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Research Article

Influence of Substrate pH and Watering Frequency on the Growth of Oyster Mushroom

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Abstract

Experiment was carried out in mushroom research shade house of Olericulture Division, HRC (Horticulture Research Centre), Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, during the period from July, 2015 to December, 2015 to evaluate the effect of pH levels of substrates and frequency of watering on the growth traits of oyster mushroom. Spawn of *Pleurotus ostreatus (Jacquin ex Fr.)* was used as test crop. The experiment consisted of two factors *i.e.*, pH level of substrates [P_0: Control (5.5), P_1: 5.0, P_2: 5.3, P_3: 5.8, P_4: 6.1 and P_5: 6.4] and frequency of watering [W_0: Control (No immersion in water and no further watering), W_1: Im + 12h, W_2: Im + 18h, W_3: Im + 24h, W_4: Im + 30h and W_5: Im + 36h]. The experiment was laid out in Completely Randomized Design (CRD) with three replications. Results revealed that most of the parameters showed the significant response due to different pH levels of substrate and watering frequency. For pH level of substrate, the treatment P_0 (5.5) exhibited the better performance on the growth characters of mushroom. In case of watering frequency, the treatment W_1 (Im + 12h) showed higher number of fruiting bodies and higher number of harvest of mushroom. Statistically, the highest number of fruiting bodies was obtained from the combined treatment $P_0 \times W_1$ *i.e.*, pH of substrate 5.5 in combination with watering frequency Im + 12 h. So, this combination may be used for higher yield of mushroom.

INTRODUCTION

Pleurotus spp.; popularly known as Oyster mushrooms under the class Basidiomycetes is cultivated and consumed by 97%, of which *Pleurotus ostreatus* alone accounts for 61%. The remaining 3% which belongs to *Agaricus* sp.; *Calocybe* sp.; *Volvareilla* sp. and *Auricularia* sp. are generally called Button, Milky, Paddy straw and Jew's ear or Ear mushrooms, respectively. It was also evident that available carbon source of the substrates for the cultivation of *Pleurotus* spp. was 76% of sawdust where frequencies of flashes were recorded more than five times in 84% cases [1]. Cultivation of oyster mushroom has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and harvested all over the year [2]. The commercial yield was obtained namely from Oyster mushrooms. On an average, 240 g from each of the spawn-packets which contained 400-500 g of substrate and subsequently on an average 264963 spawn-packets were produced per month, which accounts for 620-675 tons of edible mushrooms production in Bangladesh per annum [1]. *Pleurotus ostreatus* is one of the most popular oyster mushroom species that can grow on different agricultural wastes. *Pleurotus* have the ability to excrete hydrolyzing and oxidizing enzymes [3], which have capable of utilizing complex organic compounds that occurred agricultural wastes and industrial by-products [4], with broad adaptability varied agro-climatic conditions [5].

It requires a short growth time in comparison to other edible mushrooms [6]. The availability of growth promoting substances in the substrates of oyster mushroom depends on the pH concentration. The pH concentration influences the proper growth and development of mushroom under different substrates. The mushroom choice slightly acidic to slightly basic pH of substrates [7]. The pH has great response on nutrition and morphological development of mushrooms [8]. Hong et al. [9], reported that, the optimum range of pH for mycelium growth is about 5.5 and 6.5. Chung [10], reported that, the optimum range of pH was different in different strains of *Pleurotus* spp. Proper moisture condition of substrates verifies the performance of oyster mushroom and watering on mushroom spawn can create different moist condition per day at different frequencies [11]. Mushroom cultivation has a special relevance to Bangladesh, because sawdust and other materials are available to the farmers. So, mushroom production could keep great importance on our economy as a whole. However, the research on the effects of pH of substrates and frequency of watering on the production of oyster mushroom had not been well established.Therefore, the present experiment was undertaken to determine the most suitable pH concentration to assess the most promising combination of pH of substrates and frequency of watering for better growth of mushroom.

MATERIALS AND METHODS

Site of experimentation

The present study was carried out in the mushroom research shade house of Olericulture Division, HRC (Horticulture Research Centre), Bangladesh Agricultural Research Institute, Joydebpur, Gazipur during the period from July, 2015 to December, 2015. The geo position of Gazipur district is 23°53′ to 24°20′ N latitudes and between 90°9′ to 90°42′ E longitude and it also situated under Madhupur tract (AEZ-28).

Planting materials

Spawn of *Pleurotusostreatus (Jacquin ex Fr.)* in a bottle was collected from Savar farm and used to inoculate the substrate with 5% of spawn for each bag. Oyster mushrooms are characterized by the rapidity of the mycelial growth and high saprophytic colonization activity on cellulosic substrates. Their fruiting bodies are shell or spatula shaped white color. If the temperature increases above 32°C, its production markedly decreases.

Experimental treatment and design

The experiment consisted of two factors *i.e.*, pH level of substrates [P_0: Control (5.5), P_1: 5.0, P_2: 5.3, P_3: 5.8, P_4: 6.1 and P_5: 6.4], and frequency of watering [W_0: Control (No immersion in water and no further watering), W_1: Im + 12h, W_2: Im + 18h, W_3: Im + 24h, W_4: Im + 30h and W_5: Im + 36h]. The experiment was laid out in Completely Randomized Design (CRD) with three replications. A total of 108 plastic bags were used under study as sample for data collection.

Preparation and adjustment pH of substrates

Spawn packets was prepared with waste paper amended with wheat bran at 2:1 ratio and 0.57% calcium carbonate in polypropylene bags. The mushroom house was provided with well ventilation for easy flow of natural air. Six different levels of pH *viz.*, Control (5.5), 5.0, 5.30, 5.80, 6.10 and 6.40 were tested to determine the best levels of pH for Oyster Mushroom cultivation. For the control treatment, just pH value was measured and found it 5.5. Rest levels of pH in substrates were adjusted by using 2% (w/w) $CaCO_3$ and 1N HCl. The pH of substrate was determined using water extract of the materials with a pH meter (HORIBA M. 8_L). The substrates did not need to be chopped.They were soaked overnight in water and boiled for 5-10 minutes. The excess water was removed by drying the substrate, until the moisture content was around 60% for homogenous condition for pH adjustment.

Preparation of spawn packets

The mixed substrates were filled into 7×11 inch polypropylene bag at 500g. The filled polypropylene bags were prepared by using plastic neck and plugged the neck with cotton and covered with brown paper placing rubber band to hold it tightly in place.

Managing the frequency of watering

Method of water application was as follows: firstly, immersion in water for 15 minutes and secondly, water was sprayed on the spawn packets placed on the shelves of mushroom house. Spawn packets was immersed in a bucket of water after scraping and opening at every harvest. Water was sprayed to the spawn packets once at 12, 18, 24, 30 and 36 hours intervals and water was not applied on control treatments.

Cultivation of spawn packets

Therefore the packets were sterilized about 1 hour and then these were kept for cooling. After cooling, 5 g mother spawn was inoculated into the packets in the laminar airflow cabinet and the packets were kept at 20-22°C temperature until the packets become white with the mushroom mycelium. After completion of the mycelium running the rubber band, brown paper, cotton plug and plastic neck of the mouth of spawn packet were removed and the mouth was wrapped tightly with rubber band. Then these spawn packets were transferred to the culture house. Two ends, opposite to each other of the upper position of plastic bag were cut in "D" shape with a blade and opened by removing the plastic sheet after which the opened surface of substrate was scraped slightly with a tea spoon for removing the thin whitish mycelial layer. Then the spawn packets were soaked in water for 15 minutes and invested to remove excess water for another 15 minutes. The packets of each type were placed separately on the floor of culture room and covered with newspaper. The moisture of the culture room was maintained 80-85% relative humidity by spraying water 3 times a day. The light around 300-500 lux and ventilation of culture house was maintained uniformly. The temperature of culture house was maintained 22°C to 25°C. The first primordia appeared 2-4 days after scribing depending upon the type of substrate. The harvesting time also varied depending upon the type of substrate.

Data recorded

Oyster mushrooms matured within 2-3 days after primordia initiation. The matured fruiting body was identified by curial margin of the cap. Mushrooms were harvested by twisting to uproot from the base. The data on days required for development of pin head, days required from pin head to first harvest, number of fruiting bodies produced/packet and number of harvest/packet.

Statistics used

The data obtained for different characters were statistically analyzed following the analysis of variance techniques by using MSTAT-C computer package and the treatment means were compared by Least Significant Difference (LSD) at 5% level of probability [12].

RESULTS AND DISCUSSION

Days required for development of pin head

In respect of days required for the development of pin head due to different levels of pH was found statistically significant. A gradual decreasing trend was found with the increasing of pH level. The longest (30.52 days) period was required by the mushroom produced from P_0. The shortest (29.83 days) period was required from P_2 which was statistically similar to P_1, P_3, P_4 and P_5 (Table 1). The increasing of pH from moderate acidic to slightly basic has lengthening the period of pinning on mushroom [13]. In respect of days required for the development of pin head due to different frequency of watering was found statistically significant. A gradual increasing trend was found with the

Table 1: Response of pH levels of substrates on days required for development of pin head and pin head to first harvest, number of fruiting bodies and number of harvest.

pH level	Days required for development of pin head	Days required from pin head to first harvest	No. of fruiting bodies produced/packet	No. of harvest/ packet
P_0	30.52 a	4.11 b	42.66 a	4.35 a
P_1	29.89 b	4.22 ab	37.55 b	4.33 ab
P_2	29.83 b	4.05 b	33.33 d	4.02 c
P_3	29.88 b	3.97 b	35.55 c	4.36 a
P_4	29.88 b	4.20 ab	43.27 a	4.23 ab
P_5	29.94 b	4.47 a	36.72 b	4.15 bc
LSD (0.05)	0.29	0.32	1.12	0.18
CV (%)	1.48	11.65	4.42	6.64

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$
P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.

increasing of frequency of watering. The longest (31.50 days) period required for development of pin head was found from $W_5$and the shortest (27.91 days) was from W_2 (Table 2). Gislerod [14], pointed out that, the lower non intermittent application of water decreased the pin formation times from spawn packet. This result is in agreement with findings of present study. Significant variation was found due to different combinations of pH of substrates and frequency of watering on days required for development of pin head of mushroom from seed in packets. Results showed that, the longest (32.66 days) period required for development of pin head of mushroom from seed in packets was found from $P_0 \times W_5$ which was statistically similar to $P_0 \times W_4$ (32.00 days) and $P_3 \times W_5$ (32.00 days**)** while, the shortest (27.36 days)was found from $P_1 \times W_2$ (Table 3).

Days required from pin head to first harvest

A day required from pin head to first harvest due to different levels of pH was found statistically significant. Results showed that, the maximum (4.47 days)required from pin head to first harvest was found from P_5 which was statistically similar to P_4 (4.20 days) and P_1 (4.22 days) while, the minimum (4.11 days) was found from P_0 (Table 1). Gislerod [14], said that, the pH near about basic condition had lengthening the period of harvest of spawn. This result is in agreement with findings of present study. Days required from pin head to first harvest due to different frequency of watering were found statistically significant. A gradual increasing trend was found with the increasing of frequency of watering.

The maximum (4.58 days) required from pin head to first harvest was found from W_5 which was statistically similar to W_4 (4.52 days) and W_0 (4.41 days). The minimum (3.61 days) was found from W_0 (Table 2). Gislerod [14], also said that, the long interval application of water on spawn packets has increased the duration of first harvest of spawn from packets. No significant variation was found due to different combinations of pH of substrates and frequency of watering on days required from pin head to first harvest of oyster mushroom. But numerically, the maximum (5.20 days) required from pin head to first harvest was found from $P_4 \times W_5$ and the minimum (3.16 days and 3.16 days) was found from $P_0 \times W_1$ and $P_4 \times W_1$, respectively, (Table 3). Result was also supported by [15].

Number of fruiting bodies produced/packet

In case of number of fruiting bodies produced due to different levels of pH was found statistically significant. A gradual decreasing trend was found with the increasing of pH level from P_0 up to P_3 and thereafter increased as similar to P_0. Results showed that, the maximum (43.27) number of fruiting bodies produced/ packet from P_4 which was statistically similar to P_0 (42.66) while, the minimum (33.33) was from P_2 (Table 1). In case of number of fruiting bodies produced due to different frequency of watering was found statistically significant. A gradual decreasing trend was found with the increasing of frequency of watering. The maximum (43.90) number of fruiting bodies produced/packet was found from W_1 and the minimum (34.50) was from W_5 (Table 2). Significant variation was found due to different combinations of pH of substrates and frequency of watering on number of fruiting bodies produced/packet of oyster mushroom. Results showed that, the maximum (52.33) number of fruiting bodies produced/packet was found from $P_0 \times W_2$ which was statistically similar to $P_0 \times W_1$ (51.66) and the minimum (30.66) was found from $P_3 \times W_5$ (Table 3).

Number of harvest/packet

In respect of number of harvest due to different levels of pH was found statistically significant. Results showed that, the maximum (4.36) number of harvest/packet from P_3 which was statistically similar to P_0 (4.35), P_1 (4.33) and P_4 (4.23) while, the minimum (4.02) was from P_2 (Table 1). Litar et al. [16], said that, the acidic condition (above 5.4) has increased the duration and frequency of harvest of Nigerian edible fungi. This result is in agreement with findings of present study.In respect of number of harvest due to different frequency of watering was found statistically significant. A gradual increasing trend was found with the increasing the frequency of watering from W_0 up to W_2 and thereafter decreased towards W_5. Results showed that, the maximum (4.63) number of harvest/packet from W_1 which was statistically similar to W_2 (4.49) while, the minimum (3.80) was found from W_5 (Table 2). Ismail et al. [17], also observed that, increases in spawn harvest number with higher daily irrigation frequency compared to once-a-day irrigation when plants were grown in soil conditions. This result is in agreement with findings of present study.Significant variation was found among different

Table 2: Response of frequency of watering on days required for development of pin head and pin head to first harvest, number of fruiting bodies and number of harvest.

Frequency of watering	Days required for development of pin head	Days required from pin head to first harvest	No. of fruiting bodies produced/packet	No. of harvest/packet
W_0	31.00 b	4.41 ab	35.88 d	4.31 b
W_1	28.69 d	3.61 c	43.90 a	4.63 a
W_2	27.91 e	3.67 c	40.44 b	4.49 ab
W_3	30.03 c	4.22 b	38.05 c	4.11 c
W_4	30.83 b	4.52 ab	36.27 d	4.10 c
W_5	31.50 a	4.58 a	34.50e	3.80 d
LSD (0.05)	0.29	0.32	1.12	0.18
CV (%)	1.48	11.65	4.42	6.64

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$
W_0: Control, W_1: Im + 12h, W_2: Im + 18h, W_3: Im + 24h, W_4: Im + 30h and W_5: Im + 36h.

Table 3: Combined effect of pH levels of substrates and frequency of watering on days required for development of pin head and pin head to first harvest, number of fruiting bodies and number of harvest.

Treatment combination	Days required for development of pin head	Days required from pin head to first harvest	No. of fruiting bodies produced/packet	No. of harvest/packet
$P_0 \times W_0$	31.83 b	4.50	35.33 j-m	3.66jk
$P_0 \times W_1$	28.46 h-j	3.16	51.66 a	4.66 b-e
$P_0 \times W_2$	27.83 jk	3.66	52.33 a	4.83 a-d
$P_0 \times W_3$	30.33 ef	4.00	40.00 f-h	4.33 e-h
$P_0 \times W_4$	32.00ab	4.66	39.66 g-i	4.66 b-e
$P_0 \times W_5$	32.66 a	4.66	37.00 i-k	3.93 h-k
$P_1 \times W_0$	30.83 d-f	4.26	37.33 h-j	4.33 e-h
$P_1 \times W_1$	28.66 g-i	3.93	41.33 e-g	4.83 a-d
$P_1 \times W_2$	27.36 k	3.66	37.33 h-j	4.33 e-h
$P_1 \times W_3$	30.66 d-f	4.50	39.33 g-i	4.33 e-h
$P_1 \times W_4$	30.16 f	4.66	36.33 j-l	4.00 g-j
$P_1 \times W_5$	31.66 bc	4.33	33.66 l-n	4.16 f-i
$P_2 \times W_0$	30.33 ef	4.33	31.33 n-p	4.50 c-f
$P_2 \times W_1$	29.36 g	3.50	35.33 j-m	4.16 f-i
$P_2 \times W_2$	28.33 ij	3.50	34.33 k-m	4.00 g-j
$P_2 \times W_3$	30.16 f	4.16	33.66 l-n	3.50 k
$P_2 \times W_4$	30.50 ef	4.16	35.66 j-m	3.83 i-k
$P_2 \times W_5$	30.33 ef	4.66	29.66 p	4.16 f-i
$P_3 \times W_0$	30.83 d-f	4.33	34.33 k-m	4.66 b-e
$P_3 \times W_1$	29.16 gh	3.66	44.667cd	5.13 a
$P_3 \times W_2$	28.33 ij	3.33	38.00 h-j	4.90 a-c
$P_3 \times W_3$	28.00 i-k	4.00	34.33 k-m	4.33 e-h
$P_3 \times W_4$	31.00 c-e	4.50	31.33 n-p	4.16 f-i
$P_3 \times W_5$	32.00 ab	4.00	30.66 op	3.00 l
$P_4 \times W_0$	31.66 bc	4.00	37.00 i-k	4.33 e-h
$P_4 \times W_1$	28.33 ij	3.16	48.66 b	5.00 ab
$P_4 \times W_2$	27.33 k	3.66	42.66 d-f	4.50c-f
$P_4 \times W_3$	30.33 ef	4.33	46.66 bc	4.00 g-j
$P_4 \times W_4$	30.66 d-f	4.83	41.66 e-g	3.93 h-k
$P_4 \times W_5$	31.00 c-e	5.20	43.00 de	3.66 jk

$P_5 \times W_0$	30.50 ef	5.06	40.00 f-h	4.40 d-g
$P_5 \times W_1$	28.16ij	4.23	42.00 d-g	4.00 g-j
$P_5 \times W_2$	28.30 ij	4.23	38.00 h-j	4.40 d-g
$P_5 \times W_3$	30.70 d-f	4.33	34.33 k-m	4.16 f-i
$P_5 \times W_4$	30.66 d-f	4.33	33.00 m-o	4.03 g-j
$P_5 \times W_5$	31.33 b-d	4.66	33.00 m-o	3.90 h-k
LSD (0.05)	0.72	-	2.74	0.45
CV (%)	1.48	11.65	4.42	6.64

Numbers in columns followed by the same letter are not statistically different at $P_{0.05.}$
P_0: Control, P_1: 5.00, P_2: 5.30, P_3: 5.80, P_4: 6.10 and P_5: 6.40.
W_0: Control, W_1: Im + 12h, W_2: Im + 18h, W_3: Im + 24h, W_4: Im + 30h and W_5: Im + 36h.

combinations of pH of substrates and frequency of watering on number of harvest/packetof oyster mushroom.

Results showed that, the maximum (5.13) number of harvest/packet was found from $P_3 \times W_1$ which was statistically similar to $P_4 \times W_1$ (5.00), $P_3 \times W_2$ (4.90) and $P_1 \times W_1$ (4.83**)** while, the minimum (3.00) was found from $P_3 \times W_5$ (Table 3).

CONCLUSIONS

From the present study it was revealed that most of the parameters showed the significant response due to different pH levels of substrate and watering frequency. For pH level of substrate, the control treatment P_0 (5.5) exhibited the better performance on the growth characters of mushroom.In case of watering frequency, the treatment W_1 (Im + 12h) showed higher number of fruiting bodies and higher number of harvest of mushroom. Statistically, the highest number of fruiting bodies was obtained from the combined treatment $P_0 \times W_1$ *i.e.*, pH of substrate 5.5 in combination with watering frequency Im + 12h. So, this combination may be used for higher yield of mushroom.

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