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Evaluation of different substrates for growth, yield and nutritive value of *Hypsizygus ulmarius* (Blue Oyster Mushroom)

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Abstract
An experiment was carried out to investigate the cultivation of oyster mushroom (*Hypsizygus ulmarius)* on different substrates (wheat straw, paddy straw, card board, wheat straw + paddy straw, card board + wheat straw, paddy straw + wheat straw + banana leaves + card board). The objective of this study was to evaluate the best alternative substrate that support the growth of oyster mushroom, produces the maximum yield with highest biological efficiency and nutritional content. Total six treatments replicated eight times were taken under the complete randomized design. The minimum time taken for spawn run (14.50 days), pinhead formation (18.37 days) and fruting body formation (23.12 days) was recorded in T_0 wheat straw (control). Maximum average pileus width (7.30 cm) T_3 paddy straw + wheat straw and length (8.67 cm) T_0 wheat straw (control) and stipe size (7.05 cm) T_2 card board. Maximum number of fruiting bodies (179.87), maximum yield (1027.36 g) and biological efficiency (155.60 %) were recorded in T_3 (paddy straw + wheat straw).Maximum protein content was recorded in T_1 paddy straw (27 %). Where as maximum carbohydrate content was recorded in T_0 wheat straw (40 %).

Keywords: Oyster mushroom, substrates, biological efficiency, yield, nutritional analysis

Introduction
Mushrooms are macroscopic, spore- bearing fruiting bodies of fungi. They have been valued throughout the world for thousands of years both as food and as medicine. They contain reasonable amount of protein, minerals, vitamins and various novel compounds of medicinal value. Many studies revealed that mushrooms possess biopharmaceutical compounds that can be used for therapeutic applications. They have antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, hepato-protective, anti-diabetic, and hypotensive properties (Nandakumar, 2013) [11]. According to Shivashankar and Premkumari (2014) [17] out of the 10,000 known species of mushrooms, 2000 are safe for humans and about 300 of them possess medicinal properties. The mushrooms comprise a large heterogeneous group having various shapes, sizes and colours, all quite different in character, appearance and edibility. Of these large groups with more than 2000 edible species, about 300 species belonging to 70 genera are reported from India (Karthika and Murugesan, 2015) [7].
Blue oyster mushroom (*Hypsizygus ulmarius*) is one of the important edible mushroom in the world and it is a popular fungus cultivated in Japan, China and other Asian countries (Chang, 1999).
Hypsizygus ulmarius (elm oyster mushroom) is a high yielding mushroom for which commercial cultivation technology has been released and is gaining popularity, it is widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane *et al.*, 2007) [8].
Hypsizygus ulmarius species are popular and widely cultivated throughout the world mostly in Asia and Europe owing to their simple and low cost production technology and higher biological efficiency (Mane *et al*., 2007) [8]. The cultivation of edible mushrooms has become an attractive economic alternative over past few years, mainly due to increase in its demand and market value (Chang, 2006) [3].
Hypsizygus ulmarius (Bull.:Fr.) Redhead, commonly called as 'elm oyster' or 'blue oyster' is similar to oyster mushroom, but differ in morphology and biological efficiency. "*Hypsi*" means "high" or "on high" and "*zygus*" means "yoke", *Hypsizygus*, then referring to position of this mushroom often high in the tree. Ulm- refers to "elm" indicating one of the common substrates for this fungus. They often grow in clusters on living elm trees or elm logs in forests. It is a novel species with very large fruiting body, blue coloured pinheads becoming light white on maturity, high yielder, palatable with meaty flavour and attractive keeping quality. This mushroom variety has attractive shape and is fleshy with excellent taste.

Despite these attractive qualities, its production in tropical climate has not yet been fully explored. Mushroom cultivation recycles agro-residues, much of which is otherwise burnt in the field. Since our country has abundant agricultural residues, we can emerge as a major player in mushroom production. In the present agricultural scenario, secondary agriculture is going to play a pivotal role and mushroom fits very well in this category. Mushroom being an indoor crop, utilizes vertical space and requires only 25-30 litre water for production of one kg mushroom, thus offering a solution to shrinking agricultural land and water. The annual world production of button mushroom has reached 6.5 million tonnes and that of all types of mushrooms is estimated to be over 25 million tonnes. Our country has registered twenty-fold increase in production of mushrooms in the last four decades and still our production is only 1.2 lakh tonnes of which, button mushroom continues to occupy a prominent place and contributes about 80 % of the total mushroom production of our country (DMR, 2013) [4].

Materials and Methods

The present study entitled "Evaluation of different substrates for growth, yield and nutritive value of *Hypsizygus ulmarius* (Blue Oyster Mushroom)*"* was carried out to find out best substrate for the cultivation of *Hypsizygus ulmarius*. A brief description on location of experiment, experimental material, experiment and treatments, preparation of substrates, preparation of packets, collection of produced mushroom and data recording and their analysis and experimental procedures followed and techniques employed during the course of present investigation has been given in this chapter.

The experiment was carried out at the laboratory and Mushroom Crop Room, Department of Plant Pathology, and Department Biochemistry and Biochemical Engineering of Sam Higginbottom University of Agriculture, Technology, and Sciences, Allahabad, 211007, U.P., India during the period November 2017 to Febuary 2018.

In this experiment six treatments (different substrates) including control were applied and each treatment was replicated eight times to achived the desired objectives.

During the experiment Mushroom Crop Room was cleaned and white washed and sterilized with 0.2% formaldehyde spray and fumigation also for mushroom house and room was closed for three day.

Six substrates (wheat straw, Paddy straw, Cardboard, paddy straw +wheat straw, Cardboard+wheat straw, Wheat straw+ Banana leaves + Paddy straw + Card board) were collected from the different localites of Allahabad for the cultivation of oyster mushroom. Since these substrates were easly available, they were sun dried and broken into small pieces (2-3 cm). Fiftylitres of tap water was filled in a plastic drum of 100 litre capacity. Formaldehyde (50 ml) and carbendazim (7.5g) were mixedin 50 liter of water Vijay and Sohi (1987) [19]. This solution was stirred properly with a stick. Now 5 kg dry substrate was steeped completely in this chemical solution. The mouth of the plastic drum was closed with the lid and kept as such for 18 hours. After sterilization the excess water was drained and the substrates was spread out as thin layer on sterilized plastic sheet spread on cemented floor in a shaded area. The straw was left for 2-3 hours to get moisture capacity (60-65%). The 60% moisture content in the straw was judged by taking a handful of straw and squeezing tightly. The water should not drip out and the palm should feel the wetness of the straw (this is appropriate condition for spawning).

Polythene bag technology was used in this experiment. Polythene bags (35 x 40 cm) were used for cultivation. Spawning was done at the rate of 2% of wet substrates. Before filing into the bags the substrates were mixed thoroughly with broadcasting spawn was added and mouth of the spawned bag was tied with the help of nylon string. For perforation, 8-10 holes were made in each bag with the help of nail to allow free passage of air with in the bags. Eight replications for each treatment were maintained. These bages after inoculation were then incubated for spawn running in a mushroom house under darkness at ambient temperature. The spawned bages were kept at distance of (20-25 cm) in the mushroom crop room until the mycelium fully colonized the substrate. Temperature at (20-25℃) and humidity at (70-85 %) was maintained by spraying water twice a day on wall and floor. It take12-20 days when bags were fully covered with mycelium (spawn run).

When bags was completely covered by mushroom mycelium (spawn run), the bags was cut and polythene bags was removed. The compact mass of aggregated substrates termed as "bed" will be ready for hanging to the racks. At the same time, a small amount of light was provided inside the crop room. Fruiting requires an appropriate temperature range (20-25℃), ventilation, light moisture and humidity in these conditions was ensured in the mushroom crop room. The substracts was sprayed water 3-4 time per day. After 2-5 days of remove of poly polythene bags small pin heads appeared on all sides of bages.

The matured fruiting body was harvested by hand pick of clock wise or anti clock wise rotation before spraying of water. The harvested fruiting body was weighed and recorded all data substrates wise individually the same procedure was followed up to 2nd and 3rd harvesting.

Days for completion of spawn running (Average)

Data required for spawn running was recorded in days 100% spawn running on different substrates.

Days for pinheads formation (Average)

After the completion of spawn running the pinheads appearance of *Hypsizygus ulmarius* was observed. The data was recorded in days taken from spawning to the appearance of pinheads in each substrates.

Days for fruiting body formation (Average)

When the pinheads reached to maximum size then time period was recorded in days from preparation of bags to maturation of pinheads in all treatments.

Number of mature fruiting (Average)

Number of well-developed fruiting body was recorded. Dry and pinheaded fruiting bodies was discarded but tiny fruiting bodies was included in counting.

Size of Pileus (cm)

When the mature fruiting reached maximum size then length (cm) and width (cm) was recorded.

Size of Stipe (cm)

When the mature fruiting reached maximum size then length (cm) was recorded

Total yield (g)

The total yields of basidiocarp was measured for each treatment. The accumulations of three flushes was recorded as the total mushroom yield.

Biological efficiency

The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) of oyster mushroom was determined by the following formula. (Chang *et al.*, 1991)

$$\text{Biological efficiency } \% = \frac{\text{Weight of fresh mushroom fruiting bodies}}{\text{Weight of dry substrate}} \times 100$$

Estimation of Protein (%)

Protein analysis of *Hypsizygus ulmarius* using (Lowry 1951) method

Procedure

Protein standard stock weight – weight accurately 50 mg of Bovin Serum Albumin (BSA) and dissolve in distilled water to 50 ml in standard flask. Working standard – dissolve 10 ml of stock solution in 50 ml sterilized distilled water in flask. Weight 500 mg of dry mushroom sample and grind with moter pestle in 5-10 ml of the distilled water Centriguge in 5000 rpm for 30 minutes. Pipette out 0.2, 0.4, 0.6, 0.8 and 1ml of working standard into series of test tube. Pipette out 0.1 ml and 0.2 ml of sample of mushroom extracted on 2 other tubes. Make the volume up to 1 ml in all test tube. A test tube with 1 ml of distilled water served as blank. Add 5 ml of reagent C to each test tube including the blank. Mix well and allow to stand for 30 minute. Then add 0.5 ml of FC reagent, mix well and incubate at room temperature in dark for 30 minute Take the optical density reading in spectrophotometer at 660 nm wave length.

Estimation of Carbohydrate (%)

Carbohydrate analysis of *Hypsizygus ulmarius* using (Anthrone 1951) method

Procedure

To take 0.2 to 1ml of working standard solution of five different test tube add water to bring the volume to 1ml in each test tube add 4ml of anthrone reagent mix the contents as well and cover the test tube with bath for 10 min then cool the test tube to the room temperature measure the optical density in a photoelectric colorimeter at 620nm (or) by using a red filter. Simultaneously prepare a blank with 1ml of distilled water and 4ml of anthrone reagent. Construct a calibration curve on a graph paper, by plotting the glucose concentration (10 to 100mg) on x-axis and absorbance at 620nm on the y-axis. Compute the concentration of the sugar in the sample from the calibration curve. While calculating the sugar concentration in the unknown sample, the dilution factor has to be taken into account.

Results and Discussion

The result of present study entitled, "Evaluation of different substrates for growth, yield and nutritive value of *Hypsizygus ulmarius* (Blue Oyster Mushroom)*"*conducted at the laboratory and Mushroom Crop Room, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, 211007, U.P., India during the session (2017-2018).The observation were recorded at different stages of crop growth, days for completion of spawn run, days for pinheads formation, days for fruiting body formation, number of mature fruiting, size of stipe (cm) size of pileus (cm), total yield (g), biological efficiency, protein (%) and, carbohydrate (%).

Spawn run (days) in *Hypsizygus ulmarius* as affected by treatments

The days required for completion of spawn run of *Hypsizygus ulmarius* The minimum spawn run time was recorded in T_4 wheat straw + card board (14.87 days), T_3paddy straw +wheat straw(18.75 days), T_1 paddy straw (19.00 days), T_5 banana leaves + wheat straw+paddystraw+card board (22.50 days), T_2 card board (25.37 days),as compareT_0 wheat straw (14.50 days) to respectively. The probable reason for different in days for full mycelial running on different substrates may be due to variation in their chemical composition and C: N ratio as reported by Batti *et al.* (1987) [2]. The results of present study corbonate with the study of Maggo *et al.* (2014) [9]. They reported that completion of spawn run in wheat straw took 13 days and in paddy straw 15 days. While Shah *et al.* (2004) reported that spawn run in wheat straw was completed in 15 days. Sidhant *et al*., (2014) [14] also reporded that time taken for complete mycelium run ranged from 13 – 18 days.

Pinheads formation (days) in *Hypsizygus ulmarius* as affected by treatments

The days required for pinheads formation of *Hypsizygus ulmarius* The minimum days required for pinheads formation was recorded in followed by T_4 wheat straw + card board (19.75 days), T_3 paddy straw+wheat straw (23.37 days), T_1 paddy straw (24.00 days), T_5 banana leaves + wheat straw+paddy straw+card board (27.50 days), T_2 card board (35.12 days) as compare toT_0 wheat straw (18.37) days, respectively.

The pin head formation was observed following the invasion of substrates by mycelia growth. The pinheads appeared fasest in wheat straw (control) (18.37 days) than other substrates. Where as maximum days taken for appearance of pinhead were in card board (31.12 days). The variation in number of days for pinhead formation may be the lignocellulosic material. The result of present study is similar to results of Sahu *et al*. (2014) [13] who reported that time taken for pinhead formation ranged from 15-24 days. The similar findings have been reported by Hasan *et al.* (2015) [5]. Theyreported that time taken for pinhead formation ranged from 15-24 days which was in accordance to the present result. Kumar *et al.*, (2017). Who reported that *Pleurotussajorcaju* took 20-30 days for pinhead formation but in this present study, it ranged from 15-23 days.

Fruiting body formation (days) in *Hypsizygus ulmarius* as affected by treatments

The days required for fruiting body formation of *Hypsizygus ulmarius* The minimum days was recorded in followed by T_4 card board + wheat straw (23.25 days), T_3 wheat straw + paddy straw (28.50 days), T_1 paddy straw (28.87 days), T_5 banana leaves + wheat straw+ paddy straw+ card board (32.50 days), T_2 card board (36.50 days), as compare to T_0 wheat straw (23.12 days) respectively.

The minimum time taken from spawning to fruiting body formation in *Hypsizygus ulmarius* was recorded in T_0wheat straw (control) (23.12 days) whereas maximum time taken was recorded in T_2 card board (36.50 days). The probable reasons for such findings might be due to higher content of nitrogen in wheat straw causing early fruiting bodies. The result of present study is similar to result reported by Shah *et al.*, (2014) [13] they reported that time taken for fruiting body formation of oyster mushroom ranged from 20-30days. Ashraf *et al.*, (2013) [1] reported that time taken from spawning to fruiting body formation ranged from 20-27 days whereas Mondal *et al*., (2010) [10] reported that time taken for fruiting bodies formation ranged from 25-35 days.

Size of pileus (cm) of *Hypsizygus ulmarius* as affected by treatments lenght (cm)

The width of pileus (cm) of *Hypsizygus ulmarius* The minimum average pileus length was observed in the T_3 paddy straw (7.56 cm) followed by T_5 wheat straw + paddy straw+banana leaves+card board (7.66 cm), T_4 wheat straw+ card board (7.96 cm), T_2 card board (8.12 cm), T_1 paddy straw (8.39 cm), as compare to T_0 wheat straw (8.67), respectively.

There is difference in size of pileus on different substrates. Substrates rich in usable nitrogen after spawn run may be a factor in enhancing the mushroom size and quality, in addition to mushroom species in bioconversion and bioaccumulation efficiency. Pileus maximum length T_0 wheat straw (8.67 cm) and minimum pileus length T_3paddy straw + wheat straw (7.56 cm).

Size of pileus (cm) in *Hypsizygus ulmarius* affected by different treatments

Width (cm)

The width of pileus (cm) of *H.ulmarius* on The minimum average pileuswidth was observed in the followed by T_2card board (7.16 cm) T_4 card board + wheat straw (7.17 cm) T_1paddy straw (7.19 cm), T_5 banana leaves + paddy straw+wheat straw+card board (7.28 cm),, T_3 wheat straw + paddy straw (7.30 cm), as compare to T_0 wheat straw (7.13 cm) respectively.

There is difference in size of pileus on different substrates. Substrates rich in usable nitrogen after spawn run may be a factor in enhancing the mushroom size and quality, in addition to mushroom species in bioconversion and bioaccumulation efficiency. The maximum average pileus width was recorded in T_3wheat straw+ paddy straw (7.30 cm) and minimum pileus width T_0wheat straw

Size of stipe (cm) in *Hypsizygus ulmarius* as affected by different treatments

The stipe size of sporocarp (cm) of *Hypsizygus ulmarius* the minimum stipe length was observed on followed by T_5 paddy +wheat+banana leaves +card board (6.55 cm)T_3 paddy straw + wheat straw (6.67 cm) T_4 card board+ wheat straw (6.76 cm) T_1 paddy (6.92 cm) and T_2 card board (7.05 cm) as compare to T_0 wheat straw (6.42 cm) respectively.

There is difference in size of pileus on different substrates. Substrates rich in usable nitrogen after spawn run may be a factor in enhancing the mushroom size and quality, in addition to mushroom species in bioconversion and bioaccumulation efficiency. Stipe maximum length T_2 card board (7.05 cm) and minimum average stipe legth T_0 wheat straw (6.42 cm).

Mature fruiting bodies in *Hypsizygus ulmarius* as affected by treatments

The total number of mature fruiting bodies in three fleshes of *Hypsizygus ulmarius* The minimum number of fruiting bodies were recorded in T_2 card board (115.41)T_4 card board + wheat straw (132.65) T5 banana leaves + paddy straw+wheat straw+card board (154.82), T_1 paddy straw (169.62), T_3 wheat straw + paddy straw (179.87) by, as compare toT_0 wheat straw (173.52), respectively.

The difference in number of fruiting bodies on different substrates may be due to environmental factors, physiological requirements, controlled, semi – controlled condition e.g. constant humidity, light, temperature etc. The results of present study is similar to result of Supta *et al.*, (2017) [18] who reported that number of fruiting bodies of *Pleurotus Florida* were in the range of 100- 160.

Yield (g) in *Hypsizygus ulmarius* as affected by treatment

The data regarding total yield in three flushes of *Hypsizygus ulmarius* on The minimum yield (g) was recorded in followed by.T_2 card board (567.62 g) T_4 card board + wheat straw (697.37 g), T5wheat straw+banana laeves + paddy straw+card board (751.36 g), T_1 paddy straw (917.50 g), T_3 wheat straw + paddy straw (1027.36 g) as compare to T_0 wheat straw (932.87 g), respectively

The probable reasons may be that. Mushrooms get nutrition from cellulose, hemicellulose and lignin, which are abundantly available in cereal straws. The results of present study are similar to results of Patil (2012) [12]. They recorded highest yield in wheat straw + paddy straw (846.33g) and lowest yield in soybean straw + arharstraw (716g). Iqbal *et al.*, (2005) [6] reported that yield of oyster mushroom was maximum in paddy straw (922.76 g) and minimum yield reported of oyster mushroom was in maize straw (677.00 g).

Biological efficiency (%) in *Hypsizygus ulmarius* as affected by treatments

The biological efficiency calculated on total yield in three flushes of *Hypsizygus ulmarius* The maximum biological efficiency was recorded in T_3 wheat straw + paddy straw (155.60 %) followed by T_4 card board+ wheat straw (136.55 %), T_1 paddy straw (128.84 %), T_5 banana leaves + wheat straw+paddy straw+card board (116.73 %), T_2 card board(100.82 %), as compare to T_0 wheat straw (136.88 %), respectively.

The maximum biological efficiency was recorded in T_3 wheat straw + paddy straw (155.60 %) and minimum biological efficiency was recorded in T_2 card board (100.82 %). The variation in biological efficiency may be due to fungal species, spawn strain and spawn rate in substrate. The results of present study is similar to the finding of Iqbal *et al*. (2016) who reported that biological efficiency of wheat straw was 136% and Shah *et al*., (2014) [13] who reported that biological efficiency of *Pleurotus Ostreatus* (90 -135 %).

Carbohydrate percentage in *Hypsizygus ulmarius* as affected by treatments

The present study carbohydrate percentage in *Hypsizygus ulmarius* onthe minimum carbohydrate percentage was recorded in T_2 card board (31.00%), T_4 wheat straw +card board (32.00%) T_3 paddy straw +wheat straw (34.00%) T_5 paddy+ wheat+bananaleaves+cardboard (35%) T_1 paddy straw (36%) as compare to T_0 wheat straw (39 %) respectively.

Ashraf *et al*., (2013) [1] reported that carbohydrate content in oyster mushroom in range 34-38% and protein content in range of 24-28% which is similar to present study. Sharma *et al*., (2013) [16] reported that carbohydrate content in *Pleurotus Ostreatus* was 30-40% and protein content was 22-27%. Maximum carbohydrate content T_0 wheat straw (39%) was recorded. and minimum carbohydrate content (31%) T_2 card board was recorded.

Protein percentage in *Hypsizygus ulmarius* as affected by treatments

The present study protein percentage in *Hypsizygus ulmarius* onthe minimum protein percentage was recorded in T_2 card board (20.00%), T_4 wheat straw +card board (21.00%) T_5 paddy straw +wheat straw+ banana leaves +card board (22.00%) T_3 paddy straw + wheat straw (23%) T_1 paddy straw (27 %) as compare toT_0 wheat straw (24 %) respectively.

Ashraf *et al*., (2013) [1] reported that carbohydrate content in oyster mushroom in range 34-38% and protein content in

range of 24-28% which is similar to present study. Sharma *et al*., (2013) [16] reported that carbohydrate content in *Pleurotus Ostreatus* was 30-40% and protein content was 22-27%. The maximum protein content was observed in T_1 paddy straw (27.00%) While minimum protein content (20%) T_2card board.

Summary and Conclusion

The fruiting bodies of *Hypsizygus ulmarius* were collected from the beds maintained in the Mushroom Crop Room Department of Plant Pathology.

Studies of the fruiting bodies showed that sporocarps were medium to large in size, produced either singly or as a bunch. The pileus was fleshy, dark blue coloured in pinhead stage became creamy white on maturity having non decuurent gills with creamy white, solid, eccentric, cylindrical stipe.

Cultivation studies were conducted with substrates like paddy straw, wheat straw, card board, paddy straw+wheat straw, card board + wheat straw, paddy straw+wheat straw+banana straw+card board, to evaluate the best medium for mushroom production of *Hypsizygus ulmarius*. The minimum time for spawn run (14.50 days) and first harvest (23.12 days) was recorded for wheat straw and maximum for card board. Biological efficiency of 155.60 % was noted for paddy straw + wheat straw followed by card board (100.82%). Very low yield was recorded for card board (567.62 g) followed by paddy straw+wheat straw (1027.36g).

Hypsizygus ulmarius took an average of five days for harvesting from pinhead formation. Dimension of pileus and weight of the sporocarp increased gradually and reached maximum at the day of harvest. The stipe length increased uptothird day and thereafter decreased.

Conclusion

Minimum time for spawn run, pin head, and fruiting body formation Wheat straw (50%) + Card board (50%) substrates recorded. Maximum number of pileus length wheat straw (100%) pileus width wheat straw (100%) and stipe size card board (100%). Maximum number of yield (g /2 kg wet substrates) and biological efficiency (%) paddy straw + wheat straw (50%+50%) in *Hypsizygus ulmarius.* Maximum protein (%) paddy straw (100%) and carbohydrate (%) wheat straw (100%) in *Hypsizygus ulmarius* the present findings are of crop period (December 2017 to February 2018) under Allahabad condition as such to validate the findings more such trials should be carried out in future.

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