

# Effect of perforation size and substrate bag fruiting position on the morphology of fruiting bodies and clusters in *Pleurotus ostreatus* (Jacq.) P. Kumm

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## ABSTRACT

Perforation or fruiting hole size on substrate bags control cluster sizes and morphology in exotic mushroom cultivation. The effect of three different perforation sizes on substrate bags (factor A: 50, 100, and 150 mm) and their positioning on the shelves (factor B: Horizontal, vertical, and slant) on the crop and various morphological characteristics in *Pleurotus ostreatus* was studied. Microclimatic conditions for fruiting were 16±1°C, 87±3% RH; 230±42 lux illumination. The formula for calculating area of ellipse was modified and used for the area of mushroom cap. Results indicated that the total fruit body yield and biological efficiency (BE) in the bags set in horizontal position were 10% lower than other treatments. The effect of perforation size on mushroom cluster sizes was more on the substrate blocks in the horizontal position. There was a linear correlation between perforation size and fruiting body cluster sizes. Results suggest that the 50 mm perforation on bags in vertical and slant positions gave fruiting body clusters sizes 186–196 mm width and 122–154 mm height, with 92.36±6.48% BE. The cluster size indicated is the best fit for standard packaging containers used in commercial oyster mushroom production in Ukraine.

## 1. INTRODUCTION

The demand for exotic mushrooms is increasing every day due to their nutritional and medicinal values. The combined production of *Pleurotus* spp. ranks second after the *Agaricus bisporus*, primarily because of the availability of highly efficient cultivation technology, the nutritional and medicinal properties [1,2], and various other uses [3-5].

In 2004, fresh oyster mushroom consumption per capita was 2.72 kg; by 2018, it has reduced to 1.63 kg per capita, and the number of producers did not change; large quantities of mushrooms are now pickled. One of the reasons for the drop in per capita consumption of fresh oyster mushrooms is consumers are now more selective about the quality, appearance, and packaging of fresh mushrooms. Therefore, it has become necessary to look at consumer appeals to improve marketing and sales locally, and post-harvest processing acceptable for export. Consumers like both fresh and canned oyster mushrooms. However, most producers sell fresh mushrooms; therefore, size, appearance, and packaging have become very important.

Across Europe, consumer preferences are dictating the need to produce mushrooms with high organoleptic properties such as rich color, delicate texture, and optimal size of fruiting bodies. According to the latest estimates of Champinter, a mushroom marketer in Spain, 300 g packets of peeled mushrooms, which is enough for two servings of a delicious meal, is most preferred. In the case of oyster specifically, buyers prefer packages containing a collection of individual fruiting bodies, although oyster mushroom keeps better in sprouts clumps [6]. Humidity, lightning, and air composition are factors that can affect the size and quality of mushrooms, and they are continually being studied by scientists [7]. It has been shown that substrate blocks with a fruiting hole diameter of 90 mm resulted in the highest number of fruit bodies and overall biological efficiency (BE) [8]. Zireva *et al.* [9] reported the effect of substrate size and their location in the growing chamber on mushroom fruiting body yield. However, there are limited studies and reports on the effect of cultivation conditions (substrate bag position in the fruiting room, number of holes on substrate bags, size of each hole, etc.) on yield, BE, and organoleptic properties. We studied the effect of perforation size on substrate bags and the fruiting position of the bags on the morphology of fruiting bodies and clusters characteristics of *Pleurotus ostreatus* in a commercial production setting in Ukraine.

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## 2. MATERIALS AND METHODS

### 2.1. Spawn Preparation

*P. ostreatus* (strain 2301 IBK) used is from the culture collection of edible mushrooms at the Institute of Botany, Ukraine [10]. The culture was maintained on nutrient media (maltodextrine – 20 g, yeast extract – 2 g, and agar-agar – 20 g, in 1 L ddH<sub>2</sub>O) and stored at 1–2°C [11]. Spawn was made with barley, wheat, rape, flax, and chalk (CaCO<sub>3</sub>) combined in the ratio 60:30:8:1:1. Pre-cooked barley and wheat, and pre-soaked rapeseeds, flax, and chalk, were mixed and loaded into polypropylene bags of size (580 mm × 490 mm), PP75/BEU6/X47-57 (from Sac02, Belgium) and sterilized at 128°C, 24 PSI for 180 min. Upon cooling, the sterile grain mixture was inoculated with mother spawn (0.5% w/w), sealed and incubated at 24±1°C for 8 days before thorough mixing to allow the achieve uniform colonization of the spawn materials. The spawn was ready after 11±1 days and stored in a refrigerator (2±1°C) until use.

### 2.2. Substrate Preparation

The substrate used for cultivation contained straw substrate and sunflower husk (ratio 1:3), humidity = 72 %; pH= 8.02; carbon to nitrogen ratio (C:N) = 69:1, was pasteurized using a three-step process: Pre-fermentation, pasteurization, and fermentation [12]. During the pre-fermentation process, the combined raw substrate materials were loaded into the pasteurization chamber (volume = 45m<sup>3</sup>), hydrated to attain 72–74% moisture content, and maintained at 30–35 °C for 24–36 h. In the pasteurization step, steam was injected into the chamber to raise the temperature to 70–75°C and maintained for 12 h; then, the temperature in the chamber was reduced to 50–55°C over 24 h. Fast cooling with fresh air passed through an F-filter into the chamber was done to obtain ready to use substrate at 25–28°C [13].

A partially mechanized process involving a vibrating table, which thoroughly mixes the spawn with the substrate, was used to add spawn (3.5% w/w) to the substrate bags [14]. The substrate and spawn complex was loaded in 350 mm × 900 mm polyethylene bags with a film thickness of 70 µm. Each substrate bag had a diameter–220 ± 20 mm, height–750 ± 50 mm, and weight–12.43 ± 0.23 kg.

### 2.3. Growing Conditions

The incubation of the substrate and fruiting process was done in the same room. The room temperature during the spawn-run (incubation) period was set at 20±2 °C (which allowed the temperature in the center

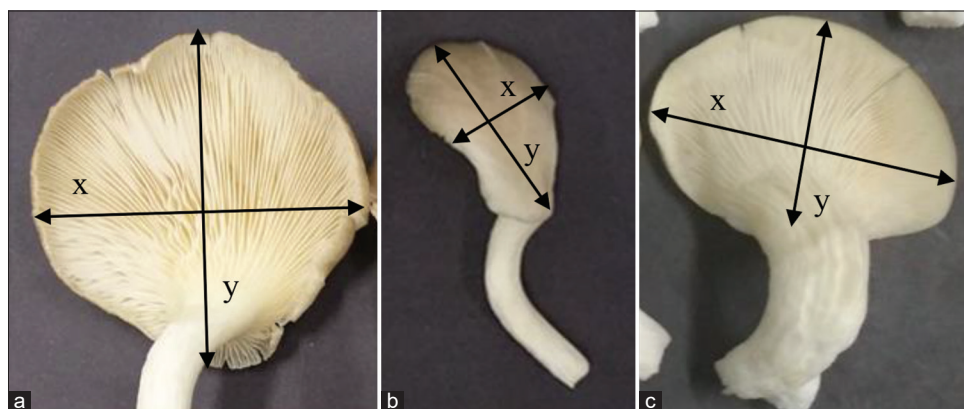
of the bag block to be 26±2°C) and relative humidity of 75±3%. For fruiting body induction, the temperature in the room was gradually reduced to 16±1°C over 48 h by ventilation system with the addition of fresh air and reduced CO<sub>2</sub> concentration in the chamber from 3200±100 to 1050±100 ppm, the relative humidity increased to 87±3%, and illumination increased from 50 ±12 (daylight) to 230±42 lux. These microclimate conditions in the growing room were maintained through fruiting body development to harvest.

### 2.4. Data Collection

The bags with substrates were placed on the shelves in a growing chamber in a complete randomized block design, based on the position of the substrate bag: 1 = Horizontal; 2 = Vertical, and 3 = Slant (factor A). There were 30 bags in each fruiting position, which were further divided into three groups of 10 bags, each representing 50 or 100 or 150 mm perforations (Factor B) and perforated. The surface on each bag perforated was an average of 0.2% of the total bag surface area. There were 12 holes on the bags with 50 mm, six holes with 100 mm, and four holes with 150 mm incisions on the substrate bags. The perforations were staggered at distances of 100–150 mm apart on the front surface of each bag. After harvest, 25 randomly selected fruiting body clusters per treatment were analyzed for width, height, weight, and the number of individual fruiting bodies per cluster. The selected fruiting body clusters were picked apart, and 100 individual fruiting bodies were randomly selected per treatment for analysis. The weight of the individual fruiting body (intact fruiting body with cap and stipe), cap weight, width (diameter), and length (the distance from the end of stipe to the opposite end of mushrooms cap), stipe length, and diameter were determined [Figure 1].

### 2.5. Data Analyses

The coefficient of cap asymmetry was calculated as the ratio of width to length; it is equal to one when a fruiting body cap is round. The elongated leaf-shaped cap of the fruiting body found in oyster mushrooms had a coefficient below one. Furthermore, the coefficient of weight loss (CWL) when the stipe is separated from the fruiting body was calculated because some markets prefer “caps-only” fruiting bodies for sale. The coefficient was determined by the ratio of the cap’s weight to the mass of the whole fruiting body. *P. ostreatus* fruiting body cap usually has the shape of an ellipse. Therefore, the formula  $A = \pi \cdot 1/2x \cdot 1/2y$  or  $\pi \cdot 1/4xy$  was used to calculate the area of the mushroom cap, where y is the distance from the end of the stipe to the opposite end of the mushroom cap and x



**Figure 1:** Three different shapes common on *Pleurotus ostreatus* fruiting bodies: (a) Round, (b) leaf, (c) oyster. Coordinates x and y were measured to obtain values for calculating area of fruiting body cap as an ellipse shape ( $A = \pi/4xy$ ).

is the distance across the width of the cap, which is perpendicular to y [Figure 1]. The BE was calculated as total mushrooms fresh weight divided by the dry weight of the substrate used per bag [15]. In this experiment, BE was calculated with values obtained from the first flush only because the strain 2301 IBK tested produced 80% of its total yield in the first flush [16]. The statistical analysis of the data obtained was performed using Microsoft Office Excel 2016, QI Macros 2020 software, and the Agrostat New software package [17].

### 3. RESULTS AND DISCUSSION

#### 3.1. Biological Efficiency

The incubation time was 15±1 days. The fruiting bodies in the first flush reached maturity in 5±1 day after primordia appearance, and there were no significant differences ( $P > 0.05$ ) among all treatments. A two-factor dispersion statistical analysis on the comparison of BE did not indicate differences between treatments. However, a U-test analysis for comparing averages determined that the level of BE in the horizontal position was 10% lower than the vertical and slant fruiting positions [Table 1]. The best results from the horizontal and slant positions may be because these are the positions that *P. ostreatus* fruit in nature [18]. Furthermore, the BE improved in the horizontal and vertical position with an increase in perforation size to give the highest BE of 77.70±6.08% and 88.04±5.74% in the horizontal and vertical position, respectively, at 150 mm perforation size. There was no such trend in the slant position, and the bags with 100 mm perforations gave the highest BE (92.36±6.48%). These results are different from Zireva et al. [9], who reported that position on the shelf had no significant effect ( $P > 0.05$ ) on mushroom yield.

#### 3.2. Cluster Characteristics

##### 3.2.1. Cluster weight

Statistical analysis (ANOVA) indicated significant differences ( $P = 0.001$ ) on the bag position's influence on fruiting body clusters' weight and sizes. The highest average mass (703±86 g) was in the horizontally positioned bags with 150 mm perforation and the lowest (404±44g) in the vertical position with 50 mm perforation [Table 1]. In general, the weight of clusters obtained from 150 mm perforations was significantly higher ( $P < 0.05$ ; LSD=161; HSD=257) than perforations of sizes 50 and 100 mm.

##### 3.2.2. Cluster width and height

The highest cluster weight (236±13 g) was recorded from the bags in the horizontal fruiting position with a perforation of 150 mm; the lowest (186±6 g) was from the vertical position and perforation of 50 mm. Two-factor dispersion statistical analysis indicated that the perforation's size had a significant ( $P < 0.001$ ) influence on the clusters' width. At the same time, the position factor is not significant ( $P > 0.05$ ). Simultaneously, both factors had a significant ( $P < 0.001$ ) influence on the height of clusters. Cluster height was significantly ( $P < 0.001$ ) in the vertical and slant positions: 194±12 and 184±10 mm, respectively. The horizontal position gave the lowest clusters height (122±8 mm). Data indicated that when perforation size increased, the cluster width and height range also increased in the horizontal and vertical positions. The range in cluster width and height also increased in the horizontal and vertical positions. The dispersion of data showed significant differences ( $P < 0.01$ ).

##### 3.2.3. Number of fruiting bodies per cluster

The number of fruiting bodies in the clusters had a positive correlation ( $R^2 = 0.94$  in horizontal, 0.98 in vertical, and 0.97 in slant positions) with the increase in the size of the perforation: In any of the positions, when the perforation was 50 mm, the average number of fruiting bodies was 25 or 26, but in the same position, when perforation was 150 mm, there was a significant difference ( $P < 0.001$ ), where it was 43±4 in the vertical position, 40±3 in vertical, and 39±4 horizontal positions. Regression analysis of these data produced a linear equation in which we can predict the number of fruiting bodies in clusters depending on the size of the perforations used [Figure 2].

#### 3.3. Fruiting Bodies

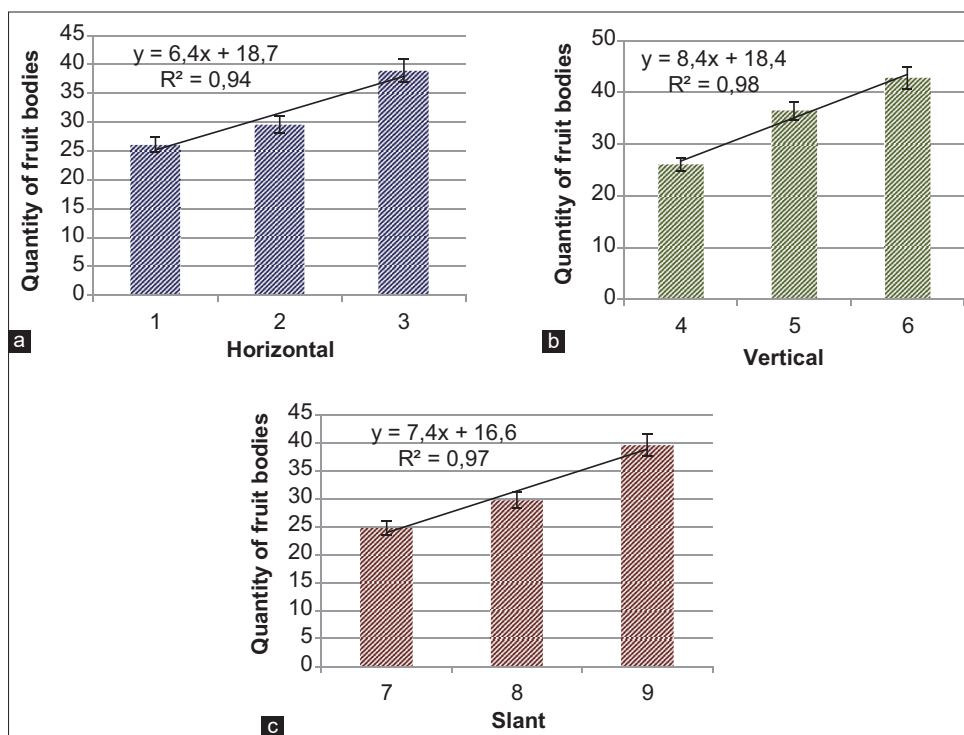
##### 3.3.1. Fruiting body yield and the CWL

The effect of bag position and perforation size was more visible in fruiting body morphological characteristics. Individual fruiting body mass was remarkably higher in the slant position compared to the horizontal and vertical position. Substrate bags having 50 and 150 mm perforations in the slant position gave the highest FB yield (21±1.2 g); the least was in the vertical position with the same perforation sizes of 50 and 150 mm (9±0.5 and 8±0.5 g, respectively). The higher fruiting body mass recorded in the slant position was significantly different from the other treatments at  $P < 0.001$  [Figure 3].

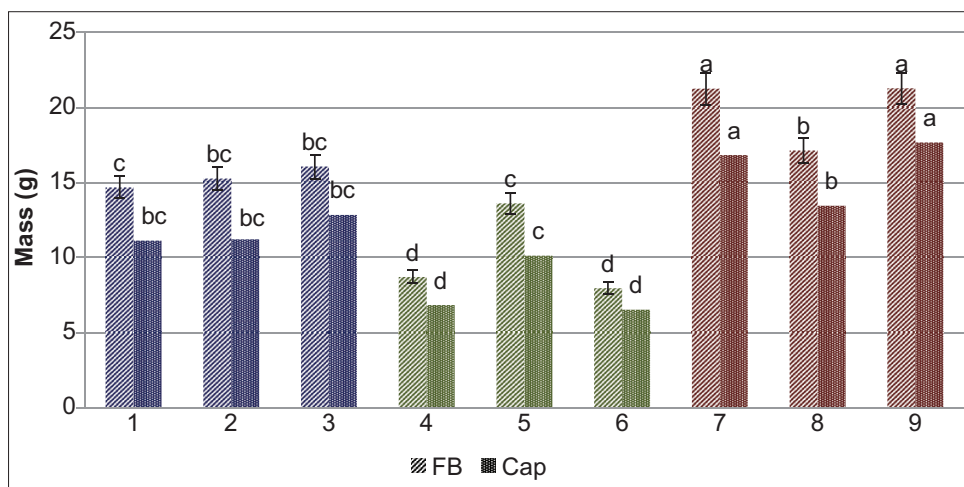
**Table 1:** Biological efficiency and cluster characteristics in *Pleurotus ostreatus* under fruiting bag position and incision diameters treatments.

Variant	Factor		Biological efficiency (%)	Cluster characteristic n=25			
	A	B		Mass (g)	Width (mm)	Height (mm)	FB quantity
1	1	1	73.01±6.81	435 <sup>b</sup> ±51	191 <sup>b</sup> ±9	122 <sup>c</sup> ±8	26 <sup>bc</sup> ±3
2		2	71.35±7.62	471 <sup>b</sup> ±44	206 <sup>b</sup> ±7	138 <sup>bc</sup> ±6	30 <sup>bc</sup> ±2
3		3	77.70±6.08	703 <sup>a</sup> ±86	236 <sup>a</sup> ±13	157 <sup>b</sup> ±11	39 <sup>ab</sup> ±4
4	2	1	77.76±3.24	404 <sup>b</sup> ±44	186 <sup>b</sup> ±6	149 <sup>b</sup> ±8	26 <sup>bc</sup> ±2
5		2	83.71±6.53	443 <sup>b</sup> ±52	188 <sup>b</sup> ±5	158 <sup>b</sup> ±8	31 <sup>b</sup> ±6
6		3	88.04±5.74	617 <sup>ab</sup> ±76	216 <sup>ab</sup> ±10	194 <sup>a</sup> ±12	43 <sup>a</sup> ±4
7	3	1	81.68±5.18	455 <sup>b</sup> ±49	196 <sup>b</sup> ±6	154 <sup>b</sup> ±7	25 <sup>bc</sup> ±3
8		2	92.36±6.48	438 <sup>b</sup> ±50	191 <sup>b</sup> ±8	151 <sup>b</sup> ±8	30 <sup>bc</sup> ±3
9		3	79.81±6.81	585 <sup>ab</sup> ±60	206 <sup>b</sup> ±5	184 <sup>a</sup> ±10	40 <sup>a</sup> ±3
LSD <sub>05</sub> (A)			11.32	161	22	18	11
LSD <sub>05</sub> (B)			10.58	157	19	12	12

Factor A – position on the shelf: 1=Horizontal, 2=Vertical, 3=Slant; Factor B – size of perforations: 1=50 mm; 2=100 mm; 3=150 mm



**Figure 2:** Effect of perforation size on quantity of fruiting bodies per cluster under the (a) horizontal position 1=50; 2=100; 3=150 mm; (b) vertical position with perforation size 4=50; 5=100; 6=150 mm; (c) slant position with perforation size 7=50; 8=100; 9=150 mm.



**Figure 3:** The average mass of FB and cap *Pleurotus ostreatus* horizontal 1=50; 2=100; 3=150 mm; vertical position with perforations 4=50; 5=100; 6=150 mm; slant position with perforations 7=50; 8=100; 9=150.

Substantial amounts of weights are lost from total yield when caps are removed from fruiting bodies and sold as “mushroom caps-only.” Fruiting body yield in the three different fruiting positions was used to calculate the CWL. Statistical analysis (ANOVA) indicated a significant difference ( $P < 0.05$ ) in CWL. The least weight lost was with cultivation in slant (CWL = 0.82) and vertical (CWL = 0.81) positions in bags with 150 mm perforations. These CWL values were significantly different from the bags in the horizontal position, where CWL ranged between 0.75 and 0.77 for bags with 50, 100, and 150 mm perforations [Table 2]. A comparison of CWL and perforation size relative to cap weight indicated that the 50 and 100 mm perforation sizes resulted in a loss of fruiting body cap weight irrespective of the fruiting position.

### 3.3.2. Size characteristics

However, fruiting bodies produced under any fruiting position and perforation size were within the size range reported (30–70 mm cap diameter) to have marketable qualities [19]. Similar results are reported for *P. ostreatus* strains HK-35, and another strain P-24, which has fruiting body diameters 60–100 mm [20]. In this experiment, in the vertical position with a 150 mm long perforation, smaller fruiting bodies ( $46 \pm 1.6$  mm width and  $52 \pm 1.3$  length) were recorded. Treatment 7 (slant, 50 mm perforations) gave the largest mushroom caps with  $75 \pm 2.2$  mm of width and  $70 \pm 1.5$  of length. The least cap development in the 150 mm perforation under vertical position may be due to the fruiting body quantity, which was the most in this treatment, and the

impedance to airflow around the developing fruiting bodies compared to the rest treatments. The fresh airflow into the fruiting house is usually from top to bottom, and it purges CO<sub>2</sub> from the fruiting house. However, the fresh air movement is impeded by fruiting bodies at the top of the column leading to the formation of smaller fruiting bodies toward the middle of the column. The slightly larger fruit bodies at the bottom of the column are usually due to fresh air hitting the floor and creating a more oxygen-rich microenvironment toward the bottom of the column. However, the slant and position airflow allows better air and even air circulation around the fruiting bodies, which eventually leads to better and even cap development across the bags' fruiting surface. The market preferences for *P. ostreatus* fruiting body size and packaging differ in many countries. In Turkey, they prefer larger caps, while in Russia, they like smaller caps, mainly because they are suitable for producing pickles. Our results indicate the possibility of regulating mushroom caps to meet market preferences.

The mushroom cap area was also calculated to determine the influence of factor A (perforation size) and B (fruiting position) on yield and fruit body morphology [Figure 4]. Statistical analysis (ANOVA) indicated significant differences ( $P < 0.001$ ) in the mushroom cap area from the slant fruiting position compared with the horizontal and vertical fruiting positions. The largest area  $4368 \pm 212$  mm<sup>2</sup> was in horizontal position and 50 mm perforation size, followed by  $4128 \pm 212$  mm<sup>2</sup>

in horizontal fruiting position and 150 mm perforation size. The least result was  $2027$  mm<sup>2</sup> in vertical fruiting position and 150 mm perforation size.

The characteristic, almost stemless, and shelf-like shape *P. ostreatus* earned it the name "oyster mushroom" [21]. The three common shapes encountered in this experiment and the dimensions measured to obtain data for the calculation of fruiting body area are shown in Figure 1. One-way ANOVA analysis on fruiting body asymmetry coefficient indicated statistical differences ( $P < 0.001$ ) between treatments. The least asymmetry coefficient ( $0.90 \pm 0.02$ ) was obtained in the vertical position and 150 mm perforation size. In this treatment, clusters had the highest numbers of fruiting bodies, which could have probably affected the FB shape and area. The highest asymmetry coefficient (1.12) was found in the caps from the horizontal fruiting position and 100 mm perforation size. In general, wider fruiting bodies were found in the slant and horizontal than the vertical fruiting positions.

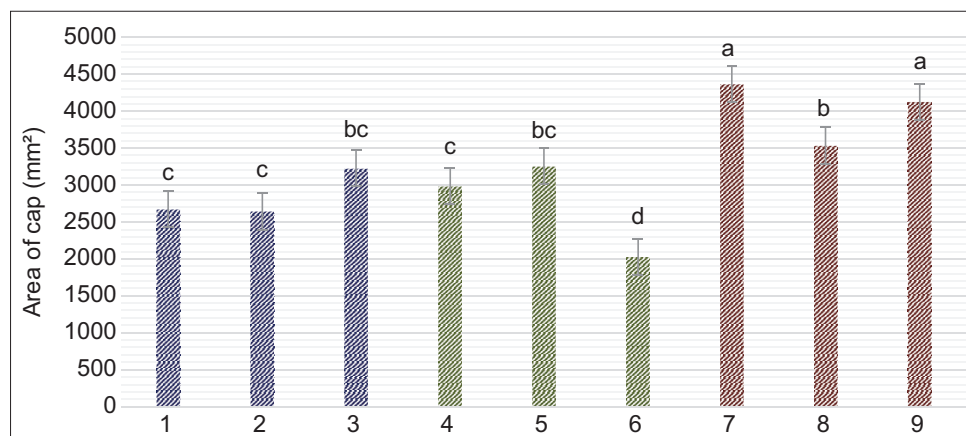
### 3.3.5. Stipe length and diameter

Stipe length depends on microclimate conditions [22]. There were significant differences ( $P < 0.001$ ) in the stipe length of fruiting bodies from the different treatments. In the horizontal fruiting position, the larger the perforation size, the higher the stipe length. The slant position

**Table 2:** Morphological characteristics of *Pleurotus ostreatus* fruiting bodies.

Treatment	Factor		Fruit bodies characteristics (mean±SD)					Stipe diameter (mm)
	A	B	CWL	Cap width (mm)	Cap length (mm)	Coefficient of cap asymmetry	Stipe height (mm)	
1	1	1	0.75 <sup>c</sup> ±0.01	58 <sup>cd</sup> ±2.4	54 <sup>c</sup> ±1.3	1.06 <sup>b</sup> ±0.02	17 <sup>d</sup> ±0.5	16±0.7
2		2	0.77 <sup>b</sup> ±0.05	59 <sup>c</sup> ±2.2	53 <sup>c</sup> ±1.4	1.12 <sup>a</sup> ±0.02	22 <sup>c</sup> ±0.8	15±0.7
3		3	0.78 <sup>ab</sup> ±0.02	62 <sup>c</sup> ±1.9	61 <sup>b</sup> ±1.6	1.03 <sup>b</sup> ±0.02	26 <sup>b</sup> ±1.3	12±0.4
4	2	1	0.79 <sup>ab</sup> ±0.01	60 <sup>c</sup> ±1.9	59 <sup>b</sup> ±1.4	1.03 <sup>b</sup> ±0.01	14 <sup>f</sup> ±0.5	11±0.3
5		2	0.76 <sup>bc</sup> ±0.01	64 <sup>bc</sup> ±2.2	60 <sup>b</sup> ±1.5	1.06 <sup>b</sup> ±0.02	25 <sup>bc</sup> ±1.2	12±0.4
6		3	0.81 <sup>a</sup> ±0.01	46 <sup>d</sup> ±1.6	52 <sup>c</sup> ±1.3	0.90 <sup>a</sup> ±0.02	16 <sup>df</sup> ±0.6	8±0.2
7	3	1	0.78 <sup>ab</sup> ±0.01	75 <sup>a</sup> ±2.2	70 <sup>a</sup> ±1.5	1.06 <sup>ab</sup> ±0.02	28 <sup>a</sup> ±0.9	12±0.3
8		2	0.80 <sup>ab</sup> ±0.01	67 <sup>b</sup> ±2.4	62 <sup>b</sup> ±1.5	1.08 <sup>ab</sup> ±0.02	22 <sup>c</sup> ±1.1	12±0.5
9		3	0.82 <sup>a</sup> ±0.01	72 <sup>ab</sup> ±2.5	69 <sup>a</sup> ±1.5	1.03 <sup>b</sup> ±0.02	22 <sup>c</sup> ±0.7	12±0.4
LSD <sub>05</sub> (A)			0.02	7.9	6.1	0.06	4.3	P=0.17
LSD <sub>05</sub> (B)			0.05	7.9	6.5	0.06	3.3	

CWL: Weight loss coefficient, SD: Standard deviation



**Figure 4:** Area of caps according to treatments: Horizontal position with perforations 1=50; 2=100; 3=150 mm; vertical position with perforations 4=50; 5=100; 6=150 mm; slant position with perforations 7=50; 8=100; 9=150 mm.

seems the reverse because the highest length was found in the fruiting bodies from 50 mm perforation size. In the vertical position, 100 mm perforation size gave the highest stipe length, which was significantly different from 50 and 150 mm perforation sizes. These unexplainable observations could be the subject of further investigations. However, the long stipe is not a desirable feature in *P. ostreatus* fruiting bodies because it takes away from the total weight of marketable yield and reduces the cap area. There was no statistical difference ( $P = 0.17$ ) in the stipe diameter in all treatments, which may mean that perforation sizes and fruiting position do not affect stipe diameter among all the morphological parameters monitored in this experiment.

#### 4. CONCLUSION

The effect of perforation size was more on the substrate blocks in the horizontal position and linear correlation exists between perforation size and the size of mushroom fruiting body clusters. The best perforation size is 50 mm, which can give clusters best suited for packaging and storage of oyster mushrooms in commercial operation in Ukraine. Studies of this nature on cultivated exotic mushrooms are needed for predicting cluster sizes, fruiting body morphological features, and optimum packaging requirements that reduce spoilage and food safety concerns.

#### 5. AUTHORSHIP

All authors contributed to the conception, design, execution, acquisition, analysis, and interpretation of data in this study; participated in manuscript preparation and approved the final version submitted for publication. Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

#### 6. CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported.

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