WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 2, 553-568. Research Article ISSN 2277–7105

STUDIES ON THE CULTIVATION TECHNIQUE OF PLEUROTUS OSTREATUS GROWN ON PADDY STRAW SUBSTRATES

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Article Received on
01 December 2024,

Revised on 21 Dec. 2024,
Published on 15 Jan. 2025

DOI: 10.20959/wjpr20252-34826

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ABSTRACT

The present investigation is based on the cultivation technique of *Plerotus ostreatus* grown on paddy straw substrates it is conducted to know the growth of the mushroom in paddy straw The culture of *Pleurotus ostreatus* mycelium in PDA medium and sub- culturing were also done after 15 days. and to prepare the mother spawn using sorghum grains substrate then the preparation of the paddy straw substrate for mushroom cultivation was done and the cultivation of *Pleurotus ostreatus* using paddy straw substrate and Analysis of yield parameters in harvested edible mushroom was identified the study focuses its attention on the yield and growth of *Plerotus ostreatus* and the potential ability of paddy straw substrate in its growth.

KEYWORDS: *Plerotus ostreatus*, paddy straw, spawn.

INTRODUCTION

A vegetable mushroom can play an important role in meeting the nutritional needs of the worldwide population. A healthy person requires 200-250g vegetable per day. But in some countries like Bangladesh, Pakistan only 40-50g Vegetable per day is available to people. To get rid of this situation, It is necessary to increase the production of vegetable which needs a huge land areas. Mushroom may be used to reduce shortage of vegetable, since it required minimum land area. Mushroom fungi have low calorie they are cholesterol free and have certain medicinal properties.

Oyster mushroom (*Pleurotus species*) belongs to the family to Tricholomataceae and is the second widely cultivated mushroom worldwide following the *Agaricul bisporus, Pleurotus*

species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures In comparison to other edible mushrooms, *Pleurotus Species* need a short growth time and their fruiting bodies are not often attacked by diseases and pests *Pleurotus species* require carbon, nitrogen and inorganic compounds as their nutritional sources. Oyster mushroom can grow on a wide variety of substrate. However, the yield and the quality of oyster mushroom depend on the chemical and nutritional content of substrates. (Hai thihoa 2015) observed the presence of Ca, Cu, Fe, K, Mg, Mn, P and Zn as mineral content of substrate in his study of the effect of different agro wastes on the growth of oyster mushroom.

Mushrooms are known as healthy foods and dietary nutrients in all over the world, because they include rich amounts of vegetable proteins, chitin, essential amino acids, vitamins, minerals and low fat and calories. On dry basis, an edible mushroom has 56.8% carbohydrate, 25.0% protein, 5.7% fat and 12.5% ash contents. However, mushrooms are considered as not only a food source but also having medicinal properties because of bioactive compounds. Also, edible mushrooms could be useful for many diseases such as hypertension. Cancer and cholesterol. For many years, cereal products have been existing in human's life and they are the most preferred food materials in the world. Because of this reason, baking industry has grown rapidly and bakery products such as bread, croissant, snack etc. have taken huge part in international food market. But obesity, cardiovascular diseases, diabetes and some types of cancers are directly related to dietary habits. In order to reduce the risks, consumers tend to try new but healthier food market. But obesity, cardiovascular diseases, diabetes and some types of cancers are directly related to dietary habits (Hu, 2002). In order to reduce the risks, consumers tend to try new but healthier food products. Hence, baking industry should follow last and healthy trends (Byrne, 2000; Kohn, 2000; Kotsianiset al., 2002). This may be succeeded through processing or reformulation of food products such as non-thermal operations or functional food (Reisch and Gwozdz, 2011; Kaurand Das, 2011; Aschemann-Witzel, 2015). Researchers have focused on substitution of various flour types for wheat flour to satisfy demands for healthier foods recently (Coelho and Sals-Mellado, 2015).

There are various type of mushrooms such as oyster mushroom, milky white mushroom, shitake mushroom and button mushroom etc. Which are cultivated in many countries. Among them, some popular species of an oyster mushroom such as, *Pleurotus ostreatus, P.djamor, P.florida, P.cystidiousus and P.geesteranus* can be cultivated in countries like Pakistan, China, India and Bangaladesh because the climate and weather of these countries is

more suitable for the mushroom cultivation. Though the weather and climate of Pakistan is suitable for year-round oyster mushroom cultivation but the farmers cannot cultivate the mushroom due to lack of autoclave sterilization of substrate. Without sterilization of spawn packets contaminations occur. In the country mushroom spawn is prepared by using sawdust which is sterilized with autoclave sterilization but corn cobs, rice straw, wheat straw, pulse straw, sugarcane baggage, water hyacinth and tea leaves may be used as substrates after sterilizing them with hot water treatment, drum pasteurization or chemical treatment for spawn production.

Substrate assumes a significant part in the yield and supplement substance of shellfish (Oyster) mushroom. The substrates on which mushroom bring forth (Commercial generated / Merely vegetative seed materials) is developed, influences the mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka grows oyster mushroom using rice straw with hot water treatment and sawdust with autoclave sterilization. Farmer of some countries like Pakistan and India cannot produce mushroom spawn due to high cost autoclave sterilization. Therefore, it is necessary to identify the less expensive sterilization technique without autoclave formushroom production. If low cost method of sterilization is introduced in place of high cost autoclave sterilization, farmers will be interested to grow mushroom in respective agriculture lands. Hot water treatment of substrate may be an effective alternative of autoclaving. With this view in mind a research project was designed with the objectives to find out the concentration of polysaccharides in oyster mushroom (*P.ostreatus)* and to determine the antioxidant activity of polysaccharides in oyster mushroom (*P.ostreatus*).

MATERIALS AND METHODS

3.1. Mushroom Spawn Production

Spawn is the mycelium of mushrooms growing in its substratum and prepared for the purpose of propagating mushroom production. In a more simple language it is defined as a medium impregnated with mushroom production. In a more simple language it is defined as a medium impregnated with mushroom mycelium that serves as the “seed” for mushroom cultivation.

Disinfect the working area and hands with disinfectant and wipe off mushroom fruiting body with 70% alcohol.

Make two equal half of mushroom with the help of sterilized but cooled knife without touching the inner surface of mushroom fruiting body.

Remove small pieces of tissue from the stipe, pileus connecting pointand place several pieces on the Malt Extract Agar plate on different locations.

Incubate plates at 34±2°C for 4 to 5 days IN BOD incubator or room temperature.

Transfer small mycelium bearing portion of medium on the fresh MEA slants followed by further incubation at 34±2°C for 4 to 5 days.

Use cultures directly in spawn substrate.

Required material for mushroom cultivation

- Mother spawn
- Preparation of mushroom bed

a) Substrate materials
b) Polythene Bags
c) Thread

Methodology of Mushroom Production TechniqueAgro-climate Requirements

Oyster mushroom was grown at moderate temperature ranging from 21°C to 30°C and humidity 55-70% for a period of 2 to 6 months in a year. It was cultivated in winter season.

Variety Cultivated.

Among all the cultivated Mushrooms Genus: *Pleurotus* has maximum number of commercially cultivated species suitable for commercial cultivation. The *Pleurotus ostreatus* was selected for the cultivation purpose and utilized for further analysis.

Sampling

The spawn culture of oyster mushroom (mushroom seed) was collected from the Tamil Nadu Agricultural University, Madurai. It was a source of inoculum for further cultural methods.

Mushroom Cultivation Techniques

- Pure culture of Oyster mushroom
- Preparation of spawn
- Preparation of substrate for commercial cultivation
- Spawn inoculation
- Incubation
- Harvesting and storage

a) Pure culture method of Oyster mushroom

The Potato dextrose agar medium was prepared and sterilized in a autoclave at 121^{o}C, 15 lb pressure for 15-20 minutes. The medium was poured into a sterilized Petri plates. After solidification, a small piece of inoculum of mycelium from spawn bottle was inoculated on a plate. The inoculated plates were incubated at room temperaturefor 2-4 days.

The Composition for PDA medium

- Potato - 200g.
- Dextrose - 18g.
- Agar - 20g
- Water - 1000ml

The well grown culture was sub cultured frequently for maintaining the viability of mycelium. They were used for further study (Plate :1).

b) Preparation of Spawn

A pure culture of oyster mushroom was inoculated on the different sterilized substrates such as Paddy straw, *Sorghum*, maize and wheat grains. These inoculated substrate bottles were incubated at room temperature for 2 to 3 weeks.

METHOD

Three hundred gram of the selected substrate (Paddy *Straw, Sorghum, maize*, wheat grains were purchased from Departmental store) was soaked in sterile water for 3-5 hrs. After soaking, the substrate was drained off and it was mixed with a 3-4 grams of (8%) calcium carbonate for the absorption of water and moisture. Then they were transferred into glass bottles. Only three fourth of the glass bottle was filled with substrate. Such bottles were sterilized in autoclave at 121^{o}C, 15 lb pressure for 15-20 minutes. After cooling, they were inoculated the pure culture of mycelium and shaken thoroughly for the proper growth of mycelium spread over the substrate. The inoculated bottles were incubated at 24^{o}C to 33^{o}C for 7-14 days. After incubation, the inoculated bottles were observed for mass colonization of oyster mushroom mycelium, It was used as a source of inoculums for further commercial cultivation (Plate:2).

c) Preparation of substrate for commercial cultivation

Four different cellulosic substrates namely news paper waste, coir waste, ground nut shell

and paddy straw were selected for commercial cultivation of oyster mushroom under aseptic condition, These substrates were sterilization in an autoclave at 121^{o}C, 15 lb pressure for 15-20 minutes. After the sterilization, these substrates were drained off and dehydrated with mixing of Calcium carbonate (8%) for absorption of moisture and water in the substrate.

d) Spawn inoculation

The sterilized substrates of different sources were packed into a polythene bag (size 40cm length x 15cm breath) in compact layer alternatively (one layer which was followed by spawning done in right angles with a crisscross pattern) inoculated with spawn culture. This process was repeated for 3 to 6 layers, it depends upon the bag size and the final layer was packed with loose substrate. Inoculated bags were tied on the top with thick thread and leaving a space above the bag for aeration. And it was incubated in incubator. These bags were performed with needles for maintaining proper aeration to initiate the mycelia grown in a substrate (Plate 3).

e) Incubation

The Spawned bags were kept for incubation at 21^{o}C to 30^{o}C and with a sufficient light and ventilation for 15-17 days for spawn growth and mycelium development. In this place the bags were hanged below the bamboo basket with thick threads and it was covered with wet cloth. Water was sprayed over the bags twice a day to maintain the moisture (Plate 5).

f) Fruiting

The mycelium was fully colonized on the substrate and the fungus was ready for fruiting. All the bags required high humidity (70-85%) during fruiting. The water was frequently sprayed over the bags in the cropping place depending upon the atmospheric humidity. Sufficient ventilation was provided during fruiting (plate 6).

Crop Protection

The crop was protection from flies, mites and other diseases by proper monitoring and pruning.

Harvesting and storage

The right time for crop harvesting was done based on the shape and size of the fruit body by hand picking. The fruiting bodies were harvested before spore release by twisting. They were harvested for three times/bag from every12-16 days of incubation. The harvested mushroom

fruiting bodies were measure per bag of substrate using electronic balance (Plate: 11& 12).

Short – term Storage

Fresh mushrooms were packed in perforated polythene bags and it was stored at low temperature (0-5^{O}) for 1-2 weeks without lose in quality. These mushroom samples were collected and studied for further analysis.

The mother were collected from Agricultural department in Aduthurai, Thanjavur District, Tamilnadu.

Substrate preparation

Oyster mushroom was grown on various substrates viz., paddy straw, cotton waste, compost etc. The substrate materials were collected from Orathanadu, in Thanjavur District, Tamilnadu.

Soaking

Paddy straw and cotton waste were chopped into 3-5 cm pieces and soaked in fresh water for 8-16 hours. Excess water from straw was drained off by spreading it on filter paper.

Heat Treatment

Heat treatment of substrate results in minimizing contamination problem and gives higher and almost constant yields. It can be done by pasteurization.

Pasteurization

Water was boiled in a wide mouth container such as tub or drum. The wet substrate was filled in gunny bags. The filled bag was dipped in hot water of 80-85C for about 10-15 minutes. To avoid floating, it was pressed with some heavy material or with the help of a wooden piece. Afterpasteurization, excess hot water was drained off from container so that it can be reused for other sets & hot water temperature was maintained at 80- 85C for all sets to achieve pasteurization.

Preparation of mushroom bed

I have selected four different kinds of the substrate to used for the cultivation of mushroom. The four different kinds of substrates like paddy straw, compost, cotton waste, and wooden chips.

The substrate materials are surface sterilized properly has been done by with the help of formaldehyde and carbon oxide. The substrate materials has been soaked the formaldehyde solution maximum 18 hours for their surface sterilization.

After the soaking the materials has been allowed for air drying. After the air drying the substrate materials are ready to poured the polythene bag.

To taken the polythene bag the end has been tied with thread tightly, rotate to create cylindrical in structure. To placed the sterilized substrate filled upto 1cm high on the bag.

After placed 10gm of the mother spawn on the peripheral side of the polythene bag. The repeated same process again the substrate after spread the mother spawn. This process continued to create maximum five layers in single bag. After the forming of five layer, the polythene bag has been filled and the end has been tied tightly. Now the entire bag look like a cylindrical structure. It has been create some pores on the entire bag for the aeration purpose.

To maintain the bags are fumigated mushroom shed under low temperature (less than 26^{o}C). The growth of the mushroom has beenobserved day by day.

Nutritional Analysis

Determination of Carbohydrates

(a) Molisch's test: 3ml of the aqueous extract was added to 2ml of Molisch's reagent and the resulting mixture shaken properly. 2ml of concentrated H_2SO_4 was then poured carefully down the side of the test tube. A violet ring at the interphase indicates the presence of carbohydrate.

(b) To 3ml of the aqueous extract was added about 1ml of iodine solution. A purple colouration at the interphase indicates the presence of carbohydrates.

Determination of Crude Fiber (CF)

A moisture-free and ether extracted sample was digested first with a weak acid solution, then a weak base solution. The organic residue was gathered in a filter crucible. The loss of weight on ignition is equivalent to the crude fiber.

Procedure

About 2gm of the dried, fat-free sample was taken into a 600ml beaker. 200ml of hot

sulphuric acid was added and the beaker was placed under the condenser and boiled gently for exactly 30 min. Distilled water was used in order to maintain volume and to wash down particles sticking to the sides. Filtered through Whatman No.541 paper in a Buchner funnel, using suction. Washing with boiling water has been provided. Residue was transferred back to the beaker and 200ml hot sodium hydroxide solution was added. Replaced under the condenser and again brought to boiling within 1 min. After boiling for exactly 30min, it was filtered through a porous crucible and washed with boiling water; 1% hydrochloric acid and then again with boiling water. Mashing was given twice with alcohol or acetone, dried overnight at 100^{o}C, cooled and weighed. Ashed at 500^{o}C for 3 hours, cooled and weighted. The weight of fiber was calculated by the difference in weight.

Determination of Lipids

This test it used to know the solubility of lipids in some solvents, according to polarity feature lipids are insoluble in polar solvents because lipids are non polar compounds, thus lipids are soluble in non polar solventslike chloroform, benzene and boiling alcohol.

Procedure

In two clean dry test tubes add 1ml of live oil for each tube. Add for the first one 1ml of chloroform. Add for the second tube 1 ml of distilled water. Shake both tubes vigorously for 2 minutes. Allow the tubes to stand and note the formation of homogenous solution with chloroform indicating that the lipid is dissolved and the formation of two layers with water indicating that the lipid is insoluble in water.

Determination of CalciumProcedure

Taken 2ml of extracted seed sample and 2ml of prepared ash solution in test tubes, added 2.0 ml of distilled water and 1.0 ml of 4% ammonium oxalate, mixed well and allowed to stand overnight. After calcium precipitation, centrifuged and removed the supernatant fluid without disturbing the precipitate. To this 3.0ml of 2% ammonia was addedalong the sides of the tube, mixed well and centrifuged again. Supernatant fluid was poured off. This was repeated until the supernatant gave no precipitate with calcium chloride solution. Added 2.0ml of 1N H_2SO_4 and mixed the precipitate well, placed in boiling water bath for few minutes. Keeping the mixture at 70-75^{o}C, titrated against 0.01 N $KMnO_4$, to a faint pink color, which persisted, which persisted for about a minute. Titrated 2.0ml of 1N H_2SO_4 as blank to the same endpoint. The difference between the titration gives the volume of 0.01N

$KMnO_4$ required to titrate the calcium oxalate. The estimation was done in triplicates and the results wereexpressed mg/g sample.

Determination of antimicrobial activity (Perez et al., 1990) Preparation of extract

The aqueous and acetone extracts of *Pleurotus ostreatus* were prepared in similar concentration of 100 µg/ml and used for antimicrobial activity by well diffusion method.

Test micro organisms

The following bacterial and fungal strains were used for the screening of antimicrobial activity. All the microbial strains of human pathogens used were procured from Indian Biotrack Research Institute and procured microbes are the Gram – negative bacteria, viz., *pseudomonas sp*, and the Gram – positive bacteria viz. *Bacillus cereus*, *Enterobacter aeromonas*, and fungi viz., *Aspergillus flavus*, *Trichoderma viride*, *Penicillium sp* and were selected for this study.

Media used

Potato Dextrose Agar (PDA) were used for testing the antifungal activity.

Agar well-diffusion method

Agar well-diffusion method was followed for determination of antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old-broth culture of respective bacteria and fungi. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. About 20, 40, 60 and 80 µl of aqueous and ethanol extracts were added using sterilized dropping pipettes into the wells and plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions the plateswere incubated in an upright position at $37^oC \pm 2^oC$ for 24h for bacterial and $28^oC \pm 2^oC$ for fungi. Results were recorded as the presence or absence of inhibition zone. Triplicates were maintained and the average values were recorded for antimicrobial activity.

Composition of potato dextrose agar (g/l)

Ingredients	**-**	**Gms / Litre**
Potato Infusion	-	200g
Dextrose	-	20.0g

Agar	-	20.0g
Distilled water	-	1000 ml
P^H	-	5.5

Composition of potato dextrose agar (g/l)

Ingredients	Gms / Litre
Peptone	5.0g
Beef extract	3.0g
Sodium chloride	5.0g
Agar	15.0g
Distilled water	1000ml
P^H	7.0

RESULTS

Analysis of yield parameters of the harvested edible mushroom

In first harvesting (after 20 days of bed preparation) the maximum fruit bodies (400 gm) were harvested in paddy straw substrate. After first harvesting is over the second harvest were also done after 25 days of bed preparation. In this period, the maximum fruit bodies were harvested in paddy straw substrate using prepared bed (350 gm) were recorded. After second harvesting is over, the third time were also harvested the fruit bodiesafter 30 days of the bed preparation. In this period the maximum fruit bodies were harvested in paddy substrate using prepared bed (200 gm) produce fruit bodies (Table 1 and fig 1).

Nutritional Analysis of *Pleurotus ostreatus*

The following nutritional values such as Carbohydrates, Crude fiber, Protein, Lipid and Calcium were analysed in harvested edible mushroom.

Antibacterial activity of dried sample

Antibacterial activity of *Pleurotus ostreatus* sample of against some bacterial species such as *Salmonella typhi, Enterobacter aeromonas* and *Pseudomonas sp* were studied by using disc diffusion method. The maximum zone of inhibition were observed in *E.coli* (17 mm) followed by *Pseudomonas aeroginosa* (16 mm) *Staphylococcus aureus* (15 mm) and *Klebsiella pnemoniae* (14 mm) at the concentration of 100 µg of dried sample. Increased concentration of the sample, the zone of inhibition increased and decreased concentration, the zone of inhibition also decreasedagainst the bacterial species (Table-4) (Fig-2).

Antifungal activity of dried sample

Antifungal activity of dried sample of *Zingiber officinate* at the concentration 25, 50, 75 and 100 µg against some fungal species such as *Aspergillus flavus, A.terreus, Penicillum* sp and *Fusarium* sp were studied by using agar well diffusion method. The maximum zone of inhibition were observed at the concentration of 100µg of dried sample against *Fusarium* sp (19mm), followed by *A.terreus* (12mm), *Penicillum* sp (10mm) and *Aspergillus flavus* (9mm). The minimum zone of inhibition were observed at the concentration of 25µg of dried sample against *Fusarium* (10mm) and N0 zone of inhibition were observed against *A.flavus, A.terreus* and *Penicillum* sp respectively (Table – 6) (Fig:4).

Table 1: Cultivation of *Pleurotus ostreatus.*

Substrate	Paddy Straw
1st Harvest	400 gm
2nd Harvest	350 gm
3rd Harvest	200 gm

Table 2: Nutritional analysis of *Pleurotus ostreatus.*

Substrate	Paddy Straw
Carbohydrate	52.7±0.07
Crude Fiber	47.9±0.04
Lipid	40.2±0.05
Protein	59.8±0.06
Calcium	0.27±0.03

Table 3: Antibacterial activity of *Pleurotus ostreatus.*

Name of the Bacteria	Zone of inhibition (mm)							
	Aqueous				Acetone			
	20 µl	40 µl	60 µl	80 µl	20 µl	40 µl	60 µl	80 µl
Salmonella typhi	4.01±0.57	5.00±0.57	8.01±0.58	10.01±0.59	1.27±0.57	1.60±0.57	3.05±0.58	0.48±0.59
Enterobacter aeromonus	5.33±0.88	7.00±0.57	9.66±0.88	13.33±1.20	4.08±1.15	7.00±1.15	11.0±1.15	14.00±0.57
Pseudomonas sp	3.33±1.20	3.66±0.88	10.33±0.88	13.66±0.89	2.05±0.57	7.02±0.58	9.00±0.58	11.00±0.60

Table 4: Antifungal activity of *Pleurotus ostreatus.*

Name of the fungus	Zone of inhibition (mm)							
	Aqueous				Acetone			
	20	40	60	80	20	40	60	80
Aspergillus	3.33±0.88	4.00±0.57	5.00±0.57	10.01±0.57	3.05±0.57	4.00±0.57	7.00±0.58	10.00±0.58
Trichoderma viride	2.00±0.57	4.01±0.57	7.00±0.88	9.00±0.57	3.01±0.57	6.66±0.88	9.00±0.57	12.00±0.57
Aspergillus terreus	4.01±0.57	5.66±0.88	9.33±0.88	13.00±0.57	2.05±0.57	5.66±0.88	9.00±0.57	11.00±0.57

CULTIVATION PHOTOS

Preparation of mushroom bed

Harvesting of mushroom

REFERENCES

1. Bradford, M.M A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, 1976; 7(72): 248-54.
2. Mandeel, Q.A., Al-Laith, A. A and Mohamed, S.A Cultivation of oyster mushrooms (Pleurotus spp.) on various lingo cellulosic wastes. World J of Microbiol & Biotechnol, 2005; 21: 601-607.
3. Alananbeh, K.M and Bouqellah, N.A Cultivation of oyster mushroom Pleurotus ostreatus on date-palm leaves mixed with other agro-wastes in Saudi Arabia. Saudi J of biol, 2014; 21(6): 616-625.
4. AOAC. Official method analysis. Association of Analytical Chemists. Washington D.C.15th edition, 1990; 1298.
5. Bahl, N. Hand book on mushrooms. Oxford &IBH Publishing co. Pvt. Ltd., 1998; 15-40.
6. Chang, S.T., Lau, W.O., Cho, K.Y The cultivation and nutritional value of Pleurotus sajor-caju. Euro J of Appl Microbiol, 1981; 12(1): 58-62.
7. Dundar, A., Acay, H and Yildiz, A Yield performances and nutritional contents of three oyster mushroom species cultivated on wheat stalk. Afri J of Biotechnol, 2008; 7(19): 3497-3501.
8. Eger, G., Eden, G., Wissig, E Pleurotus ostreatus, breeding potential of a new cultivated

mushroom. Theor. Appl. Genet, 1976; 47: 155- 163.

9. Sánchez, C Cultivation of Pleurotus ostreatus and other edible mushrooms. Appl. Microbiol. Biotechnol, 2010; 85: 1321-1337.
10. Soto-Cruz, O and Saucedo-Castaneda, G Effect of substrate composition on the mycelia growth of Pleurotus ostreatus. An analysis by mixture and response surface methodologies. Process Biochem, 1999; 35(1-2): 127-133.
11. Yalinkilic, M.K., Altun, L., Baysal, E and Demirci, Z Development of mushroom cultivation techniques in Eastern Black Sea Region of Turkey. Project of the Scientific and Technical Research Council of Turkey (TUBITAK), No TOAG-875, 1994; 287.
12. Yildiz, A and Ertekin, A.S An experimental study of mycelia development periods of some Pleurotus species. Mush. Res, 1996; 5: 81-88.
13. Zadrazil, F and Kurtzman, J.R The Biology of Pleurotus cultivation in the tropics. In: Chang, S. T. and Quimio, T. H. (eds.). Tropical Mushrooms, Biological Nature and Cultivation Methods. The Chinese University Press. Hong Kong, 1982; 493.