



Effects of Watering Frequency and Watering Method on the Growth and Yield of Oyster Mushroom (*Pleurotus ostreatus*)

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Abstract

The effects of watering frequency and watering method on the growth and yield of Oyster mushroom (*Pleurotus ostreatus*) were investigated at the Ebonyi State University Mushroom Farm, Abakaliki. The experiment was conducted as a 3 x 2 factorial in four replications, arranged in a completely randomized design (CRD). This experiment comprised two factors: watering frequency with three rates (once in two days, once and twice in a day) and two levels of watering methods (direct and indirect watering). The highest percentage spawn running at two weeks and one month; 70.70% and 88.48%, respectively, were recorded on mushrooms watered twice a day, while the least percentage spawn running at two weeks and one month; 57.21% and 73.63%, were observed by mushrooms watered once in two days, respectively. The twice daily watering had the heaviest fresh and dry weight (0.42kg/bag) and (0.40g/bag), and the highest number of branches and productive bags; 11.99 and 7.50, respectively. Watering once in two days had the least values for all these parameters. Fresh weight and number of productive bags were significantly ($P < 0.05$) improved by watering methods. The indirect watering gave the heaviest fresh and dry weights; 0.38kg and 0.04kg, highest numbers of branches and productive bags; 10.98 and 6.67, respectively. The heaviest fresh and dry weights; 0.48kg and 0.05kg respectively, highest numbers of branches (12.21) and productive bags (8.25 bags) were observed on mushrooms treated with twice a day x indirect watering. It was therefore concluded that twice in a day with indirect watering should be adopted for optimum growth and yield of oyster mushroom.

Keywords: Oyster mushroom, watering frequencies, direct watering, indirect watering

Introduction

Oyster mushrooms (*Pleurotus ostreatus*) belong to the class *Basidiomycetes* and are commonly farmed and consumed by man (Kamal *et al.*, 2009). Mushrooms contain proteins, vitamins and minerals and are widely used either as food or medicine. They are often found as saprophytes on soils, open fields, farm lands, woods and roadsides (Shah *et al.*, 2004). *Pleurotus ostreatus* is a popular Oyster mushroom species which can be grown on various agricultural wastes. It is capable of excreting hydrolyzing and oxidizing enzymes (Pathmahini *et al.*, 2008), that have the capacity of using complex organic compounds from agricultural and industrial wastes (Zadrazil *et al.*, 1981), and it greatly adapt to diverse agricultural environments (Jandaik and Goyal 1995). The work of Saputra *et al.* (2020) had confirmed that hood and diameter of mushrooms were not significantly affected by watering frequency. Badham (1989)

reported that adequate water was vital for the production of mushrooms in wild or domestic conditions. Thus, water requirements of mushrooms must be met for maximum yields. According to Chitra *et al.* (2018), humidity affects Oyster mushroom production. It performed better with high relative humidity of 74% - 79% than with 55% -68% relative humidity and low temperature during growth. Also, Uddin *et al.* (2011) also noted that oyster mushroom production was greatly improved by increased relative humidity. Furthermore, Sonnenberg and Blok (2011) stated that when relative humidity of the compost declined particularly at the first to the second flushes, mushroom growth was badly hindered. Watering frequency on the bag-log of Oyster mushrooms significantly favored the biomass of the mushrooms and that water application four times per day had profound effect on mushroom biomass when compared to the once per day watering (Saputra *et al.*,

2020). Danay *et al.* (2012) and Danay and Levanon (2013) reported that the use of drip irrigation overcame some disorders like hollow stem (uptake of excessive water and consequently rapid evaporation), early-openers (restriction of water at crucial stage in the development of mushrooms), hard gill (extreme evaporation alongside low moisture content in the substrates), and clefts and craters on the young mushrooms; which are related to insufficient water between mushroom's flushes. Fletcher and Gaze (2007) confirmed that waterlogged substrates led to anaerobic situations, which prevented degradation of lignin and caused visible abiotic disorders like false mummy (dried mushrooms), water logging (glassy patches on the mushroom surface), weepers (exudation of water from marginal edge of the cap), brown stems (waterlogged stem with bruises). Recent studies on mushrooms productions have been on either the watering frequencies or methods of watering; thus the need to combine these two factors for adequate and effective watering of mushrooms. Hence, this experiment was conducted to determine the effects of watering frequencies and watering methods on the growth and yield of Oyster mushroom.

Materials and Methods

Site of experimentation: This research was carried at the Ebonyi State University Mushroom Farm, Abakaliki, in derived savannah zone of the Southeast of Nigeria. The experimental site is located approximately between Latitude 06° 19' 407" N and Longitude 08° 07' 831" E of the equator at an elevation of 477m above sea level. The climate is characterized with daily temperature range of 22°C to 32°C. This area has a pseudo-bimodal rainfall pattern which starts from April, breaks at August and finally stops at November. The total annual rainfall ranges from 1613.8mm to 2136.27mm (Diagi, 2018).

Experimental design and treatments: A 3 x 2 factorial experiment was laid out in completely randomized design (CRD) with four replications. Factor A was watering frequency at three levels; watering once per day (f_1), watering twice per day (f_2), and watering once in two days (f_3) and factor B was watering method; direct watering into the substrate bag (m_1) and indirect watering on the substrate body (m_2). Fresh and dryweights were measured using sensitive weighing balance, height and circumference of stalk and circumference of pileus (cap) were measured with measuring tape. The surface area of oyster was calculated, and percentage spawns running, number of branches, non-productive and productive bags were counted.

Preparation of the mother culture: The outer layer of the fresh mushroom culture was removed with scapel and forceps. The middle whitest portion of the culture was collected and inoculated into agar plates and firmly sealed with parafilm wax. These plates were incubated in incubator at 25-27°C for a period of seven days. After this period, the fungal mycelia grew all over the plates.

The mycelia on the agar plates were taken into the laminar flow chamber and were cut in criss-cross manners. Again, the plates were sub-cultured by taking a portion of the culture with white mycelia and inoculated into a sterile agar plate. To avoid contaminating these plates, they were firmly sealed with parafilm wax and incubated for a period of seven days at a temperature range of 25-27°C.

Preparation of spawn: Transparent Bama bottles were used to prepare spawn because it enables good mycelial development. 15kg of millet grains was carefully washed, soaked for a period of 24 hours in water, and finally sieved. Then, 10kg of good grains were meshed in a 15litre of water and heated for 15 minutes and allowed to cool for another 15 minutes. The water was allowed to drain very well and the grains were filled in the Bama bottles up to half of the bottle to create space for growth of spores. The filled bottles were inoculated with the already prepared pure cultures and sealed with sterile cotton cloth.

Medium preparation: The medium consists of two substrates namely: rice dust and saw dust in the ratio of 60:40. A total of 168kg (100.8kg of rice hull and 67.2kg of saw dust) substrates were mixed. The substrates were mixed with 10g per 15kg of CaCO₃ dissolved in water. Water was added until the confirmation of sticky test. The mixed substrates were bagged and tightened with clip and foam inserted into the clip to avoid contamination and maintain proper aeration. Then, the media were labeled and loaded into the industrial cylinder and sterilized with heat.

Sterilization: Is the process of killing micro-organisms with high heat, ethanol or radiations. Sterilization occurs at a minimum temperature of 121°C steam at 15psi (1atm). The industrial cylinder was first discharged of any previous water through the outlet and washed/cleaned thoroughly. Water was filled into the cylinder just below the sieve level (conical level) before loading the bags into it. After loading, the loaded cylinder was covered and fired for eight hours to ensure maximum sterility.

Inoculation of the spawn: After cooling the sterilized media for 24 hours, the spawns were inoculated into the media. All the media were inoculated with the cultured spawn at rate of one spoon per bag. Thereafter, the bags were shaken to ensure that the spawn were evenly distributed on top of the media.

Incubation: All the inoculated media were incubated in a damp and dark room for one month while spawn running was observed during this period. High relative humidity was maintained in the incubator room while incubation lasts. During incubation, the incubated bags were treated with various watering frequencies and methods as stated in the experiment design.

Statistical analysis: All the data obtained were subjected to statistical analysis of variance (ANOVA)

based on the procedure for a completely randomized design (CRD) for a factorial experiment as outlined by Obi (2002), whereas separation of treatment mean effects was done using fishers least significant difference (FLSD) as described by Obi (2002).

Results and Discussion

Effects of watering frequencies on growth and yield of Oyster mushroom

Percentage spawn running of mushroom at two weeks and one month were significantly ($P < 0.05$) improved by watering frequencies (Table 1). The highest percentage spawn running at two weeks (70.70%) and one month (88.48%), were recorded on mushrooms watered twice a day, while the least percentage spawn running (57.21% and 73.63%) were observed by mushrooms watered once in two days, respectively. Height and circumference of stalk, circumference of pileus, and area of Oyster were not significantly affected by watering frequencies ($P < 0.05$). The tallest height (10.95cm), widest circumference of pileus (18.89cm), and widest surface area (39.49cm²) were obtained from twice daily watering, whereas, the shortest height (8.70cm), and least surface area (33.93cm²) were observed from once watering in two days. Once watering a day had the least circumference of pileus (13.18cm) and twice watering a day had the least circumference of stalk (4.53cm) (Table 1).

Watering frequency was significant ($P < 0.05$) for fresh weight, number of branches, and number of productive bags (Table 2). The twice daily watering had the heaviest fresh (0.42kg/bag) and dry weight (0.40g/bag), and the highest number of branches (11.99) and productive bags (7.50), while, once watering in two days had the least values for all these parameters. Watering frequency was not significant ($P < 0.05$) for dry weight and number of non-productive bags. Once watering in two days had the greatest number of non-productive bags (2.63), whereas, twice watering daily had the least value (1.00).

From the result, increased frequency of watering was evident to produce better result in oyster mushroom compared to lower watering frequencies. This may be attributed to the fact that moisture is crucial in mushroom production and that the water content of mushrooms is about 90-95%; hence, any restrictions in moisture supply will negatively affect mushroom production. This experiment concurred with the work of Saputra *et al.* (2020) which showed that hood and diameter were not significantly affected by watering frequency. The result of this present study agrees with Badham (1989), who reported that sufficient supply of water is critical for mushroom growth and yield. Also, Chitra *et al.* (2018), found better performance of oyster mushroom production under high relative humidity. High relative humidity was also reported to favour oyster mushroom production (Uddin *et al.*, 2011). Low relative humidity was also found to correlate negatively with mushroom growth (Stevani, 2011). Works on oyster mushroom showed negative effect in growth of

mushrooms with decreased relative humidity. The result of this study also agrees with the observations of Saputra *et al.* (2020) that watering frequencies on the bag-log significantly increased biomass of oyster mushrooms. Saputra *et al.* (2020) also noted that water application four times per day had profound effect on mushroom biomass when compared to the once per day watering. In recapitulation, regulation of relative humidity is extremely essential for oyster mushroom production.

Effects of watering methods on growth and yield of oyster mushroom

Percentage spawn running of mushroom at two weeks and one month, height and circumference of stalk, and circumference of pileus were significantly influenced by watering methods ($P < 0.05$) (Table 3). The highest percentage spawn running at two weeks (66.97%) and one month (82.82%), tallest height (11.46cm), widest circumference of stalk (5.35cm) and pileus (18.74cm) were recorded on mushrooms watered indirectly, while the direct watering had the least values for all these parameters. The watering method was not significant ($P < 0.05$) for surface area (cm²) (Table 3). The indirect watering had the highest surface area (44.30cm²), whereas, direct watering had the least surface area (30.98cm²). Fresh weight and number of productive bags were significantly ($P < 0.05$) improved by watering methods (Table 4). The indirect watering gave the heaviest fresh (0.38kg) and dry weight (0.04kg), and the highest numbers of branches (10.98) and productive bags (6.67), whereas the direct watering had the least values for all these parameters, except for number of non-productive bags (2.17). Watering method was not significant ($P > 0.05$) for dry weight, number of branches and non-productive bags. The indirect watering method had the highest values for most parameters studied, while the direct watering had the highest number of non-productive bags. The result showed that indirect watering on substrates outperformed the direct water application. This was affirmed by the studies of Quimio *et al.* (1990) and Urben (2004) which stipulated that mushrooms that grew on substrates without casing drew their water from the substrates leading to high moisture content. Thus, excessive water in the substrates resulted in breathing difficulty in the mycelium, inhibition of perspiration, hindered fruiting bodies' growth, despite great amount of inoculums. This finally resulted in the infestation of non-desired organisms like nematodes and bacteria. According to the work of Macdonald *et al.* (2010), for optimum yield of oyster mushroom, indirect watering should be done without contact with fruiting body and caps; hence, this concurred with the findings of this experiment which showed that indirect watering was better than direct watering in all parameters evaluated.

Effects of interaction between watering frequencies and methods on the growth and yield of oyster mushroom

Percentage spawn running of mushroom at two weeks was significantly ($P < 0.05$) promoted by watering frequencies and watering methods (Table 5). Interaction

between watering frequencies and watering methods was not significant ($P < 0.05$) for percentage spawn running of mushroom in a month, height and circumference of stalk, circumference of pileus, and area of Oyster. The highest percentage spawn running at two weeks (71.40%) and one month (91.30%). Tallest stalk (12.99cm), widest circumference of pileus (26.50cm) and largest area of oyster (46.50cm²) were recorded on the treatment with the combination of watering twice a day and indirect watering; although, the combination of once in a day and indirect watering had the widest circumference of stalk (5.59cm). Meanwhile, the lowest percentage spawn running at two weeks (51.93%), percentage spawn running in a month (71.50%), shortest height of stalk (7.38cm), smallest circumference of pileus (11.20cm), and smallest area of Oyster (24.53cm²) were observed on mushrooms treated with the once in two days x direct watering; except for the smallest circumference of stalk (3.40cm) that was observed in the twice a day x direct watering. The watering frequency x watering method was not significant ($P < 0.05$) for fresh and dry weights of mushroom, numbers of branches, non-productive and productive bags (Table 6). The heaviest fresh (0.48kg) and dry weight (0.05kg), highest numbers of branches (12.21) and productive bags (8.25 bags) were observed on mushrooms treated with twice a day x indirect watering; while, the once in two days x direct watering had the highest number of non-productive bags (3.00 bags). Nevertheless, the lightest fresh (0.26kg) and dry weight (0.02kg), least numbers of branches (6.80) and productive bags (4.00 bags) were obtained from mushrooms treated with the once in two days x direct watering. The least number of non-productive bags (0.50 bags) was observed in the twice a day x indirect watering. Watering frequencies x watering methods was not significant for the parameters evaluated, except for percentage spawn running at two weeks; the twice in a day x indirect watering had the highest values for all parameters evaluated except for circumference of stalk and number of non-productive bags. It is now of conventional wisdom that effective and sufficient water application is vital for mushroom production. Owing to this, it was reported that a decline in relative humidity during the early flushes reduced growth of mushroom (Sonnenberg and Blok, 2011). In another study, it was found that use of drip irrigation as indirect watering overcame the issues of hollow stem, early openers, hard gill, clefts and craters on the growing mushroom (Danay *et al.*, 2012; Danay and Levanon, 2013). These issues were related to inadequate water supply between mushroom flushes. Waterlogged substrates also led to anaerobic situations (Fletcher and Gaze, 2007), which prevented degradation of lignin and caused visible abiotic disorders like false mummy (dried mushrooms), glassy patches on the mushroom surface, exudation of water from marginal edge of the cap (weepers) and brown stems. All these findings were in conformity with this experiment, because the direct watering and watering once in two days caused a lot of damages on the mushroom, thus many non-productive bags were recorded with such treatment combinations.

Conclusion

The result of the experiment showed that watering frequency and watering methods had significant effect on the performance of oyster mushroom. Watering twice a day had better results compared to watering once a day or once in two days. Also, indirect watering produced the best results compared to direct water application. Hence, it is recommended that mushroom farmers should adopt the practice of twice daily watering or/and indirect watering for maximum productivity of oyster mushrooms.

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Table 1: Effects of watering frequencies on % spawn, stalk height and circumference (cm), pilens circumference (cm), and area of Oyster (cm²)

Watering frequency	Spawn running at two weeks (%)	Spawn running in a month (%)	height of stalk (cm)	Circumference of stalk (cm)	Circumference of Pilens (cm)	area of oyster (cm ²)
Once a day	65.19	78.83	9.53	4.65	13.18	39.49
Twice a day	70.7	88.48	10.95	4.53	18.89	39.49
Once in two days	57.21	73.63	8.70	4.94	14.49	33.93
<i>F-LSD</i> _(0.05)	2.00	3.15	NS	NS	NS	NS

NS = not significant, *F-LSD* = Fishers Least Significant Different

Table 2: Effects of watering frequencies on fresh and dry weight (kg), numbers of branches, non-productive, and productive bags

Watering frequency	fresh weight (kg)	dry weight (g)	number of branches	number of non-productive bags	number of productive bags
Once a day	0.32	0.03	10.78	1.36	5.88
Twice a day	0.42	0.04	11.99	1.00	7.50
Once in two days	0.29	0.02	8.16	2.63	4.63
<i>F-LSD</i> _(0.05)	0.06	NS	2.03	NS	0.86

NS = not significant, *F-LSD* = Fishers Least Significant Different

Table 3: Effects of watering methods on % spawn, stalk height and circumference (cm), pilens circumference (cm), and area of Oyster (cm²)

Watering method	% spawn running at two weeks	% spawn running in a month	height of stalk (cm)	circumference of stalk (cm)	circumference of Pilens (cm)	area of oyster (cm ²)
Direct	61.77	77.80	7.99	4.07	11.37	30.98
Indirect	66.97	82.82	11.46	5.35	18.74	44.30
<i>F-LSD</i> _(0.05)	1.63	2.58	3.33	1.51	8.22	NS

NS = not significant, *F-LSD* = Fishers Least Significant Different

Table 4: Effects of watering methods on fresh and dry weight (kg), number of branches, non-productive, and productive bags

Watering method	fresh weight (kg)	dry weight (kg)	number of branches	number of non-productive bags	number of productive bags
Direct	0.30	0.03	9.63	2.17	5.33
Indirect	0.38	0.04	10.98	1.17	6.67
<i>F-LSD</i> _(0.05)	0.05	NS	NS	NS	0.7

NS = not significant, *F-LSD* = Fishers Least Significant Different

Table 5: Effect of watering frequency x watering method on % spawn, stalk height and diameter (cm), pilens diameter (cm), and area of Oyster (cm²)

Treatment	% spawn running at two weeks	% spawn running in a month	height of stalk (cm)	circumference of stalk (cm)	circumference of Pilens (cm)	area of oyster (cm ²)
<i>F₁M₁</i>	63.38	76.25	7.71	3.71	11.64	35.93
<i>F₁M₂</i>	67.00	81.40	11.35	5.59	11.95	43.06
<i>F₂M₁</i>	70.00	85.65	8.91	3.40	11.29	32.49
<i>F₂M₂</i>	71.40	91.30	12.99	5.46	26.50	46.50
<i>F₃M₁</i>	51.93	71.50	7.38	4.90	11.20	24.53
<i>F₃M₂</i>	62.50	75.75	10.03	4.99	17.76	43.34
<i>F</i> -	2.83	NS	NS	NS	NS	NS
<i>LSD</i> _(0,0.05)						

NS = not significant, *F-LSD* = Fishers Least Significant Different, *F₁* = Once a day, *F₂* = Twice a day, *F₃* = Once in two days, *M₁* = Direct method, and *M₂* = Indirect method.

Table 6: Effect of watering frequency x watering method on fresh and dry weight (kg), number of branches, non-productive, and productive bags

Treatment	fresh weight (kg)	dry weight (kg)	number of branches	number of non-productive bags	number of productive bags
<i>F₁M₁</i>	0.29	0.03	10.35	2.00	5.25
<i>F₁M₂</i>	0.34	0.03	11.21	0.75	6.50
<i>F₂M₁</i>	0.35	0.03	11.76	1.50	6.75
<i>F₂M₂</i>	0.48	0.05	12.21	0.50	8.25
<i>F₃M₁</i>	0.26	0.02	6.80	3.00	4.00
<i>F₃M₂</i>	0.32	0.03	9.53	2.25	5.25
<i>F</i> -	NS	NS	NS	NS	NS
<i>LSD</i> _(0,0.05)					

NS = not significant, *F-LSD* = Fishers Least Significant Different, *F₁* = Once a day, *F₂* = Twice a day, *F₃* = Once in two days, *M₁* = Direct method, and *M₂* = Indirect method