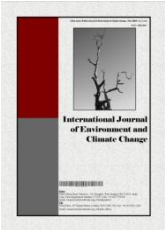

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Strategies for Improvement in Cultivation Practices of Oyster Mushrooms in North Bengal, India

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Mushrooms are used as an edible food and medicine since time immemorial. It constitutes a chief source to reduce body weight. The different agricultural wastes were used in cultivation of oyster mushroom. In West Bengal, enormous amount of different wastes are produced annually, and are of no uses. These wastes could be possibly used as a source of food i.e. substrates for cultivation of oyster mushroom. However, the most common cultivation methods of oyster mushroom were found in partial sterilized or sterilized paddy straw. Different types of agro-wastes, by-products and crop residues can be used with conventional polybag method to achieve this mushroom cultivation more profitable and popular. Oyster mushroom was cultivated on different substrates viz. paddy straw, wheat straw, sugarcane bagasse and combination of different straw to assess the suitable substrate. Use of different substrates significantly affected the number of primordia and fruiting bodies, and the amount of fresh weight or yield of mushroom. The highest number of fruiting bodies, the amount of fresh weight and the yield was procured from rice straw in combination with wheat straw followed by rice straw and the lowest with wheat straw. The biological efficiency was also higher in rice with wheat straw. The N, P and K content in straw was found higher in rice

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straw. The molecular characterization of *Pleurotus* sp. revealed that maximum similarity of the sequence with *Pleurotus ostreatus*. A 95 % coverage of the sequence resulted in 92.55% similarity with fungal strain.

Keywords: Oyster mushroom; substrates; cultivation; yield; ITS sequencing.

1. INTRODUCTION

Improving food safety and food security is imperative to adequately feed a growing population that is expected to exceed 9 billion people in this globe by 2050. Agriculture sector in India occupies a key position in the economy and provides employment to more than 60% of the working population of India and also contributes 15.4 % in GDP. The food and nutritional security of growing population is a great challenge in India, which looks for new crop as a source of food and nutrition. In this context, mushroom cultivation provides unique opportunity to take advantage of underutilized resources and produce high quality food from different wastes. Mushrooms can be grown even by landless people, that too on waste material and could be a source for high proteinaceous food [1]. Use of mushrooms as neutriteous food has been known since time immemorial and shows as an evident from the explanation in old epics of Vedas and Bible. In the past, Indian civilization had also treasured for its delicacy and therapeutic value. The cultivation of mushroom has developed all around the parts of the world and the world mushroom productions gain the growth rate of 10% during last decades. In India, owing to varied agro-climate abundance of farm waste, different types of mushrooms are cultivated throughout the country [2].

The fungi kingdom is dividing from plants, animals and bacteria. Fungi are the eukaryotic achlorophyllus organisms having absorptive mode of nutrition. According to classification, the fungi kingdom is comprised of seven phyla namely, Ascomycota, Basidiomycota, Blastocladiomycota, Chytridiomycota, Glomeromycota, Microsporidia and Neocallimastigomycota [3]. Ascomycota and Basidiomycota are considered as major phyla within the sub-kingdom Dikarya. Mushrooms are the macro fungi and classified under the phylum Basidiomycota, division Eumycota, subdivision Basidiomycotina and class Hymenomycetes [4].

Button mushroom ranked first among edible mushrooms and in the world for cultivation. Currently, oyster mushroom gain popularity and rank second in world production [5]. Oyster mushrooms are also known as 'Dhingri' in India. In Greek word, the "pleura" means lateral which refers to the lateral position of the stem in relation to the cap. Species of the genus *Pleurotus* are well recognized for their adaptability in different agro-climatic conditions and most ease way of cultivation for commercial scale production. Parameters for days of spawn run, pinhead formation, number of primordia and first harvest, total weight and total yield of mushroom were measured. With this background information, a study was undertaken to identify the most suitable oyster mushroom and its growth in different substrates available in this region.

The genetic study of mushroom can be work out by molecular markers particularly using polymerase chain reaction (PCR). It is an efficient tools used for the assessment of genetic diversity. Oyster mushroom (*Pleurotus* sp.) was differentiated on the basis of morphological (sporocarp, sporophore, colour, size), cultural, ITS sequencing (accession number) and Phylogenetic analysis [6,7].

Among the different mushrooms, oyster mushrooms are one of the most popular edible mushrooms in South Asia and its cultivation is becoming increasingly popular in India with the growing awareness of people about their food and health. Beside this, the sub-tropical and tropical climate in India favours the growth of this mushroom which thrives better in a wide range of temperature and moisture. Among the different *Pleurotus* spp., *P. ostreatus* has received increasing attention for applications in bio-bleaching and the catalysis of difficult chemical conversions in the paper industry, textile dye decolourization, and detoxification of environmental pollutants [8].

However, Oyster mushroom farming is an eco-friendly sustainable technology used for production of nutritive and medicinal fruiting bodies on agricultural waste which are commonly practices mainly in northern parts of India. Since mushroom is known for bioremediation and

bioaccumulation property, it plays an important role to detoxify toxic compounds below threshold limit by enzymatic actions. It also prefers the cultivation on agricultural waste as it is rich in cellulose, hemicellulose and lignin.

With this background information, a study was undertaken to identify the best species of oyster mushroom and its growth in different substrates available in this region with the following objectives.

1. Selection of most suitable *Pleurotus* species in the northern parts of West Bengal.
2. Identification of suitable substrates for optimum production of selected *Pleurotus* species.
3. Estimation of nutrient content in selected substrates.
4. Identification of *Pleurotus* species by ITS (Internal transcribed space) sequencing.

2. MATERIALS AND METHODS

Experimental Site

Studies on cultivation of oyster mushroom (*Pleurotus* spp.) on different agro-wastes were experimented in Research Laboratory, Department of Plant Pathology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Coochbehar, North Bengal, India.

Experimental Materials

The mother spawn of *Pleurotus* spp. were obtained from Department of Plant Pathology, UBKV, Pundibari, Coochbehar, North Bengal, India. The substrates used for cultivation are rice straw, wheat straw and sugarcane bagasse. They are used either as a whole substrate or in combination substrates with six treatments.

Design Layout and Treatments of the Experiment

The experiment was carried out in a Completely Randomized Design (CRD). Six substrates as treatments with three replications were considered. The mother spawn were produced by using wheat grain. The treatments were

T1=500g (RS)
T2=437.5g (RS) + 62.5g (SB)
T3=375g (RS) + 125g (SB)
T4=437.5g (RS) + 62.5g (WS)
T5=375g (RS) + 125g (WS)
T6=500g (WS)

Heltay and Zavodi, [9] observed that the importance of rice straw used as substrate not only for *Volvariella* but it is also used as an important component of synthetic *Pleurotus* compost containing 41% cellulose, 13% lignin, 0.8% total Nitrogen, 0.25% phosphorus pentoxide, 0.3% , 6% SiO_2, pH 6.9, C:N 58:1. Analysis on chemical composition of wheat straw which consists of cellulose (34-40%), hemicellulose (20-25%), and lignin (20%) [10]. Rice and wheat straws and cotton wastes are recommended the best productive substrates among the other substrates [11]. Park et al. [12] performed rice and wheat straw as substrates, and the yield on rice straw was slightly higher as compare to wheat straw.

Varietal Characteristics of Oyster Mushroom

The *Pleurotus* spp. was recognized for its quicker rooting hyphae and more decaying capacity on cellulosic substrates. Its basidiocarps are spatula-shaped. In this experiment, the three *Pleurotus* spp. is used which is white colored.

Preparation of Potato Dextrose Agar (PDA)

To prepare 1L PDA media, 200g washed peeled and sliced potatoes was used. 20g of dextrose and agar was added and add distilled water to make volume up to 1L. The media was then sterilized in an autoclave for 15 min at 121°C under 15 p.s.i.

Tissue Culture

A small portion of basidiocarp tissue was inoculated on sterilized PDA medium under controlled environment in a laminar air flow chamber and the Petri plates were incubated at 25°C for 7 - 10 days. These are considered as pure cultures of *Pleurotus* spp. and used in the entire experiment (Fig. 1).

Production of Tissue Mother

Two steps were followed to produce mother culture. Sterilized PDA media was used for the culture of tissue of oyster mushrooms and were produced from this tissue mother.

Morphological Identification

The *Pleurotus* sp. was identified based on various morphological characters such as mycelium growth, stipe length and diameter, pileus diameter and microscopic view of mushroom spore and spore print (Fig. 2).

Preparation of Spawn

Mushroom spawn was prepared by using wheat grains. The grains were cleaned thoroughly and inert matter, stubbles, debris were removed properly before boiling. After which the grains were washed and boiled for 50 minutes. Then dry in air on polythene sheet by spreading to remove surface moisture. 2% Calcium carbonate (lime) and 1% Calcium sulphate (gypsum) are thoroughly mixed to adjust pH and to prevent from clump formation. 200g of such grain was packed in 15×18 cm polypropylene bag or in 500 ml conical flask. It was autoclaved at 121^{0}C for 15-20 minutes at 15 p.s.i. They are inoculated with the mycelium of the fungus and incubate for 3-4 weeks at 24+1^{0}C (Fig. 3).

Preparation of Substrate

Siddiqui [13] method was followed in performance of different substrates for oyster mushroom cultivation. The wheat and paddy straw, sugarcane bagasse were dried under the sun completely and chopped into short length (2-4 cm). The bags full of dried different substrates were weighed before soaking. It was maintained in a bag of 500g for each substrate. Then soak the substrates in water for overnight to achieve 65-70% moisture content. On the next day, decant excess water gently which was boiled for 1 hour. After excess water was removed, the substrates are ready for the cultivation of mushroom using polypropylene bags and applied spawn in four layers per bag.

Pasteurization of Substrates

The substrates were primarily soaked to saturate with water. Hot water treatment of substrate was done to avoid contamination and to gain a higher yield. At first, water was boiled in a big container and then wet substrate was filled in gunny bags by dipping in hot water at 80-85^{0}C for 1 hour. It was pressed with some heavy material or with the help of a wooden piece to avoid floating. Excess hot water was drained off after pasteurization from the container. The temperature of hot water was maintained at 80-85^{0}C for all sets to achieve pasteurization.

Spawning of Substrates for Oyster Mushroom Cultivation

It was ready for filling and spawning in cooled pasteurized bags. Polypropylene bags of size 35 x 50 cm were used. 200g spawn packets were used and spawning was done in four layering per bag. Substrate was filled and pressed to a depth of 5-7 cm and broadcasted with a handful of spawn above it. This was followed similarly in 2nd, 3rd and 4th layers of substrate and the mouths of the bags were tightened with rubber band. After packing, they were stacked on cleaned racks in the culture dark room. The culture room of temperature and humidity was maintained at 25-28^{0}C and 70-85% respectively. 15-20 days are required for fully growth of white mycelium (Fig. 3).

Following the method of Chang and Miles [14], inoculated bags were taken into a dark locker room for proper initiation of white mycelium growth. When fully covered mycelial growth in the bags and emergence of pinhead were noticed, the bags were cross cut at the site of primordial initiation by sterilized sharp blade to create perforations and facilitate the development of fruiting bodies (Fig. 3).These fully colonized bags were then transferred to growing room and stored on cleaned racks that are made from wood and nylon rope at a spacing of 15-20 cm. The growth room was occasionally ventilated leaving the door open. From the pinhead formation onwards, regular inspection along with watering was followed to keep the mycelia moist for 2-3 times in a day.

Effect of Substrates on Media at Different Temperature

The prepared media of different substrates are autoclaved at 121^{0}C for 15-20 minutes at 15 p.s.i. The radial growths of mycelium were recorded periodically during 3rd, 5th, 7th, 9th days after inoculation of oyster mushroom (*Pleurotus* sp.) at 20^{0}C, 25^{0}C and 30^{0}C. (Fig. 4) Kong, [15] reported that *P. ostreatus, P. florida, P. sajor-caju* achieve their optimum growth at 25^{0}C, while *P. cornucopiae and P.cystidiosus* reach their growth at the temperature 25-35^{0}C temperature. The optimum temperature of *P. ostreatus* for the mycelia growth has been recorded at 21-26^{0}C [16].

Cropping System and its Maintenance

The optimum temperature for the mushroom growth ranges from 20-33^{0}C. The relative humidity of the room can be maintained by spraying water twice a day. It is advice to give light spray when small pin-head start appearing. The right stage for harvesting mushroom should be before the spores shed from fruiting body to maintain good quality. The next flush usually appeared after 7-10 days. Oei, [17] has evaluated the relative humidity and room temperature which was monitored with thermo-hygrometer and maintained relative humidity within 80 and 85 % by spraying fine mist of water occasionally.

Maturity of Oyster Mushroom

The prepared bags were fully impregnated with white mycelium after 20-22 days. The mushrooms are matured within 3-4 days after the initiation of pin head. Matured fruiting body was indicated by cural margin of the cap. The mushroom was harvested by twisting near the end portion base (stipe) and can be harvested 3-4 times in a packet. The mushrooms are harvested till the nutrients in each substrate were exhausted.

The days required for primordial initiation during second and third harvest are commonly between 9-10 and 7-8 days respectively. Spraying of water was continued until the mushrooms were ready to be harvested. The time required for harvesting was also varied depending upon the type of substrates reported by Sarker et al. [18].

Data Collection

Data was collected from sprouting till maturity of the mushroom and recorded periodically during the growing season as 1st, 2ndand 3rd flush. Days of spawn running, primordial initiation, number of fruiting bodies and fresh weight of mushroom were recorded. The observation on stipe length (cm) and pileus width (cm) which are considered as the main constituent of fruiting bodies were also collected. The total yield, moisture content and dry weight of each substrate were recorded from all the treatments of each substrate.

Moisture and Dry Matter

Twenty gram (20g) of freshly harvested fruit body was taken as a sample for calculating the amount of dry matter and was kept in a hot air oven for drying at 100-105^{0}C. The process of heating and cooling was continued till a constant weight was acquired. The percent moisture and dry matter were determined with the help of laboratory oven, from the differences between fresh and dry weights of the samples by using the following equation [19].

$$\text{Moisture \%} = \frac{w1-w2}{weight\ of\ sample} \times 100$$

Dry mater % = 100 – Moisture %

W1 = Fresh weight W2 = Oven dry weight

Biological Efficiency (B. E)

Khan, [20] concluded that *Pleurotus ostreatus* obtain highest yield in the first harvest and subsequently reduces in second and third flush. The highest number of pin-head and fruiting bodies of oyster mushroom was found in sterilized paddy [21]. According to the report, [11,22,23]. cultivation of oyster mushroom in paddy straw produce the maximum yield to influence its growth, yield and composition. The BE of mushroom was calculated from the equation [24].

$$\text{BE= } fresh \text{ weight } of \text{ } mushroom \text{ (g) / } dry \text{ weight } of \text{ } su\text{(g)} \times 100$$

Total yield and biological efficiency harvested mature mushrooms were weighed with analytical balance to determine the biological efficiency of mushrooms produced from substrates. The average BE of harvests was calculated and comparison was made among different substrates.

Determination of N, P and K Content of Substrates

Nitrogen in culture broth was determined by KEL PLUS nitrogen estimation system [25], phosphorous and potassium was estimated by Vanado molybdo phosphoric yellow colour method, Flame photometry respectively [26].

Quantitative Estimation of Protein in Fresh and Dry Mushroom

Total protein of mushroom was estimated following Lowry's method [27]. Oyster mushrooms contribute a high nutritional value of protein (25-50%), sugars (17-47%), cellulose (7-38%) and minerals [28].

Molecular Identification and ITS Sequencing of *Pleurotus* sp.

Mushroom species was identified by genome sequencing using ITS primer. The fungal genomic DNA was extracted from fresh mycelia culture and stored at -20°C for further process. *Pleurotus ostreatus* culture was used for ITS sequencing. The DNA was extracted with fungal DNA isolation kit (Qiagen). The DNA was checked for its quality and sent to sequencing company with the set of primers. The PCR amplicon sequence was generated from both (forward and reverse sequence) data using aligner software. The ITS region sequence was used to carry out BLAST with the database of NCBI Gen Bank (https:// blast.ncbi.nlm.nih.gov/Blast.cgi). The first ten sequences were selected based on maximum identity score and were aligned by multiple alignment software programs. The phylogenetic tree was constructed using MEGA X [29].

Primer used in the Study:

Primer name	Primer sequence (5'-3')	rRNA operation binding site
ITS1	*CTTGGTCATTT AGAGGAAGTAA*	Small subunit Gardes and Bruns, [30]
ITS 4	TCCTCCGCTTA TTGATATGC	Large subunit [31]

3. RESULTS AND DISCUSSION

Morphological Studies

According to the finding of this study, the morphological characteristics of *Pleurtous ostreatus* was confirmed in similarity with Chhetry and Pfoze, [32] who reported that the pileus was soft and smooth, light yellow with diameter of 4.5 -11 cm. It also possessed white and broad decurrent gills with regular lateral stipe, usually elongated from 5-8 cm with several fruit bodies that joined at the base forming a large common base. The fresh sample is soft, white with mild odour. Ram, [33] reported that *P. ostreatus* was characterized morphologically by white spores with an eccentric or lateral stem of fleshy texture. Cap size was 2-15 cm wide with 3-11 cm long and smooth with white upper surface, spathulae to kidney shaped, margin decurved or inrolled. Stem was generally short or stem poke base, imbricate in groups of 5-20 cm. Gills were 18-20 cm at margin, and 5-15 mm wide, decurrent, sometimes unite forming a net or pore like pattern on the stem. It appears white when fresh, yellowish when dried. Murugesan et al. [34] also dedicate a work on isolation of oyster mushroom from forest region and identified *Pleurotus* sp. based on microscopic view and phylogenetic basis.

Relative Performance of *Pleurotus* spp.

Among the three different isolates of *Pleurotus* in rice straw substrate, there is a variation of yield and biological efficiency. The highest yield (847g) was obtained in Isolate 1 and no statistical differences between Isolate 2 and 3 were recorded. Regarding spawn run, there are no significant differences (Table 1). Based on the observation, *Pleurotus* 1 isolate was selected for further experimentation.

Effect of Different Substrates on Mycelial Growth of *Pleurotus* Isolate 1

In the next step, the effect of different substrates viz. sugarcane baggase, mustard straw, polygonum weed, rice straw and wheat straw on the mycelial growth of *Pleurotus* 1 isolate were carried out at three different temperature levels (20^0C, 25^0C and 30^0C) Figs. 4. (a, b and c). The substrates were dried, powdered and agar was mixed. After sterilization, the substrate agar media was pour into petri-plates and subsequently inoculated with *Pleurotus* Isolate 1. The observation of mycelial growth in mm were recorded at 3^{rd}, 5^{th}, 7^{th} and 9^{th} day (Left to Right) after inoculation and presented in Table 2.

From Table 2 indicates that there is a strong variation of mycelial growth of *Pleurotus* sp. among the substrates at different temperature levels. Irrespective of the substrates, higher growth was observed at 25^0C and lowest growth at 30^0C. At 25^0C, the highest growth was recorded in wheat straw followed by rice straw; the lowest growth was recorded with polygonum weed though the differences are significant (at 5%). Based on the performance of different substrates, rice straw, wheat straw and sugarcane bagasse were selected for their efficacy on yield parameters of *Pleurotus* sp.

Effect on Yield Performance of Different Substrates of Oyster Mushroom

Most organic matters containing cellulose, hemicelluloses and lignin can be used as mushroom substrate i.e. rice straw, wheat straw,

sugarcane bagasse, mustard straw, banana leaves, saw dust, waste paper etc. As per information available the amount of nutrition requirements differs according to mushroom species and types of substrates used. This study was designed to identify the suitable substrate for *Pleurotus* sp. available in this region. Accordingly, six (6) different treatments consist of either single substrate or in combinations were selected and inoculation was done as described in methodology (Fig. 5). The yield and yield attributing characters of mushroom obtained from different substrates are presented in Table 3.

The substrate used in this study showed variation in spawn run, fruiting body formation, cap diameter, stipe length and fresh weight (Table 3). Among the different substrate, wheat straw (29%) required lesser time for spawn run (14 days) followed by rice + wheat straw (14.5%) and longer duration was required for rice straw in combination with sugarcane bagasse (18 days). However, primordial formation was fastest in rice straw with wheat straw (14.5%) and lowest in wheat straw.

The stipe length and cap diameter of *Pleurotus* mushroom was measured in an average up to two harvests and observed significant difference among the substrate. The highest stipe length was obtained in rice straw with wheat straw (3.2 cm) followed by T5 (3.1 cm) and lastly in wheat straw (2.4 cm). Likewise, cap diameter was also found highest from T4 i.e. 7.40 cm followed by T5 (7.27 cm) under similar environment and cultural practices among the substrates (Fig. 5).

Regarding yield of mushroom, highest yield obtained in rice straw with wheat (938g) which is closely followed by rice straw alone (914g) and lowest yield in wheat straw (469g) (Fig. 6). The dry weight was higher in rice + wheat straw followed by rice + sugarcane bagasse. The biological efficiency was highest in rice + wheat straw. The moisture content was varied from 85-87%.

Relationship between colonization duration and first harvest duration was assessed and presented in Fig. 7. A positive linear relationship was observed between first colonization and first harvest. The result shows that first harvest depends on first colonization duration and more than 26% (R^2= 0.2589) in the first harvest duration may be explained by variation of first colonization duration.

Estimation of N, P, K Content in Selected Straw

The above studies indicate that the nature of substrate has a positive effect on the yield of oyster mushroom, so it was considered to assay the nitrogen, phosphorus and potassium content in selected effective substrate viz. Rice straw alone and Rice + wheat straw combination. The standard protocols were followed which was described in methodology and the results are presented in Table 4.

Table 4 shows that the variation in nitrogen content is negligible whereas the phosphorus and potassium content were higher in rice straw than rice + wheat straw combination. The trend was also graphically presented in Fig. 8.

Impact of Substrates on Yield and Protein Content and of Mushrooms

Mushroom is known for its high protein content. As earlier studies indicate that different substrates have strong influence on the yield parameters, it was considered to investigate the relationship between the various substrates and the protein content of *Pleurotus*. Accordingly, the fresh mushroom was harvested from 1st harvesting date and the protein value was estimated as per methods described earlier. The result is presented in Table 5.

A variation in protein content and nature of substrate was recorded. The highest amount of protein was recorded in wheat straw followed by rice + wheat straw mixture (Table 5) which indicates that wheat straw is best substrate for quality protein mushroom though the yield is low.

Economic Analysis

The economic analysis were also carried out in different substrate by calculating the cost of materials and production and presented in Tables 6 and 7.

The results show that higher economic benefit can be obtained from use of rice straw as substrate for cultivation of *Pleurotus* followed by rice straw + wheat straw. However, Cost benefit ratio is higher in rice straw. The low yield in wheat straw might be due to high moisture content and quality of straw.

Fig. 1. Isolation and pure culture of *Pleurotus spp.*

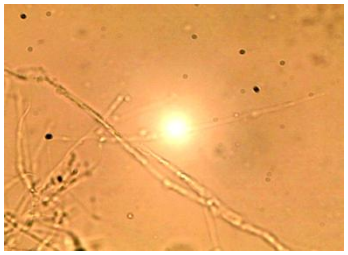

Fig. 2. Microscopic view

Fig. 3. Cultivation and white myciliam growth of *Pleurotus* sp.

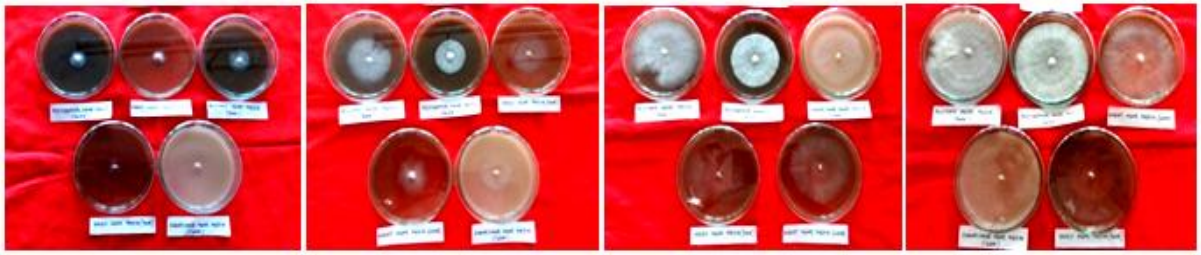

Fig. 4. a) Mycelial growth of *Pleurotus* Isolate 1 in different substrates at 20^{0}C

Fig. 4. b) Mycelial growth of *Pleurotus* Isolate 1 in different substrates at 25^{0}C

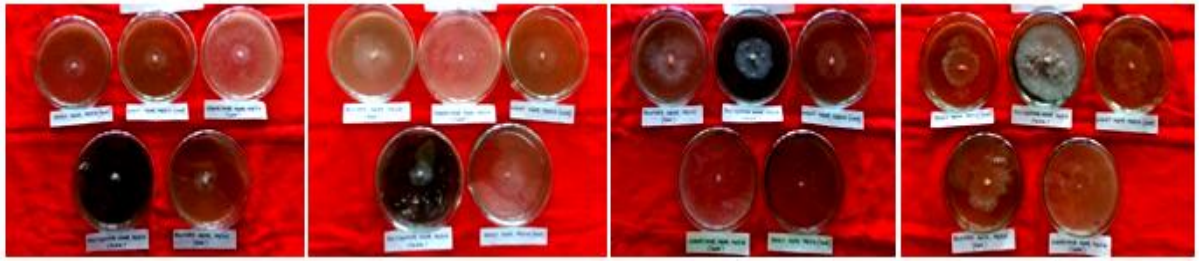

Fig. 4. c) Mycelial growth of *Pleurotus* Isolate 1 in different substrates at 30^{0}C

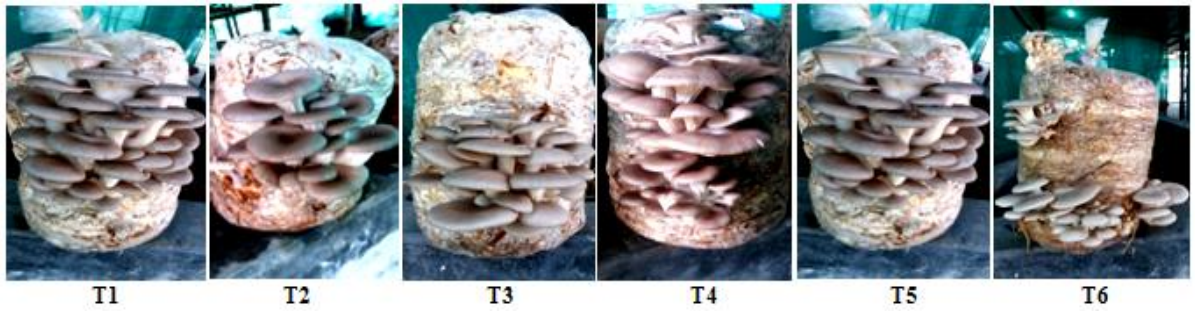

Fig. 5. Mushroom production in different substrates

Table 1. Relative performance of three different species of *Pleurotus*

***Pleurotus* spp. (Isolate)**	**Spawn run (days)**	**Yield (g) of 1st harvest**	**Yield (g) of 2nd harvest**	**Yield (g) of 3rd harvest**	**Total fresh yield (g/bag)**	**Biological efficiency (B.E) (%)**
Pleurotus 1	18	613	211	23	847	179.22
Pleurotus 2	17	523	123	31	677	167.8
Pleurotus 3	19	534	129	35	698	169.24
CD at 5%	NS	36.17	32.19	12.28	36.88	8.79

*****CD – Critical difference*

Table 2. Mycelium growth of *Pleurotus* Isolate 1 in different substrates at different temperature level

Substrates	**Mycelial growth in mm at 20^{0}C**				**Mycelial growth in mm at 25^{0}C**				**Mycelial growth in mm at 30^{0}C**			
	3rd day	**5th day**	**7th day**	**9th day**	**3rd day**	**5th day**	**7th day**	**9th day**	**3rd day**	**5th day**	**7th day**	**9th day**
Sugarcane bagasse	9	19	60	70	7	38	64	82.5	16.5	35	50	55
Polygonum weed	8	35	62.5	69	22	36	60	80.5	11	26.5	57.5	70
Rice straw	14.5	30	48	79.5	13	42.5	68	83.0	19	28.5	33.5	41.5
Wheat straw	13.5	29	74.5	85.5	11	40	72.5	84.5	20.5	33.5	37.0	39.5
Mustard straw	15	30.5	43	71.5	27	49.5	79.5	81.5	22.5	35.0	44.5	56.0
CD at 5%	1.35	2.56	4.66	2.18	1.01	2.34	2.89	2.11	1.23	1.9	2.11	2.78

*****SB - Sugarcane bagasse, PW - Polygonum weed, RS - Rice straw, WS - Wheat straw, MS - Mustard straw*

Table 3. Effect of different substrates on oyster mushroom (*pleurotus* sp.) production

Treatment	**Spawn run (days)**	**Primordial formation (days)**	**Fruiting body formation (days)**	**No. of fruiting bodies bag^{-1}**	**Stipe length (cm)**	**Cap diameter (cm)**	**Yield of 1st harvest (g)**	**Yield of 2nd harvest (g)**	**Yield of 3rd harvest (g)**	**Total fresh yield (g bag^{-1})**	**Dry wt. (20g fresh wt.)**	**B.E (%)**	**Moisture content (%)**
T1	16.33	25.00	28.67	58.33	3.0	7.17	656.67	191.00	66.33	914.00	2.63	182.80	87
T2	18.00	26.33	30.67	49.67	2.9	6.77	588.67	168.67	48.33	805.67	2.83	161.13	86
T3	17.00	25.67	29.33	52.67	2.7	7.07	591.67	174.00	50.00	813.67	2.37	162.73	88
T4	15.00	23.33	26.33	59.00	3.2	7.40	664.67	197.33	76.67	938.67	2.80	187.73	86
T5	15.67	23.67	26.67	54.67	3.1	7.27	621.67	181.33	52.00	855.00	3.00	171.00	85
T6	14.00	27.67	31.33	37.67	2.4	6.63	368.33	80.33	20.33	469.00	2.53	93.80	87
CD at 5%	3.30	4.06	4.05	9.05	0.506	0.78	78.94	52.49	43.13	95.5	0.979	19.10	4.75

Table 4. Amount of N, P and K in the substrate

Treatments	Nitrogen (%)	Phosphorous (%)	Potassium (%)
RS	1.01	0.27	1.90
RS + WS	1.02	0.19	1.55
CD at 5%	0.002	0.0234	0.035

Table 5. Effect of substrate on protein content of oyster mushroom

Treatment	Protein content (g $100g^{-1}$ fresh wt.)	Yield of mushroom (500 g bag) in Kg
T1	31.24	1.256
T2	29.68	1.012
T3	28.76	0.987
T4	31.78	1.345
T5	30.98	1.298
T6	32.36	0.876
CD at 5%	1.256	0.0257

Table 6. Cost of materials (10 kg)

Treatment	Cost of spawn	Polypro- pylene cost	Covering plastic (Rs. 30 m^{-1})	Rope	Fuel	Straw cost (Rs. 3 kg^{-1})	Labour cost	Total cost
T1	150.0	6.00	10.00	5.00	10.00	30.0	125.0	336.0
T2	150.0	6.00	10.00	5.00	10.00	40.0	125.0	346.0
T3	150.0	6.00	10.00	5.00	10.00	50.0	125.0	356.0
T4	150.0	6.00	10.00	5.00	10.00	50.0	125.0	356.0
T5	150.0	6.00	10.00	5.00	10.00	55.0	125.0	361.0
T6	150.0	6.00	10.00	5.00	10.00	60.0	125.0	366.0

Table 7. Benefit from selling of mushroom

Treatment	Total yield (kg)	Rate (Rs. Kg^{-1})	Total sale (Rs.)	Total cost (Rs.)	Benefit (Rs.)
T1	18.28	60.00	1096.80	336.0	760.80
T2	16.10	60.00	966.0	346.0	620.0
T3	16.26	60.00	975.60	356.0	619.0
T4	18.76	60.00	1125.60	356.0	769.6
T5	17.10	60.00	1026.0	361.0	665.0
T6	9.38	60.00	562.80	366.0	196.8

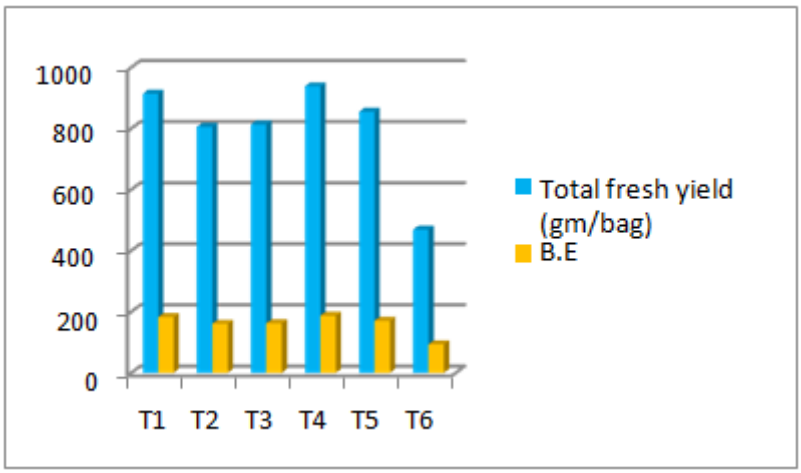

Fig. 6. Relationship between fresh yield and biological efficiency in different treatments

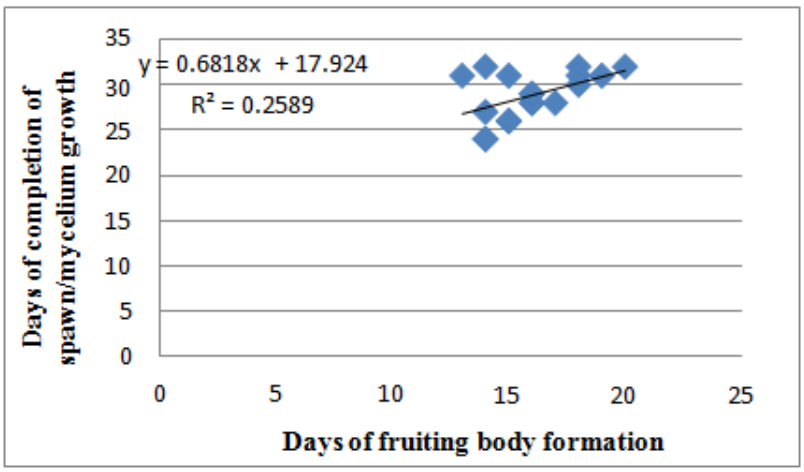

Fig. 7. Correlation between spawn run period and days to fruiting body formation in different treatments

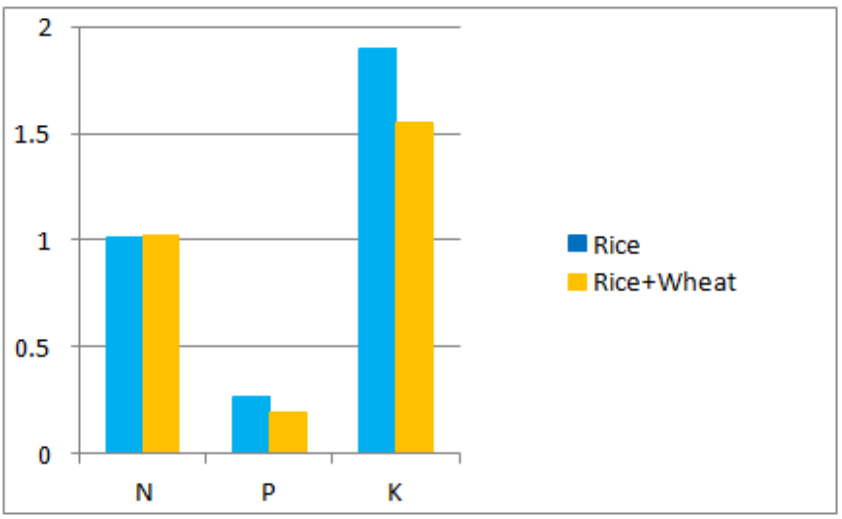

Fig. 8. Diagrammatic relationship between substrate and N, P and K content

Sequencing of *Pleurotus* sp.

To identify the species of *Pleurotus,* the sequence of the fungus was undertaken. The fungal DNA was isolated with DNA isolation kit and assessed for its quality. The DNA was amplified in PCR for ITS region using ITS primers. The 400-900 bp amplification was gel eluted and the product was sequenced by Sanger's method of DNA sequencing. The sequencing results were assembled. The 506 base pairs sequence was derived from sequencing machine and the sequence was subjected to BLAST search in NCBI data base (SOP NO. CRO - 01).

All the BLAST search results show that maximum similarity of the sequence with *Pleurotus ostreatus* (Fig. 9). A 95 % coverage of the sequence resulted in 92.55% similarity with fungal strain (Accession No. kx836129) (Fig. 9). Oyster mushroom (*Pleurotus ostreatus*) was molecularly identified by using ITS primer, which was closest match with result of [35]. They researched on twenty strains of *Pleurotus* spp. from different regions by DNA extract and for sequencing using ITS primer to analyse the genetic diversity of *Pleurotus* strains. The results observed that the major one included *P. ostreatus*, *P. cystidiosus*, *P. eryngii*, and *P. pulmonarius* among the 20 strains. Similarly, Liu et al. [36] evaluated the genetic diversity of *Pleurotus ostreatus* strains on the basis of ITS sequence, translation elongation factor (EF1α) and the second largest subunit of RNA polymerase II (RPB2). The polygenetic tree was constructed using combined results of the ITS, EF1α and RPB2. Sequence analyses showed the genetic relationship between assessed strains. They also provide important information on its relationship and identified as *Pleurotus ostreatus* by molecular method.

Oyster mushroom (*Pleurotus* spp.) categorized under the family of Tricholomataceae and is considered the worldwide second widely cultivated mushroom after the *Agaricus bisporus*. However, Obodai et al. [37] reported that the oyster mushroom is the third largest commercially produced mushroom in the world market. The *Pleurotus* spp. are well known recognized and cultivated widely throughout the world especially in Asia, America and Europe as its cultivation is simple, low cost production technology and high biological efficiency. Moreover, the interest of oyster mushroom is increasing largely due to its taste, nutrient, and medicinal properties [38]. *Pleurotus* species can efficiently degrade agricultural wastes and they grow at a wide range of temperatures [38]. *Pleurotus* species require carbon, nitrogen and inorganic compounds as their nutritional sources. As the main nutrients contain less nitrogen with more carbon, so the materials containing hemicelluloses, cellulose and lignin (i.e., rice and wheat straw, cotton seed hulls, sawdust, waste paper, leaves, and sugarcane residue) can be used widely as mushroom substrates [24]. Cultivation of oyster mushroom can be grown on a wide variety of substrates. However, the quality and the yield of oyster mushroom depend on the nutritional content and chemical of substrates [39].

The present study was conducted in the direction as follows. Among the three different isolates of *Pleurotus* available in the Department of Plant Pathology, *Pleurotus* Isolate 1 was selected for better yield. The selected *Pleurotus* was tested *in-vitro* in different substrate of locally available materials and observation indicates that 25^0C is optimum for futher investigation of this mushroom. The effect of substrates on yield parameters indicates that rice and wheat mixture is best for *Pleutotus* cultivation. The findings of our research were supported by Elattar et al. [40]. According to their observations, use of substrates such as rice straw with wheat straw mixture and single rice straw obtained the maximum yield (7600g and 6650g) respectively. The mushrooms grown on a mixture of rice straw and wheat straw showed the highest yield. It was also produced as a rich source of minerals, proteins and fibers. It concludes that the oyster mushroom developed on mixture of rice straw and wheat straw is nutritious and rich in pharmaceutical products. In another research, the highest mean value of growth and yield parameters were procured from the combination of sawdust and teff straw. While the lowest mean value of growth and yield parameters were observed from the combination of teff straw and onset waste [41]. The results confirm the earlier observation of [42]. The poor yield in sugarcane bagasse amended substrate and wheat straw is probably due to high sugar and moisture content respectively. These results confirm the earlier observation of [42]. The protein content of mushroom, however, was higher in substrate of wheat straw. The sequencing data of selected *Pleurotus* sp. using ITS primer indicates the similarity with *P. ostreatus.*

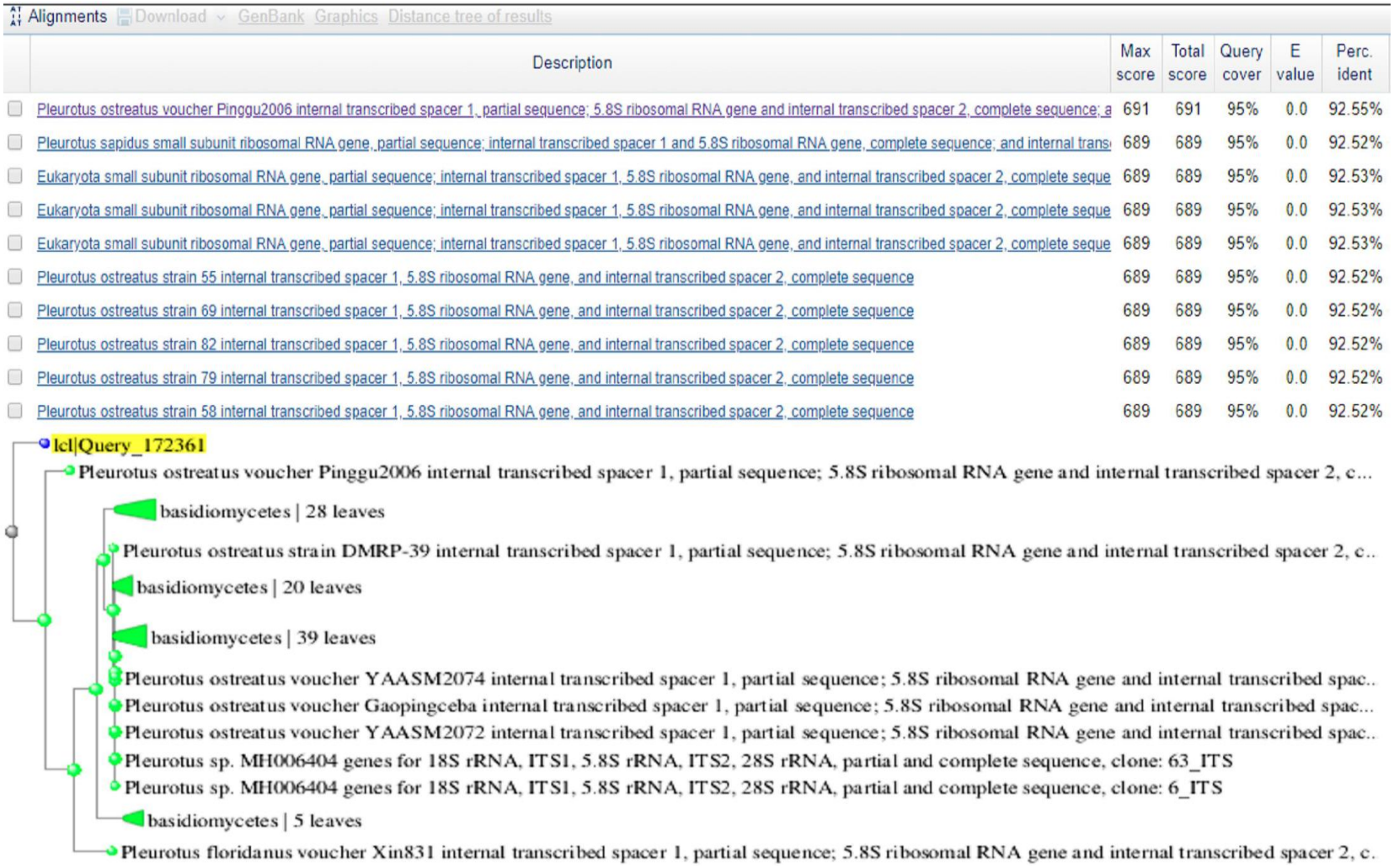

Fig. 9. Blast search of the sequence with existing database at NCBI

4. CONCLUSION

The experiment was conducted to find out the effect of different substrates on yield and other yield attributes of oyster mushroom. The study was carried out in the laboratory and culture house, Department of Plant Pathology, UBKV, Pundibari, in winter season.

The relative efficacy of three different isolates of *Pleurotus* in rice straw substrate showed Isolate 1 was better in terms of yield and biological efficiency. Further experimentation was conducted by using Isolate 1 only. The *in vitro* studies of five different substrates (rice, wheat, mustard, polygonum, sugarcane bagasse) at three temperature levels (20^0C, 25^0C and 30^0C) that higher growth was observed at 25^0C and lowest growth at 30^0C. At 25^0C, the highest growth was recorded in wheat straw followed by rice straw; the lowest growth was recorded with mustard straw. The effect of six different substrates *viz*., rice straw, wheat straw and sugarcane bagasse are used either as whole substrates or in combination substrates on yield and other parameters of *Pleurotus* sp. The rice straw mixed with wheat straw was better in terms of yield, biological efficiency as well as economics. This substrate is good with respect to N, P and K content though higher protein content was recorded with wheat straw. The sequencing data confirms the selected strain as *Pleurotus ostreatus.*

In case of substrate variation, the highest days required for complete mycelium running was recorded in T2 (18 days) and the lowest in T6 (14 days). The highest days required for pinhead initiation was recorded in T6 (27.67 days) and the lowest in T4 (23.33 days). The highest days required for first harvest was recorded in T6 (31.33 days) and the lowest in T4 (26.33 days).The highest no. of fruiting body was recorded in T4 (59) and the lowest in T6 (37.67). The highest length of stalk was recorded in T4 (3.2 cm) and the lowest in T6 (2.4 cm). The highest diameter of pileus was recorded in T4 (7.40 cm) and the lowest in T6 (6.63 cm). The highest yield was recorded in T4 (938.67 g/packet) and the lowest in T6 (469 g/packet). The biological efficiency was also showed the same trend i.e, higher yield has higher BE (%). The highest economic return was recorded in T4. The estimation of N, P and K showed that rice straw is better substrate than rice + wheat combination. The higher amount of protein in mushroom was recorded in wheat substrate though it is poor performers under the situation. The sequencing data confirmed the selected strain as *Pleurotus ostreatus.*

The study confirmed that the *Pleurotus isolate* 1 sequence was shown 92.55% similarity with *Pleurotus ostreatus*. “Shnyreva and Shnyreva [43] analyzed ten *Pleurotus* spp. based on ITS sequences of rDNA. A phylogenetic tree was constructed on the basis of 31 oyster fungi strains of different origin and 10 reference sequences from Gene Bank to fungal species identification. They were revealed the divergence between commercial strains and natural isolates of *Pleurotus ostreatus* by phylogenetic analysis”.

Therefore, it may be concluded that *Pleurotus ostreatus* is the most suitable species for cultivation of oyster mushroom in this agro-climatic region. The rice and wheat straw combination is the best substrates for higher yield, biological efficiency and economic return.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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