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GROWTH, DEVELOPMENT AND YIELD OF OYSTER MUSHROOM, *PLEUROTUS OSTREATUS* (JACQ. EX. FR.) KUMMER AS AFFECTED BY DIFFERENT SPAWN RATES

M.I. BHATTI[1], M.M. JISKANI[1*], K. H. WAGAN[1], M.A. PATHAN[1] AND M.R. MAGSI[2]

[1]*Department of Plant Pathology, Sindh Agriculture University, Tandojam, Pakistan.*
[2]*Cotton Research Institute Sakrand, Sindh, Pakistan*

Abstract

The oyster mushroom, *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer was cultivated on wheat straw in polythene bags (containing 500 g wheat straw on dry weight basis per bag) using sorghum grain spawn at different rates. The spawning was done followed by boiling of substrate and sterilization of bags. The bags were kept in mushroom growing room at 25 to 35°C with 80 to 100% humidity under regular white fluorescent light arranged by the tube lights in mushroom growing room (10'x14'x14').

The pinheads first appeared 32.33 days after spawning by using 70 g spawn rate per kg on substrate dry weight basis. The minimum period of 4.66 days after pinhead formation for maturation of fruiting bodies was recorded by using 60, 70, 80, 90 and 100 g spawn rate. The minimum period between flushes (6.33 days) was taken by using 20 g spawn rate. The maximum flushes (4.00) were harvested by using 70 g spawn rate. The maximum number of bunches per bag (7.66) were obtained by using 100 g spawn rate. The maximum number of fruiting bodies per bunch (7.30) was observed by using 70 g spawn rate. The maximum yield on fresh weight basis (45.4%) as well as on dry weight basis (4.63%) was also obtained by using 70 g of spawn rate per bag. The results were highly significant from each other. It is concluded that spawning at 70 g per kg on substrate dry weight basis found to be the best dose for obtaining early and high yielding crop of oyster mushroom, with minimum period for maturation of fruiting bodies, maximum number of flushes and fruiting bodies per bag.

Introduction

The mushrooms are naturally grown in fields, forests, on manure heaps, water channels and hilly areas, mostly during and just after rains. The most popular varieties are *Agaricus bisporus* (European or white button mushroom), *Pleurotus* spp., (Oyster mushrooms or dhingri), *Volvariella volvacea* (Chinese or paddy straw mushroom) *Lentinus edodes* (Shiitake mushrooms) and *Auricus laria* (Black ear mushroom).

The oyster mushroom is grown under natural conditions on living trees as parasite or dead woody branches of trees as saprophyte and primary decomposer. The chemical composition of the fresh fruiting bodies of oyster mushroom, *Pleurotus ostreatus* indicates a large quantity of moisture (90.8%), whereas fresh as well as dry oyster mushrooms are rich in proteins (30.4%), fat (2.2%), carbohydrates (57.6%), fiber (8.7%) and ash (9.8%) with 345 K (cal) energy value on 100 g dry weight basis; while vitamins such as thiamin (4.8 mg), riboflavin (4.7 mg) and niacin (108.7 mg), minerals like calcium (98 mg), phosphorus (476 mg), ferrous (8.5 mg) and sodium (61 mg) on 100 g dry weight basis, are also found present (Pandey & Ghosh, 1996). Rambelli & Menini (1985) reported that this mushroom is reputed to be antitumoural because of its chemical composition.

*Corresponding author E-mail: mithaljiskani@yahoo.com

The oyster mushrooms can be cultivated successfully under semi controlled conditions in a small space by using agricultural as well as industrial waste and other refuse as substrate. Badshah *et al*., (1992) have grown *Pleurotus ostreatus* on wheat straw, sugarcane bagasse, corn cobs or sawdust by mixing 120-130 g of spawn with 2 kg of substrate and placing the mixture in sterilized polyethylene bags which were kept in the dark at 25^{O}C for 2-3 weeks. Fruiting bodies were harvested at maturity with yields of 49.8 g/2 kg substrate (sawdust), 432.8 g/2 kg substrate (wheat straw), whereas control (grown in the field) yielded only 18.5 g/2 kg substrate. Bernabe-Gonzalez & Arzeta-Gomez (1994) mixed *P. ostreatus* inoculum at 4 g/100 g substrate in 4-kg plastic bags using peanut hulls and maize leaves cut to 5- or 10-cm lengths. Kausar & Iqbal (1994) used 5% spawn of *Pleurotus* (w/w basis) in 15kg paddy straw, pinheads formed 28 days after spawning. The yield varied from 18.6 to 83.5% based on different nitrogen supplements amended with straw. Cangy & Peerally (1995) used spawning rates 0.75, 1.50, 3.00 and 6.00% of substrate fresh weight for 10 species of *Pleurotus*. Results showed that 1% spawning rate was found to be adequate when using the smaller bags (yields >16% of spawned substrate weight) at mean temperature 18^{O}C (range 13-23^{O}). Marimuthu (1995) reviewed the use of crop residues as growing media for oyster mushroom (*Pleurotus*) production. Paddy and wheat straw, cotton waste, maize cobs, waste paper and cotton stalks are all suitable for high production capacity, whole grains of sorghum, bajra (*Pennisetum glaucum*) or maize are recommended. Patra & Pani (1995) cultivated five different species of *Pleurotus* in polythene [polyethylene] bags containing chopped paddy straw (2 kg) + spawn (200 g) + boiled wheat (200 g). The fungi took 13-16 days for complete mycelial run in the bags and 20-24 days for initiation of fruiting bodies, producing the heaviest (12.2 g), and the lightest (6.9 g) fruiting bodies. Singh *et al.,* (1995) recorded the maximum yield from baggase than from the paddy straw and wheat straw respectively. Mathew *et al*., (1996) have grown *P. ostreatus* on various substrates for both (spawn production and cultivation) and found that sorghum, wheat and paddy grains were equally good for spawn production. Fan *et al.,* (2000) carried out the studies with 2.5-25% spawn rates, 25% spawn rate appeared superior, but recommended 10% spawn rate in view of the process economics. The first fructification occurred after 20-23 days of inoculation and the biological efficiency reached about 90-97% after 50-60 days. Labuschagne *et al.,* (2000) reported wheat straw as main raw material for cultivation. Bughio (2001) cultivated the oyster mushroom, *Pleurotus ostreatus* on combination of wheat straw, cotton boll locules, paddy straw, sugarcane and sorghum leaves at 1:1 ratio in polythene bags (650 g/bag) using sorghum grain spawn @ 30 grams per bag, followed by boiling of substrates and sterilization of bags. The present studies were undertaken on the effect of sorghum grain spawn rate on growth, development and yield of oyster mushroom, *Pleurotus ostreatus* by using wheat straw as substrate, with a target to find out the best grain spawn rate for getting early and high yield crop with short duration.

Materials and Methods

The experiment was carried out at the Department of Plant Pathology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam from July 2001 to January 2002. The primary inoculum was prepared from the fresh fruiting body of the mushroom through tissue culture method and was maintained and multiplied by sub-culturing on sterilized PDA medium in Petri dishes and test tubes, incubated at room temperature (30±2°C).

The spawn was prepared on white sorghum grains. The grains were half boiled and filled in transparent glass bottles at 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 g/ bottle. The bottles (containing half boiled grains) were sterilized in an autoclave at 15 psi for 30 minutes. The inoculation was made on the following day under aseptic conditions provided in isolation chamber and then were incubated at room temperature (32±2°C), till the grains were covered with white mycelial growth.

The wheat straw was used as such of a wheat threshing machine. Initially, the substrate (wheat straw) was boiled for 15-20 minutes to get rid of insects and other micro- organisms. The moist straw was taken out from the water and spread in thin layers over cemented floor till the remaining excess of water was removed. When the temperature of substrate dropped down to about 25 to 30^{o}C and moisture content become about 70 to 90%, the substrate was filled in the polythene bags of 30x45 cm size @ 500 g of wheat straw in each bag on dry weight basis. The filled bags were sterilized in the autoclave at 15 psi for an hour. After cooling of sterilized bags, the spawning was done with pure sorghum grain spawn @ 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 grams per kg on substrate dry weight basis.

The spawned bags were placed on iron racks in order to provide them maximum space, in a mushroom growing room. When the bags become full of mycelial growth and or pinheads started appearing on the mycelial surface, the bags mouth were opened or required portion of the bags were cut-off with blade to facilitate the development of fruiting bodies. As soon as the fruiting bodies developed with full size, these were harvested just above surface of the substrate with sharp knife or blade.

Temperature, humidity and light are basic factors for the proper growth and development of mushroom. Efforts were made to maintain these requirements by furnishing mushroom growing room (14'x10'x14') with one desert room cooler (Woods, China) and two fluorescent lamps (Philips tube lights, 40 W/54) for the promising cultivation of mushroom. The temperature and humidity remained within the required range i.e., 25-35°C and 80-100% respectively, during the course of experiment.

The experiment was laid out as complete randomized block design with arrangement of three replications and ten treatments. The growth and development of mushroom was observed daily. The time taken for pinhead formation from the date of spawning and time taken from the date of pinhead formation to maturation of fruiting bodies (ready for harvesting) was recorded by counting the taken days, but the time taken between flushes was calculated by using the following formula:

$$\text{Period between flushes} = \frac{\text{Days taken from first harvest to last}}{\text{Total number of flushes (harvesting)}}$$

The total numbers of flushes from harvesting of first flush up to last flush were also recorded. The total number of bunches harvested from each bag (or replication) were counted. The data on number of fruiting bodies per bunch was calculated by applying the following formula:

$$\text{No. of fruiting bodies per bunch} = \frac{\text{Total no. of fruiting bodies}}{\text{Total no. of bunches}}$$

Table 1. Effect of grain spawn rate on time taken (mean days) for pinhead formation, maturation of fruiting bodies and period between flushes of oyster mushroom.

Spawn rate (g per kg substrate DW basis)	Pinhead formation	Maturation of fruiting bodies	Period between flushes
10	76.00 i	5.66	0.00 a
20	71.33 h	5.33	6.33 ab
30	67.33 g	5.33	12.42 cde
40	64.00 ef	5.00	8.16 cd
50	61.33 e	5.00	16.72 e
60	35.00 ab	4.66	16.72 e
70	32.33 a	4.66	15.11 de
80	38.00 c	4.66	14.05 cde
90	38.67 c	4.66	15.67 de
100	46.67 d	4.66	16.72 e
LSD 0.05	**3.919**	**Non-significant**	**8.004**

Similar letters do not differ from one another.

The weight of fresh mushrooms was recorded after harvesting of each flush. The dry weight of mushroom (g) was recorded by keeping the fresh mushroom in hot air oven at 70^{o}C for 48 hours. The total yield was recorded by adding the fresh as well as dry weight of mushrooms of all flushes, while the fresh and dry yield percentage (g) was calculated on substrate dry weight basis using the following formula:

$$\% \text{ Age yield (g)} = \frac{\text{Total yield of all flushes from each replication (g)}}{\text{Substrates dry weight of each replication (500 g constant)}} \times 100$$

Results and Discussion

Pinhead formation: The mean number of days taken for pinhead formation of oyster mushroom from the date of spawning exhibited significant difference between different spawn rates (Table 1). The pinheads first appeared (32.33 days) by using spawn rate at 70 g per kg substrate dry weight basis, which proved to be the best spawn rate followed by 60 g (35.00 days), 80 g (38.00 days), 90 g (38.67 days), 100 g (46.67 days), 50 g (61.33 days), 40 g (64.00 days), 30 g (67.33 days), 20 g (71.33 days and 10 g (76.00 days) respectively. Kausar & Iqbal (1994) and Kausar & Zafar (1995) reported 28 days for pinhead formation after spawning. Patra & Pani (1995) revealed that mushroom took 20-24 days but Jiskani (1999) stated 25-50 days for pinhead formation, whereas Jiskani *et al.*, (1999) concluded that pinhead formation took 51.6 days after spawning in case of using wheat straw. Fan *et al.*, (2000) observed that first fructification occurred after 20-23 days of inoculation, Bughio (2001) reported 43.25 to 53.00 days after spawning by using sorghum grains @ 30 g per 650 g in case of using wheat straw, sugarcane and sorghum leaves at 1:1 ratio on substrate dry weight basis.

Maturation of fruiting bodies: The mean number of days taken from pinhead formation to maturation of fruiting bodies exhibited significant difference between different spawn rates (Table 1). The minimum period (4.66 days) for maturation of fruiting bodies was taken by using 60, 70, 80, 90 and 100 g spawn per kg substrate dry weight basis followed by 40 and 50 g (5.00 days), 30 and 20 g (5.33 days) and 10 g (5.66 days) respectively (Table 1). Jiskani (1999) reported 30-55 days, Jiskani *et al.,* (1999) recorded 60 days after spawning for maturation of fruiting bodies, Fan *et al.,* (2000) observed that the first fructification occurred 20-23 days after inoculation, whereas, Bughio (2001) reported that maturation of fruiting bodies took 5 to 6 days after pinhead formation.

Period between flushes: The mean numbers of days between flushes are given in Table 1. The results revealed significant difference at LSD 0.05 on average basis. The minimum period (6.33 days) between flushes was taken by using 20 g per kg substrate dry weight basis, followed by 40 g (8.16 days), 30 g (12.42 days), 80 g (14.05 days), 70 g (15.11 days), 90 g (15.67 days), 70, 50 and 100 g (16.72 days). However, only one flush was harvested by using spawn at 10 g per kg on substrate dry weight basis, hence, no days were recorded between flushes. Lozano (1990) reported that seven harvesting were carried during 60 days, whereas Jiskani *et al.,* (1999) reported 7.5 days, but Bughio (2001) recorded 8.53 to 14.33 days between flushes.

Number of flushes: The observations for total number of flushes from each spawn rate are given in Table 2 as mean numbers. The results indicating highly significant difference between different spawn rates and has been proved that the oyster mushroom gave maximum (4.00 flushes) by using 70 g per kg substrate dry weight basis. It was followed by 60 g (3.66 flushes), 80 g (3.00 flushes), 90 and 100 g (2.66 flushes), 40 and 50 g (2.33 flushes), 30 g (2.00 flushes) 20 g (1.33 flushes) and 10 g (1.00 flushes) respectively. Lozano (1990) reported 7 flushes, Moorthy & Mohanan (1991), Kausar & Zafar (1995), Jiskani *et al.,* (1999) harvested 3 flushes, but Bughio (2001) reported 3.00 to 6.25 flushes.

Number of bunches: The data recorded for total number of bunches (per bag) of oyster mushroom (Table 2) indicates highly significant difference between different spawn rates. The maximum number of bunches per bag (7.66 bunches) were obtained by using 100 g spawn per kg on substrate dry weight basis. It was followed by 80 and 90 g (6.37 bunches), 70 g (5.33 bunches), 60 g (5.00 bunches), 50 g (3.33 bunches), 40 g (3.00 bunches). The spawn @ 30, 20 and 10 g gave 2.00 bunches (Table 2). No reference from different research journals, text books, reports and internet etc. could be obtained on this aspect.

Number of fruiting bodies: The mean number of fruiting bodies per bunch exhibited significant difference between different spawn rates (Table 2). The result showed that the maximum number of fruiting bodies (7.30/bunch) was recorded by using 100 g/kg substrate dry weight basis followed by 90 and 80 g (5.33 fruiting bodies), 70 g (4.98 fruiting bodies), 60 g (4.83 fruiting bodies), 50 g (4.61 fruiting bodies), 40 g (4.22 fruiting bodies), 30 g (4.13 fruiting bodies), 20 g (3.07 fruiting bodies), and 10 g (2.73 fruiting bodies) respectively (Table 2). Similar to that of number of bunches per bag no any single reference is available on number of fruiting bodies per bunch.

Table 2. Effect of grain spawn rate on number of flushes, number of bunches and number of fruiting bodies of oyster mushroom (*P. ostreatus*).

Spawn rate (g per kg substrate DW basis)	Number of flushes/bag	Number of bunches/bag	Number of fruiting bodies/bunch
10	1.00 e	2.00 b	2.73 b
20	1.33 de	2.00 b	3.07 b
30	2.00 cde	2.00 b	4.13 ab
40	2.33 cd	3.00 ab	4.22 ab
50	2.33 cd	3.33 ab	4.61 ab
60	3.66 ab	5.00 ab	5.33 ab
70	4.00 a	5.33 ab	7.30 a
80	3.00 abc	6.33 ab	5.33 ab
90	2.66 bc	6.33 ab	4.98 ab
100	2.66 bc	7.66 a	4.83 ab
LSD 0.05	**1.155**	**4.934**	**3.775**

Similar letters do not differ from one another.

Table 3. Effect of grain spawn rate on percentage yield on fresh and dry weight of oyster mushroom (*P. ostreatus*).

Spawn rate (g per kg substrate DW basis)	Fresh yield (%)	Dry yield (%)
10	10.53 f	1.15 e
20	15.13 e	1.55 e
30	15.66 e	1.62 e
40	27.20 d	2.65 d
50	32.00 c	3.30 c
60	44.26 a	4.10 ab
70	45.40 a	4.63 a
80	39.93 b	3.96 b
90	38.26 b	3.72 bc
100	33.40 c	3.70 bc
LSD 0.05	**1.903**	**0.5307**

Similar letters do not differ from one another.

Yield percentage: The results obtained for percentage yield of oyster mushroom on fresh (wet) and dry weight basis are highly significant at LSD 0.05 (Table 3). The results reveals that the maximum percentage yield (45.40% on fresh and 4.63% on dry weight basis) was obtained by using spawn at 70 g/kg on substrate dry weight basis, which is near to 60 g spawn per kg substrate (44.27% fresh and 4.10% dry). These spawn rates were found to be the best followed by 80, 90, 100, 50, 40, 30, 20 and 10 g per kg (39.93 and 3.96%, 38.27 and 3.72%, 33.40 and 3.70%, 32.00 and 3.30%, 27.20 and 2.65%, 15.67 and 1.62%, 15.13 and 1.55% and 10.53 and 1.15%) fresh and dry yield respectively (Table 3). Lozano (1990) reported 43% yield, Moorthy & Mohanan (1991) recorded 332 to 474 g/bag yield from polyethylene bags containing 1.2 kg dry

substrate/bag when inoculated with 150 g spawn/bag using a multi-layered spawning technique. Badshah *et al.,* (1992) reported 49.8 g on saw dust, 432.8 g on wheat straw and 18.5 g per 2kg substrate grown in the field (control). Kausar & Iqbal (1994) reported that yield varied from 18.6 to 83.5% on the basis of different nitrogen supplements amended with straw. Kausar & Zafar (1995) reported that average yield varied from 57.17- 73.39%. Jiskani *et al.,* (1999) obtained 24 and 7.6% fresh and dry yield on the basis of substrate dry weight, in case of using wheat straw. Jiskani (1999) reported that 100% of substrate dry weight, means one kg of fresh mushroom can be obtained from one kg of dry substrate (before soaking and boiling). According to Bughio (2001) the maximum fresh (wet) and dry yield percentage on substrate dry weight basis (29.61 to 77.91 and 5.91 to 21.70) were obtained from wheat straw using in combination with cotton boll locules, paddy straw, sugarcane and sorghum leaves at 1:1 ratio in case of using sorghum grain spawn @ 30 g per bag.

The difference between achievement with the results reported by other research workers may be due to the variation in controlled, semi controlled conditions, physiological requirements for cultivation of oyster mushroom e.g., constant temperature, humidity and light arrangements. The response of different substrates also show differences in respect of time taken for formation of pinheads, maturation of fruiting bodies, period between flushes, number of flushes and yield. However, our results are very much closer to other research workers. It is concluded that spawning at 70 g per kg on substrate dry weight basis found to be the best dose for obtaining early and high yielding crop of oyster mushroom, with minimum period for maturation of fruiting bodies, maximum number of flushes and fruiting bodies per bag.

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