

PERFORMANCE OF OYSTER MUSHROOM SPECIES IN THE SUMMER SEASON USING SPORE CULTURE AND VEGETATIVE TISSUE CULTURE METHODS

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ABSTRACT

Performance of four oyster mushroom species such as *Pleurotus ostreatus*, PO-2 (S₁), *P. djmour*, Pop-1 (S₂), *P. florida*, FLO-2 (S₃) and *Pleurotus ostreatus* PO-10 (S₄) with two culture methods like spore culture (C₁) and vegetative tissue culture (C₂) was investigated. The experiment was conducted in Mushroom Development Institute (MDI), Savar, Dhaka from April to June, 2021 in CRD method. Considerable variations on different parameters related to growth and yield attributes were recorded. The least time required (56 days) from pure culture to first harvest was recorded in S₂. The minimum days required for mycelium running completion in spawn packet (20.25 days) was observed from the treatment combination S₂C₁ whereas the maximum days (28.5 days) was observed from S₃ C₂. From pure culture to first harvest, the highest duration (77.75 days) was observed in S₁C₂ and the lowest duration (54.50 days) was observed in S₂C₁. The diameter of pilea ranged from 6.2 cm to 7.3 cm with significant difference. The maximum yield (137.25g) and biological efficiency (61%) were achieved from S₂C₁, the lowest yield (108.12g) and biological efficiency (48.05%) were being found with S₃C₁. The protein percentage was found highest (24.56%) in S₁ and fiber was maximum (20.72%) in S₃. Protein percentage was found maximum (24.82%) in S₁C₁ and minimum (21.52%) in S₃C₂. The highest yields (133.31 g, 124.47 g and 137.25 g) were achieved from S₂ species, C₁ culture method and combination treatment S₂C₁, respectively.

Keywords: Performance, Oyster mushroom, Spore, Tissue culture.

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INTRODUCTION

Mushrooms are the fruiting bodies of macro fungi. They are neither plants nor animals, but they have been placed in a kingdom of their own called the kingdom of Myceteae. The term mushroom resembles to those fungi that have stem (stipe), cap (pileus), hymenium (lamellae) and spores which are present on the underside of the cap (Masarirambi et al., 2011). They have heterotrophic mode of nutrition as they lack chlorophyll. Mushroom is an organic vegetable. A high nutritional value of dried oyster mushrooms has been reported with protein (20-27%), fat (4-7%), carbohydrate (32-48%), fibre (18-24%) and ash (8-13%) (Afsary et al., 2013). In Bangladesh, mushroom is being cultivated about 40 years. Among all, oyster mushroom (*Pleurotus spp.*) is mainly cultivated throughout the country round the year. Oyster mushroom (*Pleurotus spp.*) is very popular edible mushroom due to its excellent flavor and taste.

Mushroom production and consumption are getting popularity among the Bangladeshi people. Its production is increasing day by day in Bangladesh as evident by almost four times higher mushroom is produced in 2018-19 compared to 2009-10 (Ferdousi et al., 2020). Though the mushroom production is increased in Bangladesh but could not meet the demand yet. Mushroom Development Institute (MDI) is the sole government institute for extension and research of mushroom throughout the country. MDI cultures different oyster mushroom species. Most of them provide better yield in winter season and some in summer. POP and PO10 perform better in summer season. PO2 strain is cultivated round the year.

There are two methods of mushroom culture, the spore culture and the tissue culture technique (Thapa et al., 2019). Tissue culture method is practiced in mushroom production in our country. In this method, vegetative part is used. Here, tissues from the joint point of stalk and pilea is collected and placed to a media. It is a clonal propagation. It maintains all the characters of mother plant and chance of variation is not occurred. The process is relatively easier. Spore culture is a method of producing mushroom from spore. The method is more technical. Here, Pilea collected from mushroom is placed to a petri plate. Spore falling from the lower portion of the pilea is collected and placed to the media. In spore culture, spore can be preserved for long days. By the continuous use of tissue culture method and repeated subculture through year to year, the PO2 strain is losing productivity (Sarker et al., 2014). If we consider it, spore culture may be an alternate option. In spore culture, variation in growth and yield can be expected. The present study was designed to evaluate the growth and yield performance of different oyster mushroom (*Pleurotus spp.*) species with different culture methods.

MATERIALS AND METHODS

A 2-factorial experiment in completely randomized design with 4 replications was conducted at Mushroom Development Institute, Savar, Dhaka during the period from

last week of April to June 2021. Two different culture methods viz. spore culture (C_1) and vegetative tissue culture (C_2) and four oyster mushroom species namely *Pleurotus ostreatus*, PO-2(S_1), *P. djmour*, POP-1 (S_2), *P. florida*, Flo-2 (S_3) and *Pleurotus ostreatus* PO-10 (S_4). The factors were tested in different combinations.

Preparation of pure Culture: Pure culture of above-mentioned variety (tested variety) were prepared on Potato Dextrose Agar (PDA) medium containing infusion of 250 g of peeled and sliced potato, 20 g of dextrose and 20 g of agar. The mixture was boiled on gas burner until the agar dissolved. The medium was poured into test tube (30 ml) at 10 ml/tube. The medium in test tube was sterilized in an autoclave for 20 minutes at 120°C under 1.1 kg/cm² pressure. After sterilization and solidification, the tubes were inoculated with the test materials. Pieces of inner tissues of joint of stalk and pileus were used as inoculum. A fresh and full grown sporophore of oyster mushroom was surface-sterilized with 70% ethanol by rubbing cotton soaked in alcohol. Tissues were collected from inner region of the sporophore. The tissues were cut into small pieces and placed on the solidified test tube containing PDA. For spore culture, pilea collected from mushroom is placed to a petri plate. Spore falling from the lower portion of the pilea is collected and placed to the media. After inoculation, the tubes were covered with cork. All operations were done under sterile condition in a clean bench. The inoculated tubes were transferred to a growth chamber maintaining temperature at 20-25°C and incubated for 8-15 days. Then pure culture was used for inoculation of mother culture.

Preparation of mother Culture: Mother culture was prepared by mixing sawdust and wheat bran at the ratio of 2:1. Calcium carbonate was used at the rate of 0.2% of the mixture. The moisture level of the mixture was maintained at 55% (using automatic moisture analyzer) by adding tap water. Polypropylene bags of 18 cm × 25 cm size were filled with 300 g of the above prepared mixture and packed tightly. After sterilization, the packets were cooled for 24 hours and transferred into a clean bench. A piece of pure culture medium containing mycelium of different oyster mushroom variety according to treatments were placed aseptically in the hole of mother culture packet and the packet was again plugged as mentioned before. Then the inoculated packets were placed on a wooden rack in the laboratory at 25±2°C temperature for incubation.

Preparation of Spawn Packet: The substrate of spawn packets was prepared using sawdust and wheat bran mixture at the ratio of 2:1 (dry weight/weight basis). Water was added to make the moisture content at 55% and CaCO₃ was added at the rate of 0.2% (w/w) of the total mixture to maintain the pH level at 6.5 to 7.0. Polypropylene bags of 18 cm x 25 cm were filled with 500 g of prepared substrate. The packets were tied, plugged with absorbent cotton and covered with brown paper. Then the packets were sterilized in an autoclave for 2 hour at 121°C under 1.1 kg/cm² pressure. After sterilization the packets were cooled and transferred to an inoculation chamber and inoculated with the mother culture of test materials at the rate of one teaspoonful per packet. The inoculated packets were placed on a still rack at 25 ± 2°C temperature for incubation.

Experimental Condition: The packets were kept in a dark room at 25°C for incubation. When colonization of mycelium was completed, the spawn packets were taken to a culture house and were opened by 'D' shaped cut on the shoulder and removed the sheet. The relative humidity and temperature of the culture house were maintained at 80-90% and 20-25°C, respectively by spraying water. Diffused light, about 200 lux and proper ventilation in culture house were maintained. After harvesting of mushroom, the residues were removed from the packet and temperature and relative humidity were maintained as before. The yield was obtained from double flush in the harvest period. Yield in g/packet was recorded by weighing all the fruiting bodies in a packet after removing the lower dirty portion. It was counted for two flushes. The biological efficiency was determined using the following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{Total biological yield (g / packet)}}{\text{Total dry weight of the substrate used (g / packet)}} \times 100$$

Nutritional analysis

The nutrient analysis work was done in The Association of Official Analytical Chemists (AOAC) method.

Determination of total protein: Determination of total protein: Five gram of grinded mushroom was taken with 50 ml of 0.1 N NaOH and boiled for 30 minutes. The solution was cooled in room temperature and centrifuged at 1000 rpm by a table centrifuge machine (DIGISYSTEM: DSC-200T; Taiwan). The supernatant was collected and total protein content was measured according to the Biuret method (Raghuramulu et al., 2003).

Determination of total ash: Ash content of fresh and solar dried mushroom was determined by using dry ash method AOAC (1995) method no. 942.05. Total ash content of mushrooms was determined by accurately weighing one gram of each sample into a crucible and placing the crucible on a clay pipe triangle and at first heating over a low flame till all the material was completely charred, and later by heating in a muffle furnace for about 6 hours at 600°C.

Determination of fiber: Fiber content of mushroom was determined by using AOAC method no. 962.03.

Determination of total lipid: Total lipid was determined by AOAC (1995) method.

Total carbohydrate estimation: The content of the available carbohydrate was determined by the following equation: Carbohydrate (g/100 g sample) $100 - [(\text{Moisture} + \text{Fat} \pm \text{Protein} + \text{Ash} + \text{Crude Fiber}) \text{ g/100 g}]$

Data Collection and Statistical Analysis: The experiment was laid out following completely randomized design (CRD) with 4 replications. Data on different attributes were recorded and analyzed using STATISTICS-10 computer program. Means separation were computed following Tukey HSD Comparisons Test using the same computer program.

RESULTS AND DISCUSSION

Performance of Different Oyster Mushroom Species

Performance of different mushroom species related to various growth, yield and nutrient attributes is shown in Table 1, Table 2 and Fig. 1, respectively. It was found that value of days required for mycelium completion in pure culture, mother culture, spawn culture, days required for primordial initiation, first harvest and total days required from pure culture to harvest ranges from 10.12 days to 11.62 days, 17.87 to 23.37 days, 21.12 to 27 days, 3.50 days to 14.37 days, 2.50 days to 3.37 days and 56 to 76 days, respectively (Table 1). The least time to complete mycelium in spawn culture was minimum (21.12) in S_2 which significantly differed with other species. The highest duration required (27days) to complete mycelium formation in spawn packet was in S_3 . Baysal et al. (2003) found that in oyster mushroom (*Pleurotus ostreatus*), the fastest spawn running (mycelia development) (15.80 days), pin head formation (21.4 days) and fruit body formation (25.60 days) were realized with the substrate composed of 20% rice husk in weight. The least time required (56 days) from pure culture to first harvest was recorded in S_2 .

Table 1. Growth performance of different oyster mushroom species

Species	Days required for pure culture	Days required for mother culture	Days required for Spawn culture	Days required for primordial initiation	Days required for first harvest	Total days required from pure culture to first harvest
S_1	10.62ab	22.25a	25.50b	14.37a	3.25ab	76.0a
S_2	10.12b	17.87b	21.12c	4.37b	2.50b	56.0c
S_3	11.50a	23.37a	27.0a	5.12b	2.87ab	69.87b
S_4	11.62a	22.50a	26.12ab	3.50c	3.37a	67.12b
CV (%)	7.42	5.39	4.14	8.07	18.18	3.07

In a column, means followed by a common letter are not significantly different at 5% level by Tukey HSD Comparisons Test

S_1 = PO-2, S_2 = Pop-1, S_3 = Flo-2, S_4 = PO-10

It was found that values of average number of fruiting body, length of stalk, diameter of stalk, length of pileus, diameter of pileus, thickness of pileus ranged from 11 to 20.75, 1.2 cm to 2.41 cm, 0.73 cm to 2.05 cm, 5.75 cm to 6.83 cm, 6.25 cm to 7.15 cm, 0.52 cm to 0.71 cm, respectively. Yield per packet in two flushes was found the highest (133.31g) in S_2 and the lowest (109.94 g) in S_3 and significantly differed with each other (Table 2). Biological yield was observed the minimum (48.86%) in S_3 and the maximum (59.25%) in S_2 . Kalita et al. (1997) observed similar result that biological efficiency for different substrates varies from 35.20 to 60.90%. Obodai et al. (2003) found biological efficiency (BE) followed a pattern and ranged from 61% to 80%.

Table 2. Yield performance of different oyster mushroom species

Culture	Number of fruiting body	Length of stalk (cm)	Diameter of stalk (cm)	Length of pileus (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per packet (g)	Biological Efficiency (%)
S ₁	17.12b	1.7c	1.68b	6.83a	7.15a	0.66b	123.75b	55.0b
S ₂	22.0a	1.2d	0.73c	5.75c	6.25c	0.71a	133.31a	59.25a
S ₃	11.0c	2.41a	1.65b	6.25b	6.8b	0.69a	109.94c	48.86c
S ₄	20.75a	2.05b	2.05a	6.15b	6.45c	0.52c	121.75b	54.11b
CV (%)	7.33	7.18	10.89	2.03	2.18	1.76	3.12	3.12

In a column, means followed by a common letter are not significantly different at 5% level by Tukey HSD Comparisons Test

S₁ = PO-2, S₂ = Pop-1, S₃ = FLO-2, S₄ = PO-10

Nutrient status of different oyster mushroom species is shown in Fig. 1. The protein percentage was found highest (24.56%) in S₁ which significantly differed with other species. The least protein percentage (21.68%) was observed in S₃ (Fig. 1). The data suggests the findings from Rostom M.A. (2009) that protein content varied from 11.4-31.3% (w/w) in the mushroom grown on sugarcane bagasse with different levels of wheat bran. The Maximum percentage (5.92%) of lipid was present in S₁ which was statistically non-significant to S₄ and significant with S₂ and S₃. Accordingly, fiber was maximum (20.72%) in S₃ which remains lowest (18.02%) in S₄. Ash percentage was highest (10.81%) in S₁ and carbohydrate (43.9%) in S₄.

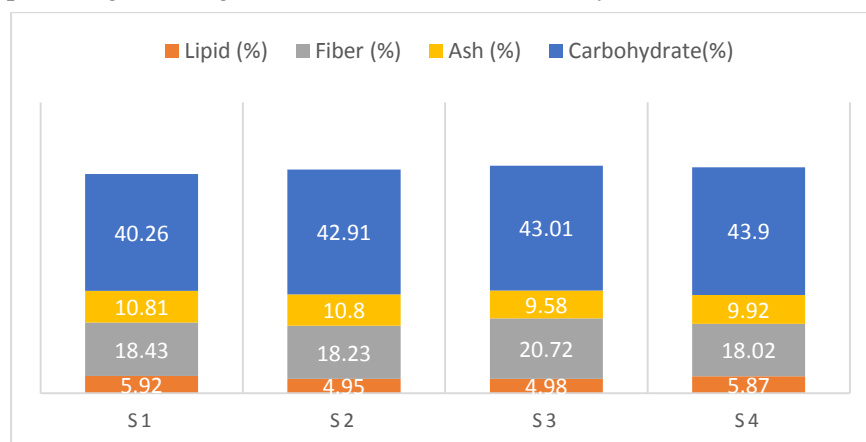


Figure 1. Nutrient status of different oyster mushroom species

Effect of Culture Methods

Effect of culture methods on different growth parameters is shown in Table 3. Ranges of days required for pure culture, days required for mother culture, days required for spawn culture, days required for primordial initiation, days required for

harvest and total days required from pure culture to harvest ranges 10.87 days to 11.06 days, 20.62 days to 22.37 days, 23.93 days to 25.93 days, 6.81 days to 6.87 days, 2.93 days to 3.06 days and 65.35 days to 69.12 days respectively (Table 3).

Table 3. Effect of culture methods on growth contributing characters

Species	Days required for pure Culture	Days for required for mother culture	Days required for Spawn culture	Days required for primordial initiation	Days required for first harvest	Total days required from pure culture to first harvest
C ₁	10.87a	20.62b	23.93b	6.87a	3.06a	65.35b
C ₂	11.06a	22.37a	25.93a	6.81a	2.93a	69.12a
CV (%)	7.42	5.39	4.14	8.07	18.18	3.07

In a column, means followed by a common letter are not significantly different at 5% level by Tukey HSD Comparisons Test

C₁= Spore culture and C₂= Vegetative Tissue culture

Effect of culture methods on different yield parameters is shown in Table 4. It was found that value of number of fruit body, length of stalk, diameter of stalk, length of pileus diameter of pileus, thickness of pileus, yield per packet and biological efficiency ranges from 17.37 cm to 18.06 cm 1.80 cm to 1.88 cm, 1.5 cm to 1.56 cm, 6.16 cm - 6.32cm, 6.55 cm to 677 cm, 0.64 cm to 0.65 cm, 119.91 g to 124.47 g and 53.92% to 55.31% respectively (Table 4).

Table 4. Effect of culture methods on yield attributes

Culture method	Number of fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Length of pileus (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per packet (g)	Biological Efficiency (%)
C ₁	18.06a	1.88a	1.50a	6.32a	6.77a	0.65a	124.47a	55.31a
C ₂	17.37a	1.80a	1.56a	6.16b	6.55b	0.64b	119.91b	53.92b
CV (%)	7.33	7.18	10.89	2.03	2.18	1.76	3.12	3.12

In a column, means followed by a common letter are not significantly different at 5% level by Tukey HSD Comparisons Test

C₁= Spore culture and C₂= Vegetative Tissue culture

Effect of culture methods on different nutrient parameters is shown in Fig. 2. The protein content was higher (23.2%) in C₁ which is significant to C₂. The rest data percentage containing lipid, fiber, ash and carbohydrate ranged from 5.23 to 5.48, 18.73 to 18.97, 10.16 to 10.39 and 41.97 to 43.06 which do not differ significantly with each other.

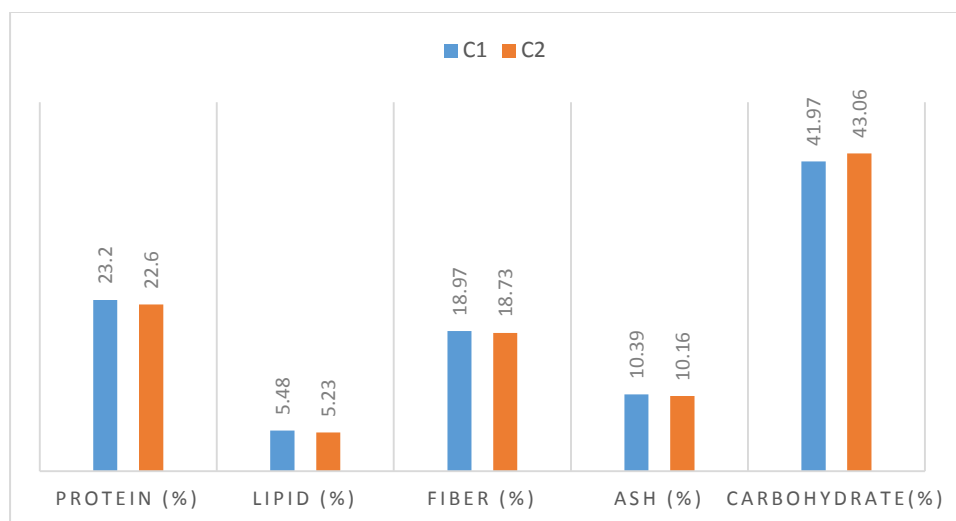


Figure 2. Effect of culture methods on nutrient components

Interaction effect of oyster mushroom species and culture methods

Effect on growth attributes: Combined effect of oyster mushroom species and culture methods on different growth attributes are presented in Table 5. which can be described as below:

Days required for pure culture completion: The maximum days (12) required from opening to first harvest was observed from the treatment combination of S_4C_2 . The minimum days (10) required from opening to first harvest were observed from S_2C_1 which was statistically dissimilar to S_4C_2 (Table 5).

Days required for mother culture completion: The minimum days (17.0) were required to complete mycelium in mother culture was observed from S_2C_1 . The maximum days (24.25) was observed from S_3C_1 which was statistically dissimilar to other treatment combinations.

Days required to complete spawn packet: The minimum days (20.25) required for completion of spawn packet was observed from the treatment combination of S_2C_1 which was statistically identical to S_2C_2 . The maximum days (28.5) required for completion of spawn packet was observed from S_3C_2 which was statistically dissimilar to other treatment combinations. The result is supported by Amin et al. (2002) who found that the different oyster mushroom species required 21.69 to 28.4 days to complete spawn packet.

Days required for primordial initiation: The highest days (14.5) required from cutting spawn packet to pinhead initiation was observed from the treatment combination S_1C_1 . The lowest days (3.5) were required from pure culture to first harvest was observed in S_4C_1 which was statistically identical to S_4C_2 . Moonmoon et

al. (2012) reported that days required to pinhead initiation for different oyster mushroom species was 3.75 days to 12.5 days.

Days required to first harvest: The minimum days (2.5) were required from primordial initiation to first harvest from the treatment combination of S₂C₁ which was statistically similar to all other treatments.

Total days required from pure culture to harvest: The least days (54.5) required from pure culture to first harvest was observed from the treatment combination S₂C₁ which was statistically non-significant to S₂C₂. The highest days (77.75) required from pure culture to first harvest was observed from S₁C₂, which was statistically dissimilar to other treatment combinations.

Table 5. Interaction effect of different oyster mushroom species and culture types on growth attributes

Treatment combination		Days required or pure culture	Day required for mother culture	Day required for spawn culture	Day required for primordial initiation	Day required for harvest	Total day required from pure culture to harvest
S ₁	C ₁	10.50ab	21.5ab	24.75b	14.50a	3.00a	74.25ab
	C ₂	10.75ab	23.0a	26.25ab	14.25a	3.50a	77.75a
S ₂	C ₁	10.25ab	17.00c	20.25c	4.50bc	2.50a	54.50e
	C ₂	10.00b	18.75bc	22.00c	4.50bc	2.50a	57.50e
S ₃	C ₁	11.50ab	22.50a	25.50b	5.00b	3.25a	67.75cd
	C ₂	11.50ab	24.25a	28.50a	5.25b	2.50a	72.00bc
S ₄	C ₁	11.25ab	21.50ab	25.25b	3.50c	3.50a	65.00d
	C ₂	12a	23.5a	27.00ab	3.50c	3.25a	69.25cd
CV (%)		7.42	5.39	4.14	8.07	18.18	3.07

In a column, means followed by a common letter are not significantly different at 5% level by Tukey HSD Comparisons Test

S₁ = PO-2, S₂ = Pop-1, S₃ = FLO-2, S₄ = PO-10, C₁ = Spore culture and C₂ = Vegetative Tissue culture

Number of fruiting body: The number of fruiting body under different treatment combinations varied significantly (Table 6). The highest number (23.0) of fruiting body was observed from the treatment combination S₂C₁. The lowest number (10.0) of fruit body was observed in S₃C₁. The findings is supported by Moonmoon et al. (2013) reported that number of fruiting body ranged from 7. to 32.50 in PO-2, Pop-1 and Flo-2.

Length and diameter of stalk and pilea: The length of stalk ranged from 1.20 cm to 2.52 cm with significant difference (Table 6). The highest length of stalk (2.52 cm) was found in S₃C₁. The lowest length of stalk was found from the treatment combination S₂C₁ (1.20) and S₂C₂. The diameter of stalk differed significantly and

ranged from 0.7 to 2.1 cm (Table 6). The highest length of pilea (7 cm) was found in S₁C₁ while it was the lowest (5.7 cm) in S₂C₂. The thickness of pileus in different treatment combinations differed significantly and ranged from 0.52 cm to 0.72 cm (Table 6). The highest thickness (0.72 cm) was found in S₂C₁ which was not statistically identical to other treatment combinations. The lowest thickness (0.52) was found in S₄C₂.

Yield per Packet (g): Significant variation was observed in yield under different treatment combinations (Table 6). The highest yield (137.25 g) was found in S₂C₁ which statistically differed to other treatments. The lowest yield (108.12) was found in S₃C₁. Alam et al. (2007) found that the biological yield of oyster mushroom ranged from 120.6 g per packet to 221.8 g per packet in three flushes.

Biological efficiency (%): Significant variation was observed on biological efficiency (BE) (Table 6). The highest biological efficiency (61%) was found in S₂C₁ and the lowest biological efficiency (48.05%) was found in S₃C₁.

Table 6. Interaction effect of different oyster mushroom species and culture types on yield attributes

Treatment combination	Number of fruit body	Length of stalk (cm)	Diameter of stalk (cm)	Length of pileus (cm)	Diameter of pileus (cm)	Thickness of pileus (cm)	Yield per packet (g)	Biological efficiency (%)	
S ₁	c ₁	18bc	1.8cd	1.6c	7a	7.3a	0.67cd	128.25abc	57abc
	c ₂	16.25c	1.6d	1.77abc	6.675b	7ab	0.65d	119.25cd	53cd
S ₂	c ₁	23a	1.2e	0.70d	5.8d	6.3c	0.72a	137.25a	61a
	c ₂	21ab	1.2e	0.77d	5.7d	6.2c	0.7ab	129.38ab	57.5ab
S ₃	c ₁	10d	2.52a	1.6c	6.2c	6.8b	0.7ab	108.12e	48.05e
	c ₂	12d	2.3ab	1.7bc	6.3c	6.8b	0.69bc	111.75de	49.66de
S ₄	c ₁	21.25a	2bc	2.1a	6.3c	6.7b	0.53e	124.25bc	55.22bc
	c ₂	20.25b	2.1bc	2ab	6cd	6.2c	0.52e	119.25cd	53cd
CV (%)	7.33	7.18	10.89	2.03	2.18	1.76	3.12	3.12	

In a column, means followed by a common letter are not significantly different at 5% level by Tukey HSD Comparisons Test

S₁ = PO-2, S₂ = Pop-1, S₃ = Flo-2, S₄ = PO-10, C₁ = Spore culture and C₂ = Vegetative tissue culture

Interaction effect of different oyster mushroom species and culture types on nutrient status

Protein percentage was found maximum (24.82%) in S₁C₁ which was statistically significant to other treatments except S₁C₂ and minimum protein percentage (21.52%) was found in S₃C₂. The Lowest lipid (4.70%) was found in S₃C₂ and the highest

(6.07%) in S₁C₂. Fiber content was the highest (21.1%) in S₃C₁, the lowest (17.87%) in S₄C₁ (Fig. 3). Similarly, the least ash and carbohydrate (9.37% and 40.07%) was present in S₄C₂ and S₁C₂ respectively whereas the highest percentage (11.17% and 44.47%) was found in S₁C₂ and S₄C₂, respectively. Alam et al. (2007) found that, the protein, lipid, fiber and carbohydrate content in 100 g of dried *P. sajor-caju* were found as 23-26 g, 4.2-4.6 g, 22.-23.6g and 37-41.5 g respectively. 100 g of dried *P. florida* contained 19-22 g of proteins, 4-4.6 g of lipids, 22-24.6 g of fiber and 40-45 g of carbohydrates.

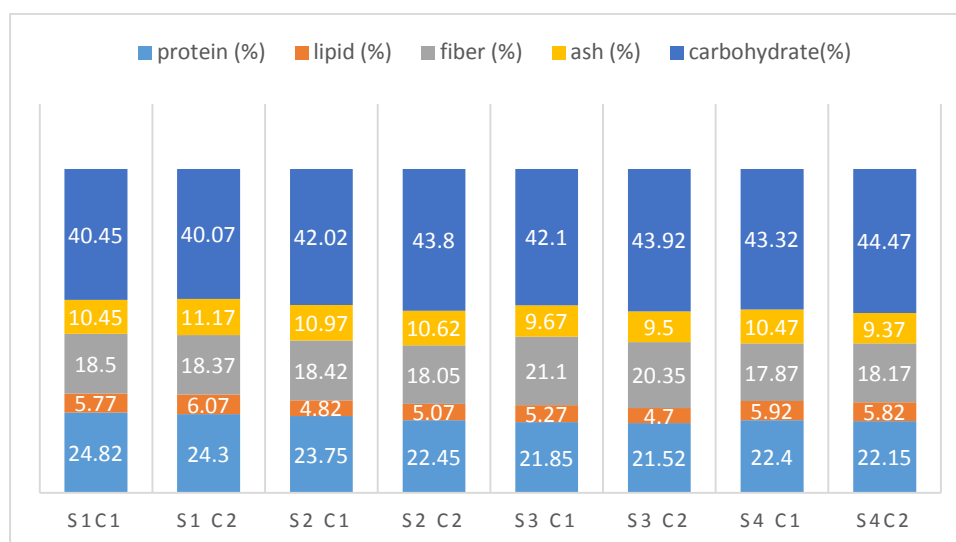


Figure 3. Nutrient status of combination of different oyster mushroom species and culture types

CONCLUSION

From the study it may be concluded that among the species, Pop-1 (S₂) performed better result in relation to time and yield context. In case of culture method, spore culture (C₁) required less time for production and provided higher yield. Within all treatment combinations, S₂C₁ required minimum duration and gained the highest yield. The highest protein was found from PO-2 (S₁) species and from spore culture (C₁). No significant variation was found between two culture methods in relation to nutrient status.

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