various tropical and sub-tropical areas of India. But commercially, it requires specific weather conditions such as temperature (14-27 ^{0}C) and relative humidity (70-85%)

(Uddin *et al*., 2011) [14]. Oyster mushroom able to grow on wheat straw, rice straw, pearl millet straw, sawdust and sugarcane bagasse. The present study was carried out to optimize the spawn rate to get early growth, higher yield and biological efficiency.

Materials and methods

The experiment was carried out during 2019-20 in Mushroom Laboratory of Department of Plant Pathology, Rajasthan College of Agriculture, Udaipur, Rajasthan. The pure culture of the strains of oyster mushroom were received from Directorate of Mushroom Research, Chambaghat, Solan. The culture was multiplied on two percent malt extract agar medium and pure culture was maintained in test tube slants having two percent malt extract agar medium by frequent sub culturing. After inoculation, slants were kept in incubator at 25 ºC till proper growth was obtained.

Spawn preparation

Spawn is referred as seed of mushroom. After obtaining pure culture mother spawn and planting spawn were raised on grains. However, mycelium of mushroom grow on grains and these grains were used for seeding.

Substrate preparation

Wheat grains were used as substrate for spawn preparation. Good quality wheat grains were soaked overnight in water and in the next morning these grains were boiled for 20-25 min. After boiling, excess of water was decanted off. Then grains were allowed to surface dry by spreading on bloating sheet for half an hour. These semi cooked grains were mixed with 1 percent calcium carbonate on the wet weight basis. $CaCO_3$ is used for the purpose of adjust the pH of grains as well as check the clumping of the grains. The advisable substrate pH is 7-7.5. Grains were properly mixed and then filled in glass bottles as well as polypropylene bags up to two-third level and mouth was plugged tightly with cotton plug and covered with aluminum foil or paper followed by tying with rubber band to hold it in place. These bottles and bags were kept in autoclave for sterilization at 20 psi (126.5 ^{0}C) for 2 hours. After sterilization these bags were kept for cooling. After cooling at room temperature, each bag and bottle were shaken vigorously and transferred in laminar air flow chamber. For the elimination of surface contaminates, these bags and bottles were exposed to UV light for 20 minutes.

Mother spawn

Bottles were inoculated with fully grown pure culture tube or mother grain spawn. Inoculation was done under aseptic condition inside the laminar air flow. One pure culture tube or 8-10 g mother spawn grains were poured into the bottles. One fully grown mother grain spawn bottle was able to inoculate 14-15 bottles.

Planting spawn

The planting spawn was used for seeding. The sterilized bags were inoculated same as mother spawn. Fully grown mother grain spawn was used for inoculation of these bags. 15-20 g of mother spawn grains were poured into the sterilized bags and bags were shaken gently to spread the inoculums uniformly. Within the 10-12 days spawn run completed and bags were full of white colored mycelium.

Preparation of substrate

The experiment was carried out by using wheat straw as substrate. Wheat straw was chopped into 3-5 cm pieces and soaked in fresh water for 16-24 hours in a 200 L capacity drum. For the sterilization of straw water was chemically treated with bavistin (4g), formalin (150ml) and nuvan (15ml) per 100 liters of water. Excess water was drained off from straw by spreading on raised wire mesh frame. When excess water decanted off, the straw was spread on poly sheet by weighing 3 kg wet straw. Optimum level of water is indicated, when water does not drip down from palm when wetted straw is squeezed in the hand. Excess moisture causes rotting of straw and low moisture results in slow spawn run.

Spawning

To know the optimum spawn rate, two strains of oyster mushroom (PL-19-05 and PL-19-06) were spawned at four different spawn rates (3%, 4%, 5% and 6%) with four replications of each in completely randomized design. Spawning was done in neat and clean place. Polybags (14x20 inches) were used for filling 3 kg wet substrate. 10-12 small holes were made in the bags as facilitate respiration by the mycelium. This experiment was conducted by using layer spawning for which a 4 cm thick layer of substrate was made in bag and pressed slightly. Spawn was spread uniformly over it which was again covered with a 4 cm thick layer of straw. This process was repeated 4-5 times and the mouth of the poly bag was tied with rubber bands.

Crop management and harvesting of mushroom

Spawned bags were stacked in racks in closed position and temperature (24±2 °C) and humidity (80-85%) were maintained by spraying water twice in a day on walls and floor. After 15-20 days spawn run completed and bags fully covered with white mycelium. Polybags were removed carefully, and one day after the removal of polythene cover watering was started. The first primordia appeared 3-4 days after removal of polybags. These primordia matured generally in 1-2 days. Matured mushroom was identified by curl margin of cap and harvested by twisting it clock wise to uproot from the base. In a single life cycle of mushroom 3-4 flushes appeared.

Results

The increasing percent of spawn rate significantly influenced the time taken in spawn run, yield and biological efficiency of *Pleurotus* mushroom (Table 1 and Table 2).

Table 1: Effect of different spawn rates on growth parameters, yield and morphology of PL-19-05 strains of *Pleurotus* mushroom

Spawn rate (%)	Spawn run (Days)	Pin head initiation (Days)	Yield (g kg^{-1} of substrate dry weight)	Biological Efficiency (%)	Average fruit body weight (g)	Average number of fruit bodies	Pilus diameter (cm)	Length of stipe (cm)	Diameter of stipe (cm)
3.00%	10.75	25.50	737.00	73.70	6.38	118.25	7.05	5.75	1.58
4.00%	9.75	24.25	754.75	75.48	7.33	99.25	7.20	4.38	2.08
5.00%	9.25	23.50	773.75	77.38	7.20	113.00	6.75	6.08	1.38
6.00%	8.25	23.75	813.75	81.38	6.80	126.25	6.53	6.03	1.78
SEm±	0.48	0.64	14.34	1.43	0.23	5.90	0.09	0.10	0.08
CD (p=0.05)	1.48	NS	44.19	4.42	0.71	18.19	0.28	0.30	0.23

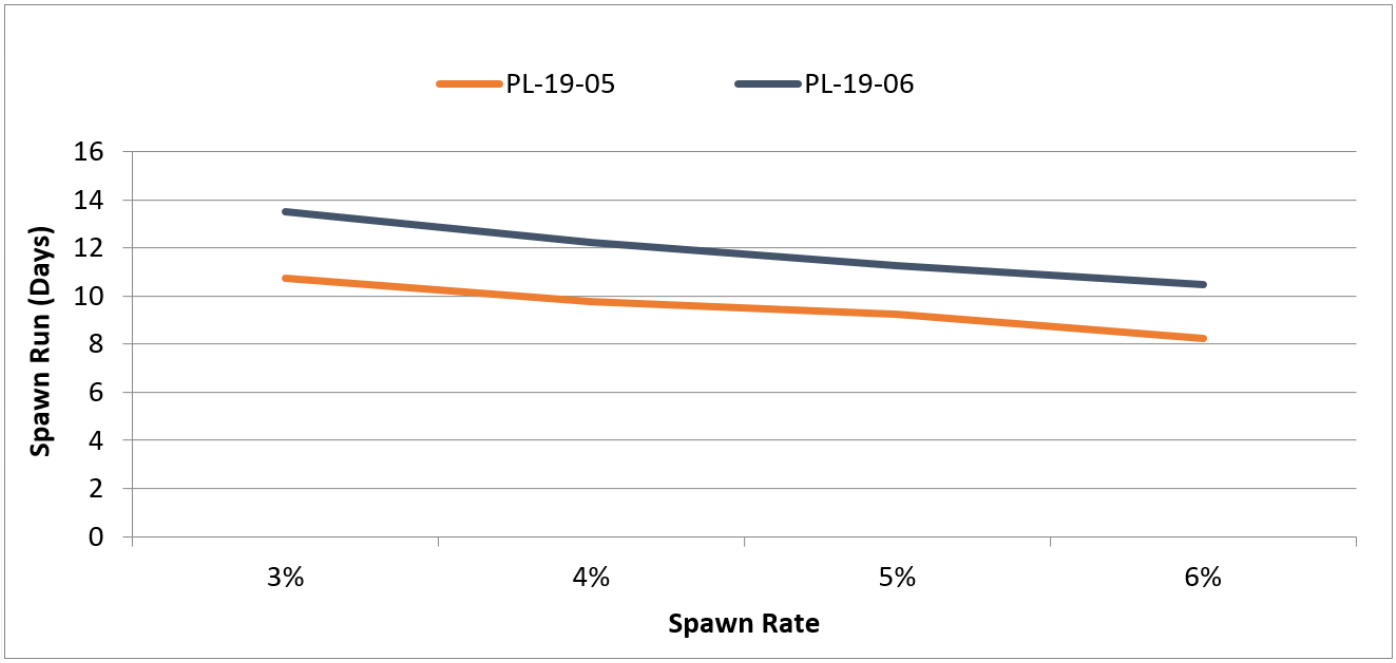

Fig 1: Average number of days taken in span run completion under varying spawn rate from the strains PL-19-05 and PL-19-06.

***Pleurotus* strain PL-19-05**

Data presented in Table 1 clearly indicates that an increase in spawn rate caused proportionate increase in yield as well as biological efficiency. Minimum days (8.25 days) for spawn run was found when the spawn rate was 6%, however, the maximum time for spawn run was found at 3% spawn rate. Pin head initiation was fastest at 5% spawn rate, however, slower (25.5 days) at 3% spawn rate. The maximum fruiting body yield (813.75 g kg^{-1} of substrate dry weight) with maximum biological efficiency of 81.38 per cent and maximum average number of fruit bodies (126.25) was recorded at 6% spawn rate, which was statistically at par with the spawn rate of 5.0 per cent. However, the minimum yield of fruit bodies yield (737 g kg^{-1} of substrate dry weight) and biological efficiency (73.70 per cent) were observed at 3% spawn rate.

Table 2: Effect of different spawn rates on growth parameters, yield and morphology of PL-19-06 strains of *Pleurotus* mushroom

Spawn rate (%)	Spawn run (Days)	Pin head initiation (Days)	Yield (g kg^{-1} of substrate dry weight)	Biological Efficiency (%)	Average fruit body weight (g)	Average number of fruit bodies	Pilus diameter (cm)	Length of stipe (cm)	Diameter of stipe (cm)
3.00%	13.50	18.75	893.75	89.38	6.50	138.25	6.00	5.25	1.05
4.00%	12.25	17.50	917.75	91.78	6.03	168.25	5.45	4.28	1.40
5.00%	11.25	17.00	939.25	93.93	5.68	152.50	6.45	5.05	1.10
6.00%	10.50	16.00	957.50	95.75	5.93	179.50	5.78	5.48	1.38
SEm±	0.53	1.00	8.48	0.85	0.15	4.45	0.13	0.12	0.07
CD (p=0.05)	1.63	NS	26.12	2.61	0.45	13.70	0.40	0.37	0.23

***Pleurotus* strain PL-19-06**

Data presented in Table 2 shows that, the minimum day for spawn run and pin head initiation was recorded 10.50 and 16.0 days respectively, at 6% spawn rate. Higher yield (957.50 g kg^{-1} of substrate dry weight), biological efficiency and maximum number of fresh fruit bodies (179.50) were also found at 6% spawn rate, which was statistically at par with the spawn rate of 5.0 per cent (939.93 g kg^{-1} of substrate dry weight) with biological efficiency of 93.93 per cent. However, the lowest fruit body yield (893.75 g kg^{-1} of substrate dry weight) was produced under 3.0 per cent spawn rate.

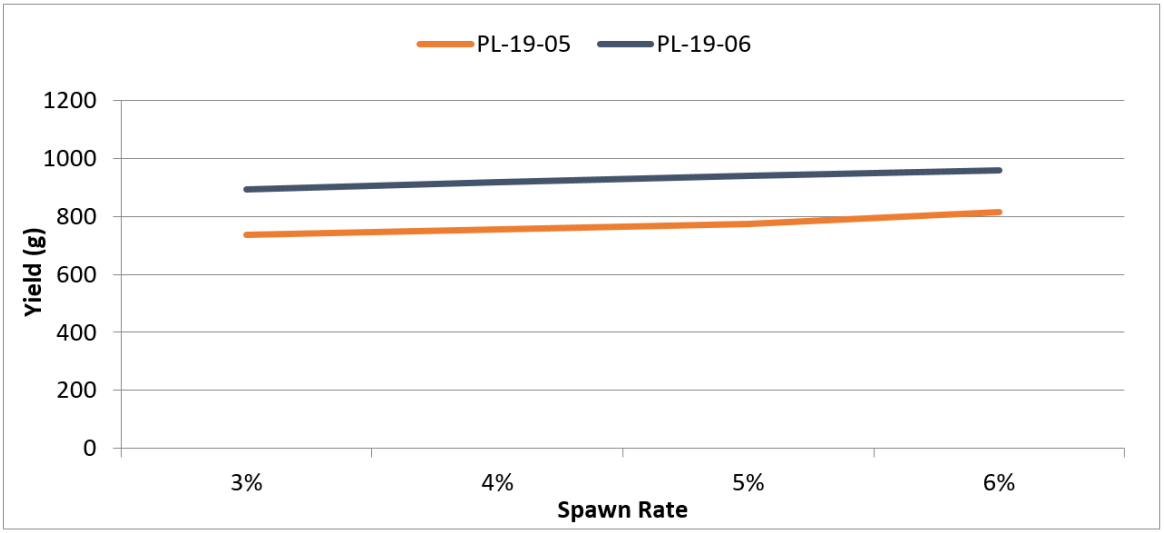

Fig 2: Yield obtained under varying spawn rate from the strain PL-19-05 and PL-19-06.

Discussion

Increase in per cent of spawn rate significantly influenced the time taken in spawn run, pin head initiation, yield, biological efficiency, number of fruit bodies, length of stipe and diameter of stipe and pilus of *Pleurotus* strain PL-19-05 and PL-19-06 (Table 1 & 2). The maximum fruit body yield (813.75 & 957.50 g) with an efficiency of 81.38 and 95.75 per cent was recorded with the spawn rate of 6.0 % by PL-19-05 and PL-19-06, respectively. Varying levels of spawn directly affects the biological efficiency of *Pleurotus* mushroom. The increase in yield is positively related with the increase in spawn rate, because there will be rapid spread of mycelia and not much of time would be required for substrate colonization which may retard other contaminants for being infected and establish in the substrate. Fan *et al.* (2000) [6] conducted the experiment with 2.5-25% spawn rate and 25% spawn rate found superior but as per economical point of view they recommended 10% spawn rate. Similarly, increasing spawn rate shorten mycelial colonization time, primordia formation, the time to the first mushroom crop (Yang *et al.*, 2013) [13] and reduced the gap of chances for entrant attack. Alananbeh *et al.* (2014) [2] opined that increasing rate of spawning significantly increased the yield, biological efficiency, and total fruiting bodies. Deora *et al.* (2021) [5] also found that increase in yield was directly proportional with increase in spawn rate up to certain level, after that with further increase in spawn rate bag temperature also rose. They concluded that 5% spawn dose was better because higher spawn dose caused higher substrate temperature which resulted into contamination of substrate. These results are closely in line with Kumar *et al.*, (2021) who also concluded that five percent spawn rate was optimum for higher yield and biological efficiency.

3% Spawn Rate

4% Spawn Rate

5% Spawn Rate

6% Spawn Rate

Plate 1: Effect of Different Spawn Rate on Fruiting

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RKVY mushroom and AICRP mushroom, Department of Plant Pathology, RCA, Udaipur.

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