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Growth Efficiency of Elm Oyster Mushroom (*Hypsizygus ulmarius*) Using Plant - Based Waste Substrates

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ABSTRACT

Mushrooms are nutritionally important organisms that grow well on agricultural plant - based waste substrates. Mushroom cultivation technique is a profitable agribusiness at a small scale. It contains different polysaccharide compounds like cellulose, lignin and hemicelluloses which could be degraded by extracellular enzymes produced by mushroom fungi. Such edible mushrooms have high nutritive values that include proteins, amino acids, carbohydrates, fats, lipids, vitamins and minerals. In the current research, the Elm (*Hypsizygus ulmarius*) mushrooms are cultivated using three different substrates: Paddy straw, corn husk, and a combination of corn husk and paddy straw under aseptic conditions. The spawn running, pin head formation and basidiocarp sprouting time period was faster (14 days) in combined substrates of corn husk and paddy straw compared to the individual substrates. The fruiting body size was larger in corn husk (cap diameter- 7.9 cm, cap length – 5.83 cm) and corn husk + paddy straw substrate (cap diameter- 6.96 cm, cap length – 5.53 cm) than in paddy straw substrate (cap diameter- 6.63 cm, cap length – 4.06 cm). The nutrient composition of the harvested basidiocarps of mushroom from the different substrates had a higher moisture content (69.86 ± 0.41 %) and maximum ash (13.06 ± 0.75 %) content in *Hypsizygus ulmarius* from corn husk + paddy straw substrate. Among these, the protein rich (44.71 ± 0.28 % and 37.88 ± 0.45 %) mushrooms were cultivated using corn husk + paddy straw and corn husk substrate which contained low levels of carbohydrates and optimum levels of fats and lipid content. Thus, corn Husk + paddy straw combination substrate and corn husk substrate were more efficient and suitable for commercial cultivation of *Hypsizygus ulmarius* than the paddy straw substrate.

Hypsizygus ulmarius produces large size basidiocarp and a higher yield than *Pleurotus* species. It is easy to cultivate it with high amount of yield. This commercial edible mushroom production technology is installed in different areas of our environment to enhance and balance the food scarcity in our state to overcome malnutrition. The present study suggested that a combination of corn husk and paddy straw could be used as a substrate for the production of nutritionally efficient mushrooms with a high yield. It would be applicable in various integrated mushroom farming along with agriculture which can lead to an increase in the Indian economy at a certain level.

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1. Introduction

Mushrooms are saprophytic macro fungi with large fruiting bodies. This fungi belongs to both class basidiomycetes and ascomycetes. Mushrooms have been consumed as food since ancient times. In 1991, it was reported that commercial cultivation of mushrooms that belonged to both Ascomycotina and Basidiomycotina families, was about 4.27 million metric tons (Miles and Chang, 1997). About 230 genera and 5,000 species of mushrooms are present. Among these more than 2000 species are edible mushrooms present throughout the world and about 283 species are present in India (Manimaran *et al.,* 2017). Out of the 10,000 known species of mushrooms, 2,000 are safe for humans and about 300 of them possess medicinal properties (Shivashankar and Premkumari, 2014).

Edible mushrooms are highly delicious due its flavor, quality, nutrient value, productivity, and better than other source of plant protein. This has been identified as first-rate food stuff to solve the problem of malnutrition in evolving countries (Eswaran and Ramabadran, 2000). They have been used as food and medicine in India in ancient medical treatise since 3000 BC (Natarajan, 1995). It has medicinal importance as it possess anticancer, antidiabetic, and anti cholesterolenic properties and has an ability to maintain the blood cholesterol at the optimum level preventing cardiovascular diseases (Rai et al., 2005).

The Oyster mushroom is an edible mushroom having good flavour and taste. It belongs to Basidiomycetes-class, Agaricales order and Agaricaceae family. Since mushroom cultivation is a profitable agribusiness that can easily grow as wild in the forests of hilly areas and can be cultivated commercially in temperate and subtropical regions of the world (Shah et al., 2004).

The Oyster mushroom is an efficient lignin-degrading mushroom and can grow well on different types of lingo cellulosic materials. Oyster mushrooms can be grown on various substrate including paddy straw, wheat straw, maize stalks/cobs, vegetable plant residues, bagasse etc. which contain lignocellulose content (Kumar et al., 2019).

The most cultivated edible mushroom is *Lentinus edodes* (about 22 % of the world supply). About 85 % of world's total supply of cultivated edible mushroom is from five genera *Lentinula, Pleurotus, Auricularia, Agaricus,* and *Flammulina* (Royse et al., 2017). *Hypsizygus ulmarius (Bull.),* commonly called as "Elm oyster‟ or "Blue oyster‟ is similar to Oyster mushroom, however, they differ in morphology and biological efficiency. It has very large fruiting body, blue-coloured pinheads formed and it becomes light white on maturity, high yield repeatable with meaty flavor and high quality. This new mushroom variety has attractive large shape and flesh has an excellent taste (Sumi and Geetha, 2016).

Many species of mushrooms provide an excellent source of natural compounds that are useful for the treatment of many diseases. Among these mushrooms, *Hypsizygus ulmaris (Bull.)* is used in many purposes due to it is flavor, texture, nutritional content and medicinal properties. Different types of *Hypsizygus ulmarius (Bull.)* extracts have been showing their activity against bacteria, diabetes, inflammation and tumor. It also provides a good source of antioxidants (Al-Faqeeh et al., 2018). The possibility of improving the quality of rice straw substrate by amending it with seaweeds and its influence on substrate biological efficiency (BE), mushroom (*H. ulmarius*) has health-related nutrients and can trace metals contents. The incorporation of 5 % seaweeds resulted in the highest total yield with 22 % higher BE than that of the control and also the highest crude protein concentration in mushrooms. The presence of the highest concentration of trace elements such as Na and K, which are beneficial to human health, was observed (Hausiku et al., 2018).

Hypsizygus ulmarius was first named *Pleurotus ulmarius* and later put under the genus *Hypsizygus* as *Pleurotus* spp. due to white rot and *Hypsizygus* spp. due to brown rot (Volk, 2003). Nutritional study revealed that elm oyster mushroom contains 23.6 % protein, 2.2 % fat, 52.4 % carbohydrates, and 12.9 % fiber on dry weight basis and source of natural antioxidant and antibiotics. (Shivashankar and Premkumari, 2014).

Mycochemical analysis of *Hypsizygus ulmarius* confirmed the presence of compounds such as polysaccharides and phenolic compounds which are responsible for the medicinal properties of such mushrooms. *Hypsizygus ulmarius* (Elm oyster) could be used as a pharmaceutical, medical, and food additive. It is known for anti-tumor, cholesterol controlling and cardiovascular properties (Panavalappil et al., 2016).

The present work was designed to study the effect of different plant-based substrates on the growth of Elm Oyster mushroom. Plant-based wastes containing the cellulose materials such as paddy straw, corn husk, and paddy straw with corn husk could be used as the substrate for the cultivation of elm oyster under household conditions. In this study, the morphological and nutritional status of elm oyster mushroom *(Hypsizygus ulmarius*) derived from the above-mentioned substrates were analyzed under laboratory conditions.

The initial study (preliminary study) dealt with the cultivation of selective elm mushroom (*Hypsizygus ulmarius*) under household condition with the following objectives: study the morphological characterization of Elm Oyster mushroom (Culturing of mushroom under laboratory condition), Mushroom cultivation technology (Sterilization and Preparation of substrate bags, Spawning of substrate, Incubation of spawn inoculated substrate bags under household environment, Harvesting of basidiocarps and its management. The further study was focused to study the nutrient screening of elm mushroom (*Hypsizygus ulmarius*): moisture

determination, total ash determination, total fat, total lipid, carbohydrates and estimation of protein. The study of morphology of basidiocarp (fruiting body) of elm oyster mushroom (*Hypsizygus ulmarius*), which included measuring the diameter and length of the cap and stipe and observation of colour of pinhead and basidiocarp, was performed.

2. Materials and Methods

2.1. Preliminary study: Mushroom cultivation technology

2.1.1. Favorable Climatic conditions

Elm mushroom (*Hypsizygus ulmarius*) was grown at temperature ranging from 21 ºC to 30 ºC for a period of 2 to 6 months per year. The winter season is favorable for cultivation.

2.1.2. Sampling

The elm mushroom: *Hypsizygus ulmarius* was selected and the spawn culture (mushroom seed) was collected from Dr. Mohan Mushrooms Research and Training Centre of Madurai.

2.1.3. Cultivation of Mushroom Techniques

2.1.3.1. Pure culture technique

A small inoculam of mushroom seed was cultured on the potato dextrose agar medium and incubated at room temperature for 2-4 days (plate: 1). The grown culture was sub cultured frequently for maintaining and checking the viability of mycelium.

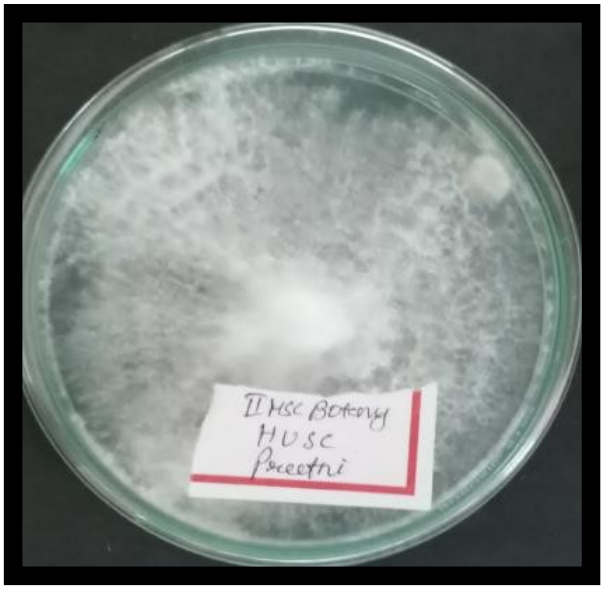

Plate: 1. Elm oyster mushroom (*Hypsizygus ulmarius*) cultured under Microbiological Laboratory condition

2.1.3.2. Substrate preparation

Two different biodegradable substrates namely paddy straw and corn husk were utilized for cultivation of elm mushroom (*Hypsizygus ulmarius*) commercially under controlled condition. Before sterilization, various substrates were water soaked for 14-16 hours. These substrates were sterilized in boiling water at 80 ºC for 30 minutes. After the sterilization, these substrates were dried under shade area to remove excess water (plate: 3).

2.1.3.3. Inoculation of Spawn

The sterilized substrates were packed in a polythene bag with 16 cm length x 9 cm width in size. They were packed as compact layer and formed alternatively by crisscross pattern. Then they were inoculated with spawn culture. Three different bags were packed (paddy straw, corn husk, and combination of paddy straw and corn husk). It was repeated for 4 to 8 layers, it depended upon the size of the bag. The inoculated bags were tied with thick thread and leaving air space above the bag. Such bags were perforated (12-16 holes per bag) with needle for promoting aeration for mycelial growth (plate: 3).

2.1.3.4. Incubation

The prepared mushroom bags were hanged in wooden bench with strings and the wooden bench was moisturized with wet gunny bag and clothes. It was maintained regularly (plate: 4).

2.1.3.4.1. Fruiting

The fully colonized mycelium was observed on the substrate and such condition was ready for the development of basidiocarps. Frequent spraying of water over the bags in the incubating place depending upon the atmospheric condition. The cultivation place was provided with sufficient ventilation during fruiting body formation (Plate: 5-7).

2.1.3.4.2. Fruiting body protection

The mushroom fruiting body was protected from mites, flies and other microbial diseases by regular monitoring and removing contaminated bags.

2.1.3.5. Fruiting body harvesting and maintenance

The harvesting of basidiocarp was done based on the size and shape of the fruit body by hand picking. They were harvested before spore release and it was done for three times/bag.

Fresh mushrooms were packed in perforated poly-bags and stored at low temperature (4 ºC) for 4 to 5 days.

2.2. Secondary study

2.2.1. Moisture content determination (Raghuramulu et al., 2003)

The moisture content of the harvested sample was determined by measuring the weight before and after. And water content was evaporation (plate: 10).

Percentage of moisture = (Initial fresh weight - final weight) x 100 /weight of sample

2.2.2. Total Ash content determination (AOAC, 1990)

Three grams of mushroom was taken heated in a hot air oven at 170 ºC for 30 min and cooled (Plate:10). Ash content was calculated using the formula:

Ash content (g/100g sample) = Weight of ash/weight of sample taken x 100

2.2.3. Total Fat content determination (AOAC, 1990)

Ten grams of mushroom was weighed and extracted with petroleum ether for 16 hours. The extract was dried, cooled and measured (plate: 11). The percentage of fat was determined using the formula:

Percentage of fat = 100(Weight of Soxhlet flask with extract fat – Weight of empty Soxhlet flask)/Weight of sample

2.2.4. Total Lipid determination (Folch et al., 1957)

The 5 g of mushroom sample was suspended in fifty milliliter of 2:1 chloroform: methanol mixture then mixed well and left for 3 days. It was filtrated and centrifuged at 3,000 rpm in centrifuge. The methanol upper layer was removed by micropipette and the chloroform was made to evaporate in a hot air oven. The remaining dried sample was measured as crude lipid (plate: 11).

2.2.5. Protein Estimation (Lowry et al., 1951)

Five grams of mushroom were weighed and ground with water with the help of a pestle and motor. Then it was filtered using a muslin cloth. The filtrate was collected in a centrifuge tube and centrifuged for 5 minutes at 3,000 rpm. The supernatant was collected and pellet was discarded. The collected supernatant was treated by adding equal volume of 10 % Trichloro acetic acid solution. Then it was again centrifuged at 3,000 rpm for 5 minutes. The supernatants were discarded. The collected pellets were taken and dissolved in 15 ml of 0.1 N of sodium hydroxide solution. It was then centrifuged again. The supernatant was taken and made up to 20 ml with 0.1 N of sodium hydroxide solutions. This was taken as the test solution. 1 ml of the test solution and 5 ml of alkaline reagent was added and allowed to stand for 10-15 minutes in a dark place. After that 0.5 ml of folinphenol reagent was added to it. The optical density of the solution was measured at 660 nano meter by using the UV-Visible spectrophotometer (plate: 11). Bovine serum albumin was used as a standard for calculating the protein content of the sample.

2.2.6. Carbohydrate determination

The carbohydrate content of the harvested mushroom was calculated by the formula:

The percentage of carbohydrate = (100 - total protein + total ash + total lipid + total fat)

2.2.7. Measurement of the size of cap and stipe

The size of the cap and stipe of the fruiting body was measured by a meter scale and expressed in terms of cm. Both length and diameter of cap and stipe were measured.

2.2.8. Colour of pinhead and basidiocarp

The color of the pinhead and basidiocarp of each bag was noted.

2.2.9. Data analysis

The measured data collected in the study have subjected to mean descriptive statistics.

3. Result and discussion

Mushrooms lack chlorophyll and are non-green organisms. They have extensive enzymes which degrade lignocellulosic materials for their nutrition and growth. Different mushrooms have different lignocellulolytic enzymes (cellulases, ligninases, endoglucanases and cellobio hydrolases) profile required for substrate bioconversion (Buswell and Chang, 1994; and Buswell et al., 1996).

Mushrooms are a good source of protein, vitamins and minerals and are known to have a broad range of uses, both as food and medicine. A high nutritional value of oyster mushrooms has been reported with protein (25-50 %), fat (2-5 %), sugars (17-47 %), mycocellulose (7-38 %) and minerals (potassium, phosphorus, calcium, sodium) of about 8-12 % (Syed et al., 2009). More than 3,000 mushrooms are said to be “the main edible species” of which only ten of those are used in industrial purpose. Its global economic value is nevertheless now staggering and an initial reason for the increase in consumption is the combination of their food value as well as their medicinal values. A variety of mushroom species constitute cost-effective food-stuff for both supplementing the nutrition to human kinds (Chang and

Miles, 2004) and other purposes.

Chang and Buswell (1996) reported the extractables derived from either the mushroom mycelium or fruiting body which are known as nutraceuticals. There are a very important for the expanding mushroom biotechnology industry. It has been shown that constant intake of either mushrooms or mushroom nutraceuticals (dietary supplements) can make people fitter and healthier. In addition, mushroom cultivation can also help to convert agricultural and forest wastes into the useful matter and reduce pollution in the environment. Therefore, this technique of mushroom cultivation could make three important contributions to society: production of healthy food, manufacture of nutraceuticals and reduction of environmental pollution.

The improvement and development of modern technologies, such as computerized control, automated mushroom harvesting, preparation of compost, production of mushrooms in a non-composted substrate, and new methods of substrate sterilization and spawn preparation, will increase the productivity of mushroom culture. All these aspects are crucial for the production of mushrooms with better flavor, appearance, texture, nutritional qualities, and medicinal properties at low cost (Sánchez, 2004).

Chang (2007) reported that many mushrooms possess a range of metabolites of intense interest to pharmaceutical e.g. antitumour, immunomodulatory, antigenotoxic, antioxidant, anti-inflammatory, hypo cholesterolaemic, antihypertensive, antiplatelet aggregating, antihyper glycemic, antimicrobial and antiviral activities (antitumour, immunomodulation agents, and hypocholesterolemia agents and food (ex: flavor compound) industries. The above data demonstrated there are 660 species from 182 genera of mushrooms containing antitumor or immune stimulating polysaccharides. The wild mushroom species contain higher contents of protein and lower fat concentrations than commercial mushroom species. But commercial species have higher concentrations of sugars, while wild sp. contained lower values of MUFA but also higher contents of PUFA. There were no differences between the antimicrobial properties of wild and commercial species (Barros et al., 2008).

Mushrooms degrade lignin cellulosic substrates and can be produced on natural waste materials from agriculture, woodland, animal husbandry and manufacturing industries (Rinker, 2002). Many people consumed mushrooms as comestibles for their nutritional value and folk medicine for their supposed medicinal importance. Besides their excellent flavour mushrooms have attracted much attention due to their proven healthy properties (Chiron and Michelot, 2005).

The preliminary study focused on the pure culture of mushroom seed (spawn) which was initially collected from Dr. Mohan Mushrooms Research and Training Centre, Madurai, and was sub-cultured in potato dextrose agar medium under an aseptic condition in the microbiology laboratory. After 3-4 days of incubation, white cottony mycelium was grown on the potato dextrose agar plate (Plate: 1). In the present study, massive growth of mycelium observed in all the *Hypsizygus ulmarius* spawn inoculated substrates (Paddy straw, corn husk, corn husk + paddy straw) within 4 to 6 days (Plate: 2). The pin head formation (Plate: 2) occurred at various time interval viz. Paddy straw in 19 ± 1.0 days, Corn husk in 17± 1.0 days and Corn husk + Paddy straw in 13 ± 1.0 days. Favorable climatic condition determined the fruiting body formation in the above-mentioned substrates. But the fruiting body sprouting was observed on the 20th day in Paddy straw bag, on the 19th day in Corn husk, and on the 14th day in Corn husk + Paddy straw (Table 1 and Plate: 2). This result indicated that the mycelium colonization, pin head formation and fruiting body development in all plant-based substrates depended upon the environment factors and composition of substrates used for cultivation and it might be optimum for the edible elm mushroom: *Hypsizygus ulmarius*. The harvested mushrooms from each substrate were collected in polythene covers aseptically and were stored in a refrigerator for further analysis work.

The cultural studies of *Hypsizygus ulmarius* revealed that out of nine media tested, MEA and WEA media supported maximum (90 mm) growth followed by PDA medium. Optimum temperature was 25 °C and pH level was 7 for the mycelial growth of the fungus. There was gradual decline in growth of fungus when temperature and pH level increased or decreased (Kushwaha et al., 2011). But the other study showed (Jonathan et al., 2012) that the cultivation of *Pleurotus ostreatus* on different agricultural wastes such as *Oryza sativa* straw, *Gossypium hirsutum* wastes and *Milicia excels* sawdust with the addition of *Oryza sativa* bran additive to enhance the mycelial growth. *Gossypium hirsutum* substrate with rice bran additive showed high moisture content (93.43 %), crude protein (28.02 %), fat contents (8.72 %) and fiber contents (17.42 %) of *P. ostreatus*.

Fan et al. (2008) observed that mushroom production could convert the huge lignocellulosic waste materials into a wide diversity of products (edible or medicinal food, feed and fertilizers), protecting and regenerating the environment. And also, mushroom production could generate equitable economic growth which already had an impact at national and regional levels. This impact was expected to continue increasing and expanding in the future, because more than 70 % of agricultural and forest materials are nonproductive and have been wasted in the agro-industrial processing or even consuming period.

The present investigation reported that the morphology study of *Hypsizygus ulmarius* was determined by measuring the diameter, length of cap and stipe of the fruiting body (Basidiocarp) and colour of pinhead and Basidiocarp.

Plate: 2. Elm oyster's mushroom (*Hypsizygus ulmarius*) spawn running process and basidiocarp formation stage

Table 1
Effect of different substrate on the growth of elm oyster mushroom (*Hypsizygus ulmarius*)

Sl. no	Substrate	Spawn running (Days)	Pin head formation (Days)	Basidiocarp formation (Days)
1.	Paddy straw	6.3 ± 1.5	19 ± 1.0	20.6 ± 1.5
2.	Corn husk	5 ± 1.0	17 ± 1.0	19.6 ± 1.5
3.	Corn husk and paddy straw	4 ± 1.0	13 ± 1.0	14.6 ± 1.5

Values are mean of three replicates ± SD

The Diameter and length of cap and stipe of basidiocarp from the Paddy straw substrate bag was 6.63 ± 0.66 cm; 4.06 ± 0.23 cm; 1.23 ± 0.11 cm; 3.86 ± 0.35 cm, from corn husk substrate bag was 7.9 ± 3.5cm; 5.83 ± 2.36 cm; 1.06 ± 0.11cm; 4.36 ± 1.01 cm and from corn husk + paddy straw substrate bag was 6.96 ± 3.10 cm; 5.53 ± 1.70 cm; 1.6 ± 0.55 cm, 3.36 ± 0.90 cm (Table 2). The colour of pinhead and basidiocarp of elm oyster mushroom from three substrate bag was also observed and noted. The colour of the pinhead and basidiocarp of

H. ulmarius in a corn husk, Corn husk + paddy straw and paddy straw substrate bag was observed to be same colour. The colour of the pinhead of *H. ulmarius* was greyish blue in colour. At maturity, the colour was gradually changed into light white colour from greyish blue colour. The colour of basidiocarp of *H. ulmarius* was white in colour.

Buah et al. (2010) investigated the cultivation of *Pleurotus ostreatus* on various substrates (corn cob and sawdust). There were different steps involved in the cultivation methods like composting the substrates, bagging the substrates, sterilizing the bagged compost, spawning, incubation and cropping. The result showed that the corn cob used as a substrate for oyster mushroom cultivation performed better than saw dust in terms of the growth and yield of the mushroom. The Corn cob could therefore substitute the saw dust since it is cheap and available during a year, unlike sawdust, where the demand is between mushroom growers and poultry farmers.

The investigation (Pokhrel et al., 2016) on the growth of oyster mushrooms in easily available substrates such as corn cob, vegetable residue and waste paper was examined with the supplementation of rice bran and chicken manure separately. The observation showed that best mycelial extension, early pin head formation and better yield in corn cob substrate followed by paper waste and vegetable residue. Among these substrates used, corn cob showed the highest yield with range from 99.08 to 109.50 % biological efficiency, whereas 69.81 to 88.36 % and 52.26 to 65.22 % biological efficiency was observed in paper waste and vegetable residue respectively.

Sumi and Geetha (2016) analyzed the morphological study on *Hypsizygus ulmarius* showed that the sporocarps were medium to large having a dark blue colour in the pinhead stage which became creamy white on maturity with an irregularly shaped, convex pileus with gills attached to the stem, but not decurrent and cylindrical, smooth and eccentric stipe and their studies on developmental morphology showed that *H. ulmarius* took an average of five days from the day of pinhead formation to complete maturity. When compared with *Pleurotus florida*, *H. ulmarius* took more time (18 days) for complete spawn run in paddy grains and the yield was higher on paddy straw (1.096 g/kg) than *P. florida* (976 g/kg). Nutritional studies showed that *H. ulmarius* contained an appreciable amount of carbohydrate (29 %), protein (32 %), and fiber (17.69 %).

Munna et al., (2019) reported that Banana leaves substrates in combination proved to be best for the cultivation of *Hypsizygus ulmarius*. Cultivation of elm oyster mushroom on combination with paddy straw and different substrates were investigated. According to this study, the minimum time taken for a complete mycelium run (19.33 days) was in T3 (wheat straw + banana leaves) and maximum time was observed in T6 (wheat straw + doob grass) (23.16 days). Minimum time from the primordial stage to the harvesting stage was recorded in T3 (wheat straw + banana leaves) (25.83 days) and maximum time was recorded in T6 (wheat straw + doob grass) (27.33 days). A higher yield was obtained in T3 (wheat straw + banana leaves) (936.6 g) with the highest biological efficiency (93.66 %). Maximum protein content was recorded in T3 (wheat straw + banana leaves) (35 %) and maximum carbohydrate in T3 (wheat straw + banana leaves) (25.33 %).

The growth and biomass of *Hypsizygus ulmarius* were studied based on different media, temperatures, light duration, pH level and Relative humidity. Among the tested media potato dextrose agar medium was found most suitable medium for the growth (89.00 mm) and biomass (fresh mycelium weight: 13.93 gm and dry mycelium weight 0.57 gm) of *H. ulmarius*. The optimum temperature required 26 °C was most suitable. Maximum relative humidity for radial growth was observed at 75 % relative humidity. Complete darkness or zero hours of light was excellent for mycelial growth and biomass of *H. ulmarius*. Maximum growth of *H. ulmarius* was obtained at pH 8.0 on potato dextrose agar medium (Baghel et al., 2019).

Khade et al. (2019) reported that the addition of organic and inorganic supplements to the substrate increase the yield of elm oyster mushroom. The study reported that the yield performance varied due to different treatments like neem cake at 2 % produced maximum yield of mushroom (841.11 g/kg dry) substrate followed by treatment, maize flour at 2 % with a yield of 831.11 g/kg dry substrate, whereas the lowest yield of 320 g/kg dry substrate was recorded in treatment soybean flour at 2 %. A significant variation in average fruit body weight (2.72 to 10.64 g per fruit), pileus diameter (4.09 to 6.72 cm), stipe length (2.62 to 4.43 cm), and stipe size (2.73 to 3.84 cm) were noted due to different treatments.

Kumar et al. (2019) conducted a study on the cultivation of *Hypsizygus ulmarius* mushroom on the different substrate such as banana leaves, casuarina needle, coir pith, ground nut shell, paddy straw, sugarcane trash, sugarcane bagasse, saw dust and water hyacinth and supplements on the sporophore production. Paddy straw (489.6 g/bed) was most efficient in enhancing the yield of *H. ulmarius* and Followed by water hyacinth (474.4 g/bed) and sugarcane trash (472.7 g/bed).

Cyriacus et al. (2020) investigated the cultivation and yield performance of *Hypsizygus ulmarius* grown on agricultural waste from *Musa sapientum* (MS), *M. paradisiaca* (MP), *M. accuminata* (MA), MS+MP, MS+MA, MP+MA, MS+MP+MA. Such a result showed that MS+MA had the shortest fruiting time of 12 days while MP, MP+MA had the longest, which was 14 days. The largest capsize was obtained in MP while the smallest capsize was in MA. The longest stipe length was produced by MS+MP while MS+MA has the shortest

Table 2
Morphological study of fruiting body (basidiocarp) of elm oyster mushroom (*Hypsizygus ulmarius*) from different substrates

Sl. no	Substrate	Cap diameter (cm)	Cap length (cm)	Stipe diameter (cm)	Stipe length (cm)
1.	Paddy straw	6.63 ± 0.66	4.06 ± 0.23	1.23 ± 0.11	3.86 ± 0.35
2.	Corn husk	7.9 ± 3.5	5.83 ± 2.36	1.06 ± 0.11	4.36 ± 1.01
3 .	Corn husk and paddy straw	6.96 ± 3.10	5.53 ± 1.70	1.6 ± 0.55	3.36 ± 0.90

Values are mean of three replicates ± SD

stipe. MP+MA substrates gave the highest biological yield while the least was recorded by MS+MA. Biological Efficiency was best at 76.58 % produced by MP+MA and lowest at 56.48 % by MS+MA.

In the secondary investigation, the nutrient analysis of the harvested *Hypsizygus ulmarius* was done and measured data were tabulated (Table 3). According to this the moisture content was maximum (69.86 ± 0.41 %) in corn husk + paddy straw substrate when compared to other substrates (66.06 ± 0.70 % in paddy straw; 61.66 ± 0.50 % in Corn husk) (Table 3). The ash content of the harvested basidiocarps of *Hypsizygus ulmarius* (Table 3) from Corn husk + Paddy straw substrate showed maximum at 13.03 ± 0.75 % and it was the least level in a Corn husk (6.33 ± 0.29 %) when compared to paddy straw substrate (8.20 ± 0.40 %). Shivashankar and Premkumari (2014) analyzed the total ash content, water-soluble extractive value, alcohol soluble extractive value and moisture content of *Hypsizygus ulmarius*. Qualitative phytochemical screening was also studied that showed the presence of alkaloids, phenolics, saponins, tannins, glycosides, carbohydrates and proteins. Usha and Suguna (2015) analyzed that two strains of *H. ulmarius* CO_2 and *H. ulmarius* IIHR revealed that the protein, carbohydrate and fiber contents were high. Lipid content in two strains of *H. ulmarius* ranged from 3.65 to 5.35 % and fat content ranged from 3.55 to 4.80 %, respectively.

Sethi et al. (2012) experimentally showed that cultivation of the *Hypsizygus ulmarius* in three different substrates [wheat straw, paddy straw and wheat straw: paddy straw (1:1)] was pretreated with hot water (80 ºC) and chemicals such as caebendazim and formaldehyde. Among these wheat straw pretreated with hot water (80 ºC) showed maximum biological efficiency, least spawn run period and pin head appearance in 27-33 days. Mishra et al. (2013) evaluated the total phenolics, radical scavenging activity (RSA) on DPPH, ascorbic acid content and chelating activity on Fe^{2+} of *Pleurotus citrinopileatus, Pleurotus djamor, Pleurotus eryngii, Pleurotus flabellatus, Pleurotus florida,Pleurotus ostreatus, Pleurotus sajor-caju* and *Hypsizygus ulmarius*. Agglomerative hierarchical clustering analysis on basis of seven parameters revealed that studied mushroom species fall into two clusters; Cluster I included *P. djamor, P.eryngii* and *P. flabellatus*, while Cluster II included *H. ulmarius, P. sajor-caju, P. citrinopileatus, P. ostreatus* and *P. florida.*

Sadhana and Sivakumar (2020) previously analyzed the different biodegradable substrates such as newspaper waste, coir waste, ground nut shell coat waste and paddy straw for the comparative cultivation technique of *Pleurotus ostreatus* mushroom. They reported that the nutrient values of all the harvested basidiocarps of mushrooms derived from the various substrates have been determined the higher protein content noted in both newspaper wastes (51.93^{b} ± 3.066 % and ground nut shell (43.06^{ab} ± 6.025 %) wastes substrates. The results showed that the edible mushrooms could be cultivated by using biodegradable wastes from different sources and this technology was applied in small-scale industry for the production of efficient *Pleurotus* species for our health at low cost.

Mushrooms have been treated as a special kind of functional food during ancient times but in recent decades they are known as “the ultimate health food” because of their unique nutritional status. Based on in this view, many mushrooms have been reported to have rich nutritional value with high content of proteins, minerals, fibres, trace elements and low/ no cholesterol (Breene, 1990). The carbohydrate content of mushrooms represents the bulk of fruiting bodies counting for 50 - 65 % on dry weight basis. They contain low fat and oil content (Barros et al., 2008) compared to that of proteins and carbohydrates.

In the present study, the lipid content of harvested *Hypsizygus ulmarius* was measured the lowest at 0.20 ± 0.02 % in corn husk + paddy straw and high lipid content in Paddy straw substrate-based mushroom was 0.38 ± 0.02 %. The lipid content of the *H. ulmarius* from Corn husk substrate showed an approximate level as 0.29 ± 0.02 % (Table 3). The protein content of harvested *Hypsizygus ulmarius* was maximum at 44.71 ± 0.28 % in Corn straw + paddy straw and 37.88 ± 0.45 % in Corn husk substrate and it was minimum in (17.41 ± 0.22 %) paddy straw substrate (Table 3). But minimum fat content was measured at 10.03 ± 3.51 % in Corn husk + paddy straw substrate and maximum was observed at 26.20 ± 3.06 % in Paddy straw substrate. But the fat content (Table 3) of the *Hypsizygus ulmarius* from Corn husk showed approximate level (21.50 ± 5.56 %). When compared to others substrates, the carbohydrate content of elm oyster mushrooms (Table 3) was higher at 47.81 ± 0.20 from paddy straw substrate and approximately same level of carbohydrate content from both corn husk substrate and Paddy straw substrate at 34.00 ± 1.05 % and 32.00 ± 0.50 %. This variation in nutritional level of *Hypsizygus ulmarius* derived from different plant based substrates determined by the energy sources like

Table 3
Effect of different substrates on the moisture content (%) and nutritional content of elm oyster mushroom (*Hypsizygus ulmarius)*

Sl. No	Substrate	Moisture content (%)	Ash Content (%)	Lipid Content (%)	Protein content (%)	Fat content (%)	Carbohydrate content (%)
1.	Paddy straw	66.06 ± 0.70	8.20 ± 0.40	0.38 ± 0.02	17.41±0.22	26.20 ± 3.06	47.81±0.20
2.	Corn husk	61.66 ± 0.50	6.33 ± 0.29	0.29 ± 0.02	37.88±0.45	21.50 ± 5.56	34.00±1.05
3.	Corn husk and paddy straw	69.86 ± 0.41	13.06 ± 0.75	0.20 ± 0.02	44.71±0.28	10.03 ± 3.51	32.00±0.50

Values are mean of three replicates ± SD

cellulose, lignocellulosic, hemicelluloses and pectinoses rich substrate within it. This study concluded that *Hypsizygus ulmarius* utilized the plant-based waste substrate for their metabolic processes and produced massive mycelium over the substrate along with sprouting of basidiocarp within a short period of growth. A similar study was reported by Biswas and Kuiry (2013). It showed minimum spawn period, maximum size of sporophores, maximum yield and biological efficiency in *Hypsizygus ulmarius* when compared with *Pleurotus* species such as *Pleurotus florida, Pleurotus sajor-caju, Pleurotus ostreatus* and *Pleurotus flabellatus*.

A recent study (Sen et al., 2020) evaluated the effect of nutritional medium, temperature and colour on mycelial growth behavior of *Hypsizygus ulmarius* in vitro. According to this study dense and white mycelium was grown on potato dextrose agar (PDA) medium and it required least days for full growth (10.50 days) followed by Malt Extract Medium (11.25 days). Out of different colour polythene wrapping, black colour polythene treatment gave the best result as it required the least days for spawn run (16 days) followed by blue colour (16.25 days) which provided uniform growth of the medium.

Totally three substrates were analyzed for the present study. Among these substrates the combination of corn husk and paddy straw substrates was appropriate for *Hypsizygus ulmarius* cultivation technique when compared to other utilized substrates. The nutrient composition was fractionally resolute the distribution of different level components at significant percentage for each substrate applied in this study. The plant-based waste substrates applied in this work comprised different energy sources like cellulosic, lignocellulosic and hemicellulosic compounds which could be utilized by the selective mushroom fungi for the growth and metabolic activities. The present work confirmed that the protein content was higher and low level of lipids, fats and carbohydrates in such selective elm oyster mushroom grown on plant- based waste substrate which could be suggested to include this mushroom in regular dietary food for diabetes and other ill patients.

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Efikasnost rasta gljive *Hypsizygus ulmarius* u prisustvu biljnih otpadnih supstrata

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INFORMACIJE O RADU

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Ključne reči:
Bukovača
Supstrat od slame i kukuruzne ljuske
Hypsizygus ulmarius

I Z V O D

Uzgoj gljiva, koje predstavlju nutritivno važne organizme koji imaju uspešan rast na otpadnim supstratima poljoprivrednih biljaka, jeste profitabilan agrobiznis. Pečurke sadrže različita polisaharidna jedinjenja, kao što su celuloza, lignin i hemiceluloza, koja se mogu razgraditi u prisustvu ekstracelularnih enzima koje gljive proizvode. Takve jestive pečurke imaju visoku hranljivu vrednost i sadrže proteine, aminokiseline, ugljene hidrate, masti, lipide, vitamine i minerale. U ovom eksperimentu, pečurke *Hypsizygus ulmarius* su uzgajane na tri različita supstrata: slami, kukuruznoj ljusci i kombinaciji ova dva supstrata pod strogo kontrolisanim uslovima. Period razvijanja, formiranje klobuka i nicanje su bili brži (14 dana) na kombinovanom supstratu od slame i kukuruzne ljuske. Veličina plodišta je bila veća na suspstratu od kukuruzne ljuske (prečnik klobuka – 7,9 cm; dužina kape – 5,83 cm) i na kombinovanom supstratu (prečnik klobuka – 6,96 cm; dužina kape – 5,53 cm) u poređenju sa onim na supstratu od slame (prečnik klobuka – 6,63 cm; dužina kape – 4,06 cm). Hranljivi sastav ubranih pečuraka sa različitih supstrata je imao najveći sadržaj vlage (69,86 ± 0,41 %) i maksimalni sadržaj pepela (13,06 ± 0,75 %) kod *Hypsizygus ulmarius* sa kombinovanog supstarta. Ove pečurke su takođe bile bogate proteinima (44,71 ± 0,28 % i 37,88 ± 0,45 %), sadržale su niske nivoe ugljenih hidrata, kao i optimalne nivoe masti i lipida. Dakle, kombinovani supstrat i supstrat od ljuske kukuruza su bili efikasniji i pogodniji za komercijalnu kultivaciju *Hypsizygus ulmarius* od supstrata slame.

Hypsizygus ulmarius ima veći klobuk i daje veći prinos od vrste *Pleurotus*. Lako ga je uzgajati i imati visok prinos. Ova komercijalna tehnologija proizvodnje jestivih gljiva je primenjena u različitim oblastima kako bi se poboljšala i uravnotežila ishrana u Indiji, a isto tako i prevazišao problem neuhranjenosti. Ovaj rad sugeriše da bi se kombinacija kukuruzne ljuske i slame kao supstrata mogla koristiti za proizvodnju nutritivno efikasnih pečuraka sa visokim prinosom. Mogao bi da se upotrebi kod uzgoja pečuraka i tako doprineti povećanju ekonomije na određenom nivou.