Influence of substrates and growing techniques on yield performance of Indian oyster mushroom *Pleurotus pulmonarius*

A Thesis submitted to the Orissa University of Agriculture and Technology in Partial fulfilment of the Requirements for the Degree of Master of Science in Agriculture (Plant Pathology)

By

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CERTIFICATE - I

This is to certify that the thesis entitled **“Influence of substrates and growing techniques on yield performance of Indian oyster mushroom *Pleurotus pulmonarius*”** submitted in partial fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** to the Orissa University of Agriculture and Technology is a faithful record of *bona fide* and original research work carried out by ***Pagoti Hemalatha*** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by her from various sources during the course of investigation has been duly acknowledged.

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CERTIFICATE II

This is to certify that the thesis entitled **"Influence of substrates and growing techniques on yield performance of Indian oyster mushroom *Pleurotus pulmonarius*"** submitted by ***Pagoti Hemalatha*** to the Orissa University of Agriculture and Technology, Bhubaneswar, in partial fulfilment of the requirements for the degree of **Master of Science in Agriculture** (**Plant Pathology)** has been approved/disapproved by the student's advisory Committee and the external examiner.

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"Life is not so short but that
There is always time enough for courtesy"

- R.W. Emerson

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ABSTRACT

Among the cultivated mushrooms, oyster has maximum number of commercially cultivated species suitable for diverse agro-climatic zones of the state with varying yield potential, shape, size, colour and aroma. The present investigation was undertaken to evaluate the performance of *Pleurotus pulmonarius*, the Indian oyster mushroom in Odisha.

Studies on growth pattern and fruit body characteristics indicated that the mycelium was dense strandy on PDA medium with pale white colony colour.

Biological efficiency of *P. pulmonarius* was significantly highest (99.30 %) on paddy straw substrate among the nine substrates evaluated.

Stem treatment of substrate at 60 - 80^{o}C for one hour was found superior in terms of days to spawn run (14.5) days to first harvest (21.75) and biological efficiency (98.90 %).

Among the bag dimensions, 30 x 25 cm bag accommodating 1500 g substrate was superior in respect of biological efficiency (101.18 %).

Both the layer spawning and thorough mixing methods were statistically at par yielding 97.66 and 96.87 per cent biological efficiency.

No definite role of organic supplements in improvement of the biological efficiency of *P. pulmonarius* could be established. However, boiled wheat supplementation was superior (93.37 %) among the nine additives tried.

The biological efficiency was found highest in bags uncovered after solarisation (97.34 %) which was at par with bags maintained as such with 1.0 cm size holes (96.47 %) as recorded in the investigation.

December and January spawning were appropriate in realizing superior yields (99.5-100.17 %) in *P. pulmonarius*.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBRIVIATIONS

µg	:	Microgram
^{o}C	:	Degree centigrade
%	:	Percentage
Conc.	:	Concentration
AD	:	Anno domin
BC	:	Biological Efficiency
CD	:	Critical difference
Cfu	:	Colony forming unit
cm	:	Centimetre (s)
CTMRT	:	Centre of Tropical Mushroom Research and Training
CV	:	Coefficient of variation
DNA	:	Deoxy ribo Nucleic Acid
et al.	:	Co-workers
FAO	:	Food and Agriculture Organization
Fig	:	Figure
Ft	:	Foot
g	:	Gram(s)
hr	:	Hour
ICAR	:	Indian Council of Agriculture Research
Kg	:	Kilogram (s)
l	:	litre
m	:	meter (s)
mg	:	Milligram
min	:	Minute(s)
ml	:	Milliliter
mm	:	Millimeter(s)
mt	:	Million tons
N	:	Normal
NAM	:	Nutrient agar medium
No	:	Number
NS	:	Non-significant
PD	:	Potato Dextrose Broth
PDA	:	Potato Dextrose Agar
pH	:	H ion conc
ppm	:	Parts Per Million
psi	:	Pounds per square inch
RBD	:	Randomized Block Design
SC	:	Soluble concentration
Sl	:	Serial
UV	:	Ultra violet
viz.	:	Example

INTRODUCTION

Mushrooms are biota characterized by wonder. After Yeast fermentation, mushroom production has been considered as second most amongst the esteemed commercial microbial technologies. These are the fruiting bodies of macro fungi which are devoid of leaves and of chlorophyll-containing tissues. Yet, they grow and produce new biomass. Mushroom mycelia secrete enzymes that break down compounds such as cellulose and lignin which are then absorbed by the hyphae. Mushrooms are very nutritious products. It can serve as food, tonic and as medicine. Cultivation of mushroom does not require fertile land as they can grow in the sheltered rooms degrading altogether various agro-residues.

The mushroom contains low calories and provide essential minerals thus regarded as a valuable health food. A number of edible mushroom species like *Agaricus, Auricularia, Calocybe, Flammulina, Lentinus Pleurotus, Volvariella, etc*. are commercially cultivated in different parts of the globe. They are rich in crude fibre, proteins and vitamins but low in fat and calories. They possess multi-functional medicinal properties.

Theophrastus (372-287 BC) wrote that mushrooms gathered from farmlands, fields and meadows were valued as food. According to the medical treatise of Indian, Sumhita of the Atreya charak period dating back to probably 3000BC, mushrooms were classified into three categories : edible, non-edible or poisonous and medical (Pandey and Singh,1978).

Literature references indicate that *Auricularia auricula* (black ear mushroom) was first cultivated in china in 600AD while *Flammulina velutipes* (winter mushroom) was grown around 800-900 AD (Chang and Miles, 1987). Cultivation of *Pleurotus* (oyster mushroom) on tree stumps and logs was first described at the beginning of the twentieth century (Falck, 1917). The first known cultivation of European button mushroom (*Agaricus bisporus*) began in France during the period 1550-1650 (Atkin, 1981). Paddy straw mushroom *(Volvariella volvacea)* was first cultivated in China in 1822 (Chang, 1969). Cultivation of *Lentinula edodes* (shiitake mushroom) originated in China during 1000 AD. Out of 10,000 species of fleshy fungi, more than 2000 species throughout the world are reported to be edible and about 300 species belonging to 70 genera are reported from India. Out of 2000 species of prime edible mushrooms, about 80 have been grown experimentally, 20 cultivated commercially and 4 to 5 species produced on industrial scale throughout the world.

As a result of widespread mushroom cultivation, there has been a consistent increase in mushroom production amounting to a world total of 5.0 MT (Kues and Liu, 2000). Asian countries produce more than 74.64% of world mushroom markets followed by Europe (19.63%) respectively in 2014 (FAO, 2015). Around 32.0% of the total production is contributed by button mushroom followed by shiitake mushroom (25.0%), oyster mushroom (14.0), black ear mushroom (8.0%), winter mushroom (5.0%), paddy straw mushroom (3.0%) and others (13.0%).

Mushrooms are good source of vitamins such as vitamin B1 (Thiamine), vitamin B2 (Riboflavin). Vitamin B12 (Niacin) and vitamin C (Ascorbic acid). Mushrooms are also rich in minerals such as potassium, phosphorus, calcium, iron, copper and magnesium. Mushrooms are free of cholesterol and low in calories (Lee and Chang, 1975). Basing upon these food values, FAO has recommended mushroom as a food to supplement the protein need of the under nourished population of the developing countries. Cochran (1978) has compiled the mushroom based pharmaceuticals and their components. Annual business of medicinal mushrooms in India has reached Rs.150 crores in recent years (Tewari, 2004).

In India, about 72-76% of human population lives in 6, 00,000 villages and is mainly engaged in agriculture and allied activities. Large quantities of renewable lingo cellulosic residues are generated every year as result of extensive agriculture practices. Mushrooms have the ability to transform nutritionally useless waste in highly acceptable nutritious food. Modern classification has placed the fungi in a group distinct from the traditional kingdom Plantae (Alexopolous and Mims, 1979). Most of the cultivated edible mushrooms belong to the order Agaricales of the class Hymenomycetes under the subdivision Basidiomycotina. This order is composed of fungi forming fleshy, usually umbrella like fruit bodies. The term mushroom refers to this fruit body which is otherwise called as the basidiocarp. *Pleurotus pulmonarius* is an **edible white rot** fungus commonly known as Indian oyster, Italian oyster, Phoenix mushroom, or the lung oyster, belonging to the family Pleurotaceae and order Agaricales under the class Agaricomycetes (Alexopolous *et al.*, 1996, and Jonathan *et al*., 2012c).

Pleurotus mushroom, generally referred to as ‘Oyster mushroom or Dhingri’ in India is relatively new to the mushroom industry but has gained popularity at a tremendous pace and today it is cultivated in about 25 countries of far-East Asia, Europe and America. It is the third largest cultivated mushroom in the world and its annual production is around 8.75, 000 tonnes (Chang and Miles, 2004). China alone contributes 88% of the world production. The other major producing countries are South Korea, Japan, Italy, Taiwan, Thailand and Philippines. At present India produces only small quantities (10,000 tonnes) of oyster mushroom in Orissa, Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu, Bihar, Madhya Pradesh, Chhattisgarh, Jharkhand, West Bengal and in the North-Eastern states of Meghalaya, Manipur, Mizoram, Tripura and Assam.

There are about 38 species described under the genus *Pleurotus* from different parts of the world and more than 25 species have been reported from India. Presently about 25 species are commercially cultivated in different parts of the world which include. *P. ostreatus, P. pulmonarius, P. flabellatus, P. florida, P. sajor-caju, P. cirtinopileatus, P. sapidus, P. cystidiosus, P. eryngii, P. fossulatus, P. opuntiae, P.cornucopiae, P. yuccae, P. platypus, P. djamore, P. tuber-regium, P. australis, P. purpureo-olivaceus, P. populinus, P. levis, P. columbinus and P. membranaceus* etc. Unlike other cultivated mushrooms, species of *Pleurotus* exhibit much diversity in their adaptability to varying agro-climatic conditions.

Block *et al.* (1958, 1959) appeared to be the first to write on extensive account on the requirements of the mushroom for sawdust cultivation. They used a mixture of oat meal and saw dust for cultivation and found best results on eucalyptus followed by pine saw dust. They observed some growth abnormalities in fruiting bodies due to insufficient light conditions and no mushroom production when the temperature was less than 10^0C and more than 30^0C.

In Odisha, oyster mushroom cultivation has been commercialized since 1992 with the establishment of Centre of Tropical Mushroom Research and Training in Orissa University of Agriculture and Technology. Besides the availability of paddy straw mushroom in abundance, other agro wastes such as ragi straw, maize stalks and cobs, paddy husk, coir pith, jute stick, groundnut haulms and niger sticks are found in the state.

The production of tropical mushrooms like oyster (*Pleurotus spp.*), paddy straw (*Volvariella volvacea*) and milky mushroom (*Calocybe indica*) utilizing locally available agricultural wastes viz., paddy straw, wheat, soybean, chickpea, mustard, lathyrus, cotton wastes and lignocellulosic wastes are exploited. The production of oyster mushroom in India is estimated to be around 15-20,000 metric tonnes. However, the production of paddy straw and milky mushroom is about 10,000 tonnes each. Oyster mushroom farming is largely done by the women of the self help groups in a small to medium scale. It constitutes an important source of their income. Paddy straw (*Volvariella volvacea*) mushroom is very popularly grown in Odisha. Milky mushrooms can be cultivated throughout the year in the entire plains of India. It is hoped that the advocation of mushroom farming will become a very important cottage industry activity in the Integrated Rural Development Programme (Thakur, 2013).

In India, Odisha is a leading state in terms of oyster mushroom production. As many as 10 species are under cultivation in the state with annual production of 6310 metric tonnes contributing to about 40% of the total mushroom production of the state. The condition in most of agro-climatic situation in Odisha are suitable for growing oyster mushroom from month of July to February comprising of the rainy and winter season. However, people of Odisha prefer growing oyster mushroom during winter season i.e. from November to February when productivity of paddy straw mushroom declines owing to low temperature. *Pleurotus florida* and *Pleurotus sajor-caju* are the preferred species in the state. Besides these two species, *P. eous* is also gaining popularity both in the coastal as well as inland situations.

The oyster mushroom is produced every year in the state during the winter season. It is cultivated under thatched roof (indoor cultivation) in both coastal and inland districts. Cultivation is done largely on non pasteurized paddy straw substrate without organic supplements. The yield obtained varies from 1.0 to 1.5 kg /bag (66 – 100% biological efficiency). The reasons for such wide variations in the level of productivity are attributed to use of non pasteurized straw without supplements and lack of aftercare. But there is scope for yield increase by effective substrate management besides using productive strains of the species. Raising bags under appropriate environmental conditions is also important.

Pleurotus pulmonarius is a potential protein source especially in developing countries, besides having minerals and vitamins. Besides its edibility it has also got pharmaceutical properties. Hence, *Pleurotus pulmonarius* could well be incorporated in to the mushroom farming system during winter season besides the ruling species, as this species was not in cultivation earlier. Hence the effective of various conventional as well as non-conventional substrates and various growing techniques need to be standardized for the purpose of wide spread cultivation. Therefore, an attempt has been made to investigate the suitable package of practices for *Pleurotus pulmonarius* cultivation to be followed by the growers to achieve good yield, as under:

1. Growth pattern and fruit body characteristics
2. Influence of substrates on biological efficiency
3. Effect of substrates pasteurization methods on biological efficiency
4. Effect of bag dimension on biological efficiency
5. Biological efficiency as influenced by method of spawning
6. Influence of organic supplements on biological efficiency
7. Role of post-spawning practices on productivity
8. Influence of weather parameters on productivity.

REVIEW OF LITERATURE

Oyster mushrooms are one of the most popular edible mushrooms belong to the genus *Pleurotus* and the family Pleurotaceae. It is a lignocellulolytic fungus and grows naturally in the temperate and tropical forests on dead and decaying wooden logs and organic matter. It is one of the most suitable fungal organism for producing protein rich food from various agro-wastes without composting. This mushroom is cultivated in about 25 countries of far-east Asia, Europe and America. It is the 3rd largest cultivated mushroom in the world and its annual world production is around 876,000 tonnes (Chang, 1999). At the present India produces 10,000 tonnes of oyster mushroom. Cultivation on a variety of substrates, availability of large number of species, simple cultivation method, longer shelf-life and above all highest productivity among all edible mushrooms has made it so popular among the growers. Besides these, mushroom cultivation is an eco-friendly enterprise and has got the least adverse effect on the environment. A review of available literature was taken-up particularly in the areas of research included in this thesis work, has been briefed here under.

2.1 History of cultivation

The history of oyster mushroom cultivation is of recent origin in comparison to *Auricularia* (600 A.D.), *Lentinula edodus* (1100 A.D.) and *Agaricus bisporus* (1650). A very primitive form of growing *Pleurotus* spp. was adopted by Lumberman in Europe during 19th century. He used to carry long logs/stumps in which oyster mushroom mycelium was growing naturally and set these logs in cool and damp place, which enabled them to periodically collect oyster mushroom from these logs.

Falck (1917) for the first time successfully cultivated *Pleurotus ostreatus* on tree stumps at the beginning of twentieth century.

Liese (1934) stated about successful cultivation of *Pleurotus ostreatus* on beech turnks.

Block *et al.* (1958) made an important innovation in experimental cultivation of *Pleurotus ostreatus* on saw dust under laboratory conditions. They used a mixture of oat meal and saw dust for cultivation and recorded best results on Eucalyptus followed by pine saw dust.

Bano and srivastava (1962) reported about cultivation of *Pleurotus* spp.(most probably *P. flabellatus)* on paddy straw.

Junkova (1971) laid foundation for substratum preparation and fruit bodies production on commercial scale.

Quimio (1978b, 1979, 1981a) studied and published the cultural requirements and cultivation technology for *Pleurotus ostreatus var. florida* an isolate obtained from japan.

Chang *et al.* (1981) successfully developed a method of cultivation of *Pleurotus sajor-caju* using cotton wastes from cotton industries.

Zadrazil and Brunnert (1981) *Pleurotus* species are found to be efficient lignocellulose decomposing types of white rot fungi. Therefore, many agricultural and industrial wastes can be utilized as substrates for the production of *Pleurotus* species.

Ortega *et al.* (1992) reported that the practise of oyster mushroom cultivation not only produce medicinal and nutritive food but also improves the straw quality. This takes place by reducing lignin, cellulose, hemicelluloses, tannin and crude fibre content of straw making it ideal for animal feed.

Cangy and Peerally (1995) used spawning rates 0.75, 1.50, 3.00 and 6.00% of substrate fresh weight for 10 species of *Pleurotus*. Results showed that 1% spawning rate was found to be adequate when using the smaller bags (yields >16% of spawned substrate weight) at temperature 18^0(range 13-23^0C).

Singh *et al.* (2001) cultivated *Pleurotus* species in Manipur.

Kalm and Sargn (2004) studied the use of olive mill waste water (OMWW) as a moisture source for mushroom cultivation.

Gregori *et al.* (2007) successfully utilized different technique and substrates for mushroom cultivation and biomass production with emphasis on the production of fruiting bodies and the production of mycelium.

Kumari and Achal (2008) reported that the main function of rice straw is to provide a reservoir of cellulose, hemicellulose and lignin which is used during growth and fructification of *Pleurotus ostreatus.*

Sanchez (2010) studied growing of oyster mushroom to convert a high percentage of the substrate to fruiting bodies and increasing profitability.

Buah *et al.* (2010) investigated the cultivation of oyster mushroom on different substrate where composting of substrate, sterilizing the bagged compost, spawning, incubation and cropping were done.

Sher *et al.* (2011) described the production facility for *Pleurotus ostreatus* under two different agro ecological regions with emphasis on growth parameters.

2.2 Importance of *Pleurotus*

The genus *Pleurotus* (oyster mushroom) comprises some most popular edible mushrooms due to their favourable organoleptic and medicinal properties, vigorous growth and undemanding cultivation conditions. It can be cultivated on log and a wide variety of agroforestry (by-)products, weeds and wastes for the production of food, feed, enzymes and medicinal compounds, or for waste degradation and detoxification Gregori *et al.* (2007).

JinWen Shen *et al.* (2013) studied on exopolysaccharides from *Pleurotus pulmonarius* fermentation optimization, characterization and antioxidant activity.

Nurul Azwa Abd Wahab *et al.* (2014) studied on characterisation of potential antidiabetic-related proteins from *Pleurotus pulmonarius*. The presence of four antidiabetic-related proteins which are profilin-like protein, glyceraldehyde-3-phosphate dehydrogenase-like protein, trehalose phosphorylase-like (TP-like) protein, and catalase-like protein. Hence, *P. pulmonarius* basidiocarps have high potential in lowering blood glucose level, reducing insulin resistance and vascular complications.

2.3 Morphological (growth pattern and fruit body) characteristics

Nelson Menolli Junior *et al*. 2011. Morphological and molecular identification of four Brazilian commercial isolates of *Pleurotus* spp. and cultivation on corncob. two substrates prepared from ground corncobs supplemented with rice bran and charcoal were tested for mycelium growth kinetics in test tubes and for the cultivation of four *Pleurotus* commercial isolates in polypropylene bags. The identification of the isolates was based on the morphology of the basidiomata obtained and on sequencing of the LSU rDNA gene. Three isolates were identified as *P. ostreatus*, and one was identified as *P. djamor*. All isolates had better in-depth mycelium development in the charcoal-supplemented substrate. In the cultivation experiment, the isolates reacted differently to the two substrates. One isolate showed particularly high growth on the substrate containing charcoal.

Shubhra Shukla and A. K. Jaitly (2011) studied on morphological and biochemical characterization of different oyster mushroom (*Pleurotus spp.*) about seven different species were collected. Out of the seven, five species, naming *Pleurotus citriopileatus, Pleurotus djamor, Pleurotus Florida, H. ulmarius and Pleurotus sajor-caju* were selected. Five different morphological traits i.e., mycelial growth (mm), stipe length(cm), cap diameter (cm), margin of fruit body, colour of fruit body, total yield (kg), carbohydrate content (%) and protein content (%) were recorded. Results indicate that all the five species of *Pleurotus* shows great diversity in their morphological characters and biochemical parameters. Thus all these species have a great genetic diversity.

Guadarrama-Mendoza *et al* (2014) studied on morphology and mycelial growth rate of *Pleurotus* spp. strains from the Mexican mixtec region. Direct relationship between mycelial morphology and growth rate was observed. Cottony mycelium presented significantly higher growth rates ($p < 0.01$) in comparison with floccose mycelium. Thus, mycelial morphology can be used as criterion to select which pairs must be used for optimizing compatible-mating studies. Hybrids resulting from cottony neohaplonts maintained the characteristically high growth rates of their parental strains with the hybrid $R_{1-n}xB_{1-n}$ being faster than the latter.

2.4 Effect of substrate composition on mushroom yield

Kumar *et al.* (2000) tried some weeds for cultivation of *Pleurotus sajor-caju* and reported *Ageratum* twigs to be suitable substrate.

Sharma *et al.* (2001) cultivated four species of *Pleurotus* on mycorrhizal inoculated/un inoculated castor stem and thin branches of all *Pleurotus spp.* From castor stem and mulberry branches as compared to wheat and paddy straw substrate.

Royse (2002). Studied on influence of spawn rate and commercial delayed release nutrient levels on *Pleurotus cornucopiae* (oyster mushroom) yield, size, and time to production. *Pleurotus cornucopiae* 608 was grown on a mixture of pasteurized cottonseed hulls (75% dry wt), 24% chopped wheat straw, and 1% ground limestone. . By using a spawn rate of 3.75% of the wet substrate wt, it was possible to reduce the time to production by a mean of 9.2 days compared with a spawn rate of 1.25%.

Madan *et al.* (2002) studied the efficacy of different wastes in yielding *Pleurotus sajor-caju* and found that the yield obtained from the leaves of *Morus alba* was comparable with the yield obtained from paddy straw among the four substrates tried.

Baysal (2003) reported that increase in the ratio of rice husk within the substrate accelerated spawn running, pin head and fruit body information and resulted in increased mushroom yield while more peat and chicken manure had a negative effect on yield.

Salmones *et al.* (2005) studied on comparative culturing of *Pleurotus* spp. on coffee pulp and wheat straw: biomass production and substrate biodegradation. Greater metabolic activity was observed in the wheat straw samples, with a significant increase between 4 and 12 days of incubation. The degradation of polysaccharide compounds was associated with the fruiting stage, while the reduction in phenolic contents was detected in both substrates samples during the first eight days of incubation. A decrease was observed in caffeine content of the coffee pulp samples during fruiting stage, which could mean that some caffeine accumulates in the fruiting bodies.

Cristina Soler-Rivas (2005) studied on microbiological effects of olive mill waste addition to substrates for *Pleurotus pulmonarius* cultivation, olive mill wastes (OMWs) and vegetation waters (VWs) obtained during the manufacture of olive oil were added as substrate supplements for the cultivation of *Pleurotus pulmonarius.* The material modified growth of the mushroom and the endemic microbiota of the substrate, in particular the mushroom-pathogenic bacterium *Pseudomonas tolaasii*, which is responsible for bacterial blotch disease in mushrooms.

Pathmasini *et al.* (2008) examined locally available grains of kurakkan (*Eleusine coracana*), maize (*zea mays*), sorghum (*Sorghum bicolour*) and paddy (*Oryzae sativa*) for use a spawn substrate for *Pleurotus osteatus. Among the substrates, Eleusine* spawn produced an acceleration of spawn running, pin head formation, fruit body formation and increase yield compared with other types. The fastest spawn running of 21 days, pinhead formation of 35 days, and highest mean yield of 55.37 g and maximum fresh mushroom yield percentage of 30.67% were realized from *Eleusine* spawn.

Rani *et al.* (2008) evaluated various lignocellulosic wastes for production of edible mushroom *Pleurotus eous* and *Lentinus connotus.* Biological efficiency of 55-65% was observed in paddy straw followed by sorghum stalk (45%) and banana pseudo stem (33%) for both fungal species.

Naraian *et al.* (2008) investigated the effect of different nitrogen rich supplements in cultivation of *Pleurotus florida* on corn cob substrate. Among six additives tried the cotton seed cake was the best supplement producing 93.75% biological efficiency followed by soybean meal (93.00% biological efficiency).

Bernabé-González and Cayetano-Catarino (2008) *Pleurotus pulmonarius* was cultivated on dry banana leaves (*Musa paradisiaca*) or dry “palmareca” leaves (*Chrysalidocarpus lutescens*), using two substrate treatments. . Biological efficiency in the other treatments varied between 41.4-81.2%. Substrates and treatments studied are suitable for low-cost and small-scale production of oyster mushrooms.

Ahmed *et al.* (2009) evaluated different agro wastes viz. Soybean straw, paddy straw, wheat straw and their combination in 1:1 proportion for cultivation of *Pleurotus florida.* Soybean straw showed significantly highest yield (87.56% BE) with maximum crude protein (23.50%) and maximum phosphorus content (920/mg/100g of dry mushroom). The combination of soybean straw + paddy straw contributed for significantly highest fat (2.60%), calcium (310 mg/100g) and iron (13.06 mg/100g) content.

Dunkwal and Jood (2009) evaluated wheat and Brassica straw for cultivation of *Pleurotus sajor-caju* and found non-significant difference in respect of crude fibre, crude fat, ash and energy contents, where as significant difference was noticed in crude protein (25.30 and 26.99%) and total carbohydrates (52.34 and 50.52%). Both type of mushroom exhibited good amount of vitamins, amino acids and dietary fibres.

Onuoha *et al.* (2009) studied on cultivation of *Pleurotus pulmonarius* (oyster mushroom) using some agrowaste materials. . Mixture of sawdust and oil palm fibre and cassava peels and oil palm fibres produced scanty growth. The fungus did not growth at all on oil palm fibre alone.

Adebayo *et al.* (2009) studied on evaluation of yield of oyster mushroom *Pleurotus pulmonarius* grown on cotton waste and cassava peel cotton waste was a better substrate for cultivation of *P. pulmonarius* than cassava peel. However, with the high availability of cassava peel in Nigeria, the potential use of this waste as substrate adjunct (at 20% of substrate) can be suggested based on the findings of this study. It can be deduced from this study that cassava peel may be used for mushroom cultivation if supplemented with a good nitrogen source.

Sebnem and Buyukalaca (2009) studied on yield performances and changes in enzyme activities of *Pleurotus* spp. (*P. ostreatus* and *P. sajor-caju*) cultivated on different agricultural waste and studied for their ability to produce laccase and carboxymethylcellulase (CMCase) enzymes on different agricultural wastes under solid state fermentation. *P. ostreatus* and *P. sajor-caju* grown on substrates containing wheat bran had higher biological efficiencies and total yields as well as higher CMCase and laccase activities.

Ingale and Remteke (2010) used different substrates for *Pleurotus* in which he found 85.5 percent yield on rice straw substrate.

Kadam *et al.* (2010) tried two organic supplements i.e. soybean cake, neem cake with sugarcane bagasse at different concentration. Highest reduction in C:N ratio substrate after *P. sajor-caju* cultivation was found in sugarcane bagasse + 6% soybean cake and lowest ratio was found in sugarcane bagasse + 6% neem seed cake.

Kulshrestha *et al.* (2010) used different substrates for *Pleurotus* in which maximum yield was obtained with handmade paper + wheat straw substrate.

Ruiz-Rodriguez (2010) studied on effect of olive mill waste (OMW) supplementation to Oyster mushrooms substrates on the cultivation parameters and fruiting bodies quality. Seven Oyster mushroom strains were cultivated in wheat straw (WS) bags supplemented with 0 up to 90% olive mill waste (OMW). Total phenolic content and antioxidant activity were also similar and no phenolic compounds from OMW were detected in the fruiting bodies.

Liang *et al.* (2011) studied on cultivation of the culinary-medicinal lung oyster mushroom, *Pleurotus pulmonarius* (Fr.) Quél. (Agaricomycetideae) on grass plants in Taiwan. The effects of various combinations of substrates on mushroom mycelial growth and yield calculated as biological efficiency (BE) were determined. Among 9 experimental substrates, the most suitable substrate for mycelial growth was 45ZMS:45S, followed by 45PRS:45S; their mycelial growth rates were obviously quicker than that of the control substrate

Melo De *et al.* (2012) studied on applicability of the Use of Waste from different banana cultivars for the cultivation of the oyster mushroom. The highest organic matter loss (OML) was obtained from pseudo-stem + leaf wastes. Therefore, the use of those wastes showed itself viable for *P. ostreatus* cultivation due to its availability and low cost, besides decreasing discards to environment.

Chitamba *et al*. (2012). Studied on evaluation of substrate productivity and market quality of oyster mushroom (*Pleurotus ostreatus*) grown on different substrate. Six substrates; cotton lint waste, maize stover, jatropha cake, corn cobs, wood shavings and wheat straw were evaluated for their productivity and impact on mushroom market quality of *P. ostreatus.*

Sharma *et al.* (2013) reported that rice straw was found as a best substrate with yield (381.85g) and BE (95.46%) followed by rice + wheat straw, rice straw + paper waste for the production of mushroom.

Jonathan *et al.* (2013) studied on yield performance of *Pleurotus pulmonarius* (Fries.) Quelet, monitored on four agro-industrial wastes, (coir fibre, oil palm waste, sawdust of *Gmelina arborea* and rice straw). The most abundant mineral element in *P. pulmonarius* was K (30.20mg/100g).This was obtained on rice straw at 10% concentration; while the least mineral element was Cu (0.006mg/100g). Rice straw produced the highest yield with total mean weight of 93.33±3.

Krishnaveni and Saranya (2014) studied on cultivation of *Pleurotus florida* and *Calocybe indica* using various agrowaste. The yield of milky mushroom was found to be more with sugarcane bagasse waste while the oyster mushroom showed good yield with sorghum straw and the yield was low with banana leaf waste. Milky mushroom showed higher protein, carbohydrate content.

Sofi *et al.* (2014) studied on effect of different grains and alternate substrates on oyster mushroom (*Pleurotus ostreatus*) production,. By using various grains for spawn production, and waste paper, wood chips were used in comparison with wheat husk for mushroom production. The results of the analysis of variance showed that diameter of colony extension in various grains are different and were significantly affected by substrate type. The maximum and minimum growth rates were seen in the corn and millet substrates, respectively. It is concluded that wheat straw in combination with wood chips are best substrate for oyster mushroom cultivation.

Sharma *et al.* (2014) studied on yield enhancement of different species of oyster mushroom (*Pleurotus spp.)* by animal waste products and the effect of addition of nutriwash and coelomoic fluids in the substrate on yield of different species of *Pleurotus* mushroom. Very encouraging results were obtained in repeated trials. Period of spawn run and pin head initiation was found to be reduced in *P. sajor-caju* with 1% coelomic fluid treatment, however *P. cornucopiae* and *P. opuntiae* did not show any effect on spawn run and pin heads initiation period. The data also revealed that the addition of coelomic fluid and nutriwash @ 1 and 5%, respectively in the wheat straw significantly increased the yield of (*Pleurotus florida*, *P. sajor-caju*, *P. cornucopiae* & *P. opuntiae*) in the range of 11.7 to 18.4% in different species of oyster mushroom in comparison to control.

Adenipekun and Omolaso (2015) studied on comparative study on cultivation, yield performance and proximate composition of *Pleurotus pulmonarius* Fries. (quelet) on rice straw and banana leaves. Banana leaves with wheat bran additives, irrespective of their percentage concentration, had better mushroom quality/size, yield, biological efficiency and proximate composition than rice bran and its performance in rice straw.

Bhol *et al.* (2015) worked on coconut (*Cocos Nucifera*)-based farming system: A viable land use option for small and marginal farmers in coastal Odisha. An investigation was carried out during 2012-2013 in Puri district of Odisha to study the composition, structure and role of coconut (*Cocus nucifers* L.) based farming in 15 different holding sizes. The coconut based agroforestry system of size 0.8 acre was found to be the best among the holding sizes studied with regard to viability of land use.

Yang *et al.* (2016) studied on *Pleurotus Ostreatus*; Tea Waste: an effective and economic substrate for oyster mushroom cultivation. Tea waste is the residue that remains after tea leaves have been extracted by hot water to obtain water-soluble components. The waste contains a re-usable energy substrate and nutrients which may pollute the environment if they are not dealt with appropriately. Other agricultural wastes have been widely studied as substrates for cultivating mushrooms. In the present study, we cultivated oyster mushroom using tea waste as substrate. To study the feasibility of re-using it, tea waste was added to the substrate at different ratios in different experimental groups. Three mushroom strains (39, 71 and YOU) were compared and evaluated. Mycelia growth rate, yield, biological efficiency and growth duration were measured. Substrates with different tea waste ratios showed different growth and yield performance. The substrate containing 40–60% of tea waste resulted in the highest yield.

2.5 Effect of pasteurization methods on mushroom yield

Harnandez *et al.* (2003) studied the use of wooden crates for composting a mixture of 70% grass and 30% coffee pulp, combined with 2% $Ca(OH)_2$, as a method for preparing substrate for the cultivation of *Pleurotus ostreatus.*

Banik and Nandi (2004) studied that disinfection of straw and manure by means of 0.1% $KMnO_4$ + 2% Formalin solution in hot water caused 42.6% increase in yield of *Pleurotus sajor-caju* over control (disinfection with hot water).

Siqueira *et al*. (2012) studied on cultivation of *Pleurotus* mushrooms in substrates obtained by short composting and steam pasteurization. preparation for short composting and steam pasteurization was described in illustrative figures in order to provide expertise to small producers who wish to initiate economic and sustainable mushroom cultivation making use of regional lignocellulosic residues.

2.6 Effect of type of spawning on mushroom yield

Fan *et al.* (2000) carried out the studies with 2.5-25% spawn rates, 25% spawn rate appeared superior but recommended 10% spawn rate in view of the process economics. The first fructification occurred after 20-23 days of inoculation and the biological efficiency reached about 90-97% after 50-60 days.

Bughio (2001) cultivated oyster mushroom *Pleurotus ostreatus* on combination of wheat straw, cotton boll locules, paddy straw, sugarcane and sorghum leaves at 1:1 ratio in polythene bags (650g/bag) using sorghum grain spawn @ 30 grams per bag followed by boiling of substrates and sterilization of bags.

Royse (2002) cultivated *Pleurotus cornucopiae* on a mixture of cotton seed hulls (75%), wheat straw (24%) and lime stone (1%) and spawned at various levels (5%, 25%,125%,375% wet weight). Investigation revealed that there was a negative co-relation between spawn rate and crop duration.

Sanger *et al.* (2006) reported that both thorough spawning and layer spawning are equally effective in giving good yields in oyster mushroom.

Ram (2007) advocated both thorough spawning and layer spawning for obtaining good yields of oyster mushroom varieties. In both the cases spawn run was complicated in 15-20 days.

Singh *et al.* (2010) evaluated locally available substrate with their best spawn rate combination on yield of oyster mushroom in natural condition of western U.P. Paddy straw with 3% spawn rate was the suitable substrate in terms of the days taken for completion of spawn run and bio-efficiency.

Dahmardeh *et al.* (2010) compared the substrate (wheat and barley straw) and level of spawn (50, 70, 90, 110, 130, 150 and 170 g/bag) for *Pleurotus ostreatus.* Maximum yield was obtained on barley straw substrate at 150g/bag spawn level.

2.7 Influence of organic supplements on biological efficiency

Ngezimana (2007) studied on the use of organic supplements and composted substrates in oyster mushroom (*Pleurotus ostreatus*) production. Wheat straw performed best at 14 % level (104.2 % BE), with significant difference from lower supplement levels. Composted cotton residues and wheat straw gave significantly (P<0.001) higher yields than maize stover and their controls. First two flushes contributed more to the total yields (approximately 70 %) than subsequent flushes.

Ngezimana and Mtaita (2008) studied onimproving biological efficiency of Oyster mushroom, *Pleurotus ostreatus* Fr. (Polyporaceae), through composting and use of organic supplements. Supplements were equally effective for improving the performance of Oyster mushroom in almost all the substrates used. Composting of the substrates was beneficial in cotton residues and wheat straw substrates with biological efficiency of 145.7 and 28.2% respectively compared to their controls (32.3 and 5.3 respectively). It was concluded that both supplement can be used to enhance production and composting was not beneficial with maize stover.

Mateus Dias Nunes *et al.* (2012) Studied on nitrogen supplementation on the productivity and the chemical composition of oyster mushroom. The fungi were grown in various substrates supplemented with urea or rice bran, and the biological efficiency, mineral composition, protein and β-glucan content were evaluated. The growth of *P. ostreatus* in substrates with nitrogen supplementation increases the mushroom's productivity and nutritional value.

2.8 Influence of weather parameters on biological efficiency of *Pleurotus pulmonarius*

Hassan Sher *et al.* (2010) studied on Effect of environmental factors on the yield of selected mushroom species growing in two different agro ecological zones of Pakistan. Mild winter temperatures of Peshawar region, and low summer temperatures in Swat, were found most suitable for growth and yield of *Pleurotus ostreatus*.

Yingyue Shen *et al.* (2014) studied on effects of cold stimulation on primordial initiation and yield of *Pleurotus pulmonarius.* The findings of this study suggested that an appropriate cold stimulation may enhance the performance of the primordial initiation and yield of *Pleurotus pulmonarius* cultivation during the summer season. The best performance among the 12 treatments was recorded following a 12 h cold stimulation at 5°C.

MATERIALS AND METHODS

The present investigations were undertaken to study the effect of the different substrate composition on mushroom yield, pasteurization methods, types of spawning, organic supplements (additives) and exploring the cultivation of oyster mushroom in different seasons . The materials used and methods followed in the present study are described below.

Test fungus

Different studies were undertaken during the course of investigation by taking only one species of oyster mushroom fungus , *Pleurotus pulmonarius* , the test fungus was procured from the Centre of Tropical Mushroom Research and Training (CTMRT). Department of Plant Pathology. Collage of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar.

Maintenance of culture

The pure culture of the fungus was maintained on potato dextrose agar (PDA) Slant throughout the period of investigation. The fungus was sub cultured at an interval of two months and stored at $25\pm1^{0}C$. Fifteen days old pure mycelia cultures of test fungus were used in various studies.

Cleaning and sterilization of glass wares

Borosil glass wares such as culture tubes, pertridishes, conical flasks, beakers etc., were used throughout the period of investigation. Standard procedures for cleaning and sterilization of glasswares adopted. All the glasswares were cleaned in dilute solution of potassium dichromate and sulphuric acid (60g potassium dichromate per litre distilled water, 60 ml of concentrated sulphuric acid added slowly to it) followed by thorough washing with tap water and subsequent rinsing with distilled water before use. Petri dishes, pipettes etc. wrapped with paper were sterilized in hot air oven at $160^{o}C$ for two hours.

Sterilization by flame

The scalpels, inoculating needles, glass rods, nichrome wire loops etc. were sterilized by dipping them in 70% ethanol followed by flaming over sprit lamp.

Inculcation and incubation

Aseptic conditions were maintained in the inoculation chamber at the time of inoculation. The inoculum consisted of small piece of the medium with pure culture taken out of the culture slants with the help of inoculating needle. The inoculated cultures were incubated at 25 ± 1^0C, unless otherwise specified.

Preparation of potato dextrose agar medium

Potato dextrose agar, the routine laboratory medium for growing oyster mushroom fungus, was prepared as follows.

Two hundred gram of peeled and sliced potato was boiled in 500ml of distilled water till potatoes were soft. Then the extract was filtered through cheese cloth and was collected in a graduated cylinder. Twenty gram of agar powder was boiled in 500ml of distilled water till the ager was dissolved completely. Both the solutions were subsequently mixed. Twenty gram of dextrose was added and the volume was restored to 1000ml by adding fresh distilled water.

Before sterilization, aliquots of 10 ml were taken in culture tubes for preparation of agar slants. Media to be poured into petri dishes were taken in Erlenmeyer conical flasks. The culture tube and conical flasks were plugged with non-absorbent cotton and autoclaved at 15 p.s.i. for 15-20 minutes. Streptomycin sulphate was added to the medium at the rate of 250 mg per 1000ml before autoclaving for suppression of bacterial contamination. Slants were prepared by putting still hot tubes in slating position for solidification.

3.1 Spawn preparation

The propagating material used by mushroom growers for planting is mushroom spawn. Spawn is also known as mushroom seed. A number of materials, alone or in different combination are popular as spawn substrates. However, wheat grain was used as the base for material for multiplying the mycelium in the spawn bottles.

The protocol adopted for wheat grain substrate is mentioned below.

1. Bold and healthy wheat grains were cleaned and washed several times to remove the suspended particles or foreign materials.

2. The grains were boiled with water in a container for about 30 minutes till they become soft.

3. The boiled grains were spread on a sieve under shade to decant excess water.

4. The cooled grains were mixed with 2% calcium carbonate on dry weight basis to avoid clumping of grains and improve alkalinity.

5. The grains were filled up 2/3 portion of the available space of the spawn bottles and plugged with non-absorbent cotton neither very tight nor very loose and sterilized in an autoclave at 126^0C for 22lbs p.s.i. for 2 hours followed by cooling.

6. For inoculating the bottles hygienically, the inoculation chamber was sterilized by putting 35ml of formalin (37-41% formaldehyde) and 17.5 g of potassium permanganate in a glass container and closing the room for overnight. Alternatively, the chamber was exposed to ultra violet rays for 30 minutes prior to inoculation.

7. The sterilized and cooled bottles were aseptically inoculated under laminar flow with mycelia bits of 15-days –old mycelium culture and properly labelled.

8. These inoculated bottled were incubated at 25 ± 1^0C in B.O.D. incubator for two weeks.

9. The bottles were shaken at 4 days interval to allow proper spread of mycelium between the grains.

10. The bottles were then completely colonized by the mushroom mycelium in about two weeks and that time the spawn was ready for cultivating mushroom in large scale.

PREACUTIONS

1. Cleaned and healthy wheat grains were selected for spawn making.
2. Grains were not boiled for longer period.
3. Mycelia bits of 15-days-old culture were preferred for inoculation.
4. Microbial contamination of the spawn during incubation was checked regularly and contaminated bottles were discarded.

3.2 Cultivation of pleurotus

Materials used in bag preparation

Straw from improved or tall Indica varieties of rice was preferred for preparation of bag and growth of oyster fruiting body. Well dried, hand threshed and not more than one year old rice straw was taken. The straw bundles were stored in protected condition in order to avoid wetting. The straw was chopped to a size of 1.5 –2.0” with the help of chaff cutter machine. For preparation of a single bag, 1.5 kg, dry chopped straw was required.

The farm house

All the experiments were conducted in the farm house of Centre of Tropical Mushroom Research and Training, Department of Plant Pathology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneswar.

The farmhouse structure was as follows:

Length	:	50’
Breadth	:	20’
Roof	:	asbestos
Floor	:	cemented
Windows	:	Wider, covered with fine wire net

Light and air

To regulate the light and air in the farm house of CTMRT, the wide windows were covered with gunny bags which were opened in east-west direction so that light in the morning and evening percolates through the open windows. Further, the farm house was well-ventilated.

Humidity

To maintain proper humidity, the gunny bags screened were soaked with water during cultivation. Moreover, aerial spraying of water was done and the sand put on the floor was also kept moist besides watering the substrate.

Temperature

Pleurotus pulmonarius required an optimum temperature range of 22 to 26^{0}C for mycelial growth as well as fruiting. Therefore, all the experiments were conducted during the period from November 2015 to February 2016.

Construction of raised platform/ shelve

Racks consisting of three shelves at 2.5' apart were raised in the incubation room for mycelial growth of the test fungus. Fully colonized bags were hanged in the bamboo sticks in three tiers in the farm house for fruit body induction.

Preparation of Substrate

A single bag (80 cm × 40 cm) needed three bundles of paddy straw which weighed approximately 1500g. The hand threshed, uncrumpled straw devoid of leafy materials was preferred. The straw was chopped into 1.5-2" size through a chaff cutter, packed in gunny bags and was soaked in water containing 125ml of formalin and 7.5g of Bavistin per 90litres of water for a period of six hours. Straw was pressed and covered with a polythene sheet. The straw was taken out and excess water was drained by spreading the straw on a clean cemented floor. Prior to raising bags, the moisture content of substrate was maintained at 65% which was confirmed through palm test.

Spawning of the Substrate

Freshly prepared grain spawn (20-30 days old) was procured for spawning. The spawning was done in a pre-fumigated room (48hours with 2% formalin). The spawn requirement was 10% of the dry weight of substrate (150g of spawn per bag having 1.5kg of dry substrate). The spawn was removed from the bottle with a clean and sterilized iron rod and divided into four parts. Each part of spawn as well as the supplement were put inside the polythene bag of 80cm × 40cm size on each layer of substrate having 5-6" thickness close to the edge. Four layers of substrate were seeded with spawn along with supplement and the upper end of the bag was tied up. Ten to 15 small holes (0.5-1.0cm dia) were made on all sides of the bag including two to four holes in the bottom to leach out excess water and to facilitate gas exchange.

Crop Management

Incubation

Spawned bags were kept on shelves in the incubation room for mycelial colonization of the substrate. During mycelial growth bags were not opened and no ventilation was needed. Moreover, water was not sprayed in the room as there was no need of maintaining high relative humility. Daily maximum and minimum temperature of incubation room was recorded.

Fruit Body Induction

Once the mycelium fully colonized the substrate and formed thick mycelial mat, the bags were removed from the incubation room and made naked and arranged on wooden shelves with a minimum distance of 15-20 cm between two beds in tiers in the cropping room. Appropriate light (200 lux for 8-12 hours a day), temperature (20-30^{0}C) and relative humidity (70-80%) were maintained to facilitate fruiting. The bags were sprayed with water twice daily during morning and afternoon hours to maintain moisture status of the substrate.

Fruiting and harvest

Three to four days after opening of bags, mushroom primordia (fruit bodies) started to appear. Fruiting bodies were harvested in about three days after their appearance. Harvesting was done by grasping the stalk and gently pulling or twisting the mushroom from the substrate level. Mushroom fruiting continued after harvesting of first flush at an interval of 7-10days up to 3-4 flushes covering a crop period of 45-60 days.

Yield

The yield of *Pleurotus pulmonarius* varied depending on kind of substrate, supplement, temperature and relative humidity during cropping. However, the biological efficiency ranged from 80 to 100 per cent of the dry weight of substrate.

Precautions

1. The cereal straw for the preparation of substrate should be dry and free from rotting and inert matters.

2. Very old contaminated spawn should not be used.

3. Watering on bags should not be excessive.

3.3 Morphological characterization of *Pleurotus pulmonarius*

Morphological characterization of *Pleurotus pulmonarius* was done in order to ascertain its identity.

For this purpose, the test species was grown in bag method as standard protocol. After mycelial run was over, the bags were uncovered and put on the shelves in the mushroom growing room. Watering of bags was done as and when required. Fruit bodies were harvested from the first flush at the appropriate time. A random of 10 fruit bodies were collected and brought to the laboratory of the pileus, stipe length, stipe thickness, margin and texture of fruit body were recorded.

3.4 Effect of substrate composition on mushroom yield

The trial on substrate composition was conducted to find out the appropriate substrate for obtaining higher yield. Nine different types of substrates such as maize stalk, maize cobs, sugarcane bagasse, coconut coir, rice bran, paddy husk, green gram, saw dust and paddy straw were procured for cultivation as the substrates were available in abundance across the State. All the substrates were chopped into pieces of 1.5-2.0" size and soaked for 6 hours in clean and cold water. Further the moist substrates were steamed under pressure inside the autoclave at 10 lbs for 30minutes for pasteurization.

The substrates were taken out and allowed to cool down at room temperature. Excess water was allowed to drain out and bags of 80cm × 40cm size were raised as per the procedure mentioned earlier and incubated. After the bags were colonized by the fungus, they were placed on shelves in tiers in the cropping room for induction of fruiting. Each treatment was replicated thrice. The treatment having paddy straw as the substrate served as control. Appropriate light, temperature, humidity and substrate moisture were maintained in the cropping room. Observations on time taken for spawn run, first harvest, average weight of fruiting body and mushroom yield (biological efficiency) were recorded.

3.5 Effect of pasteurization methods on mushroom yield

The efficacy of different substrate pasteurization methods were evaluated in terms of mushroom productivity.

Four pasteurization methods along with the untreated control were employed in the investigation. In the boiled water treatment, the chopped and moistened substrate was soaked in hot water (70%) for one hour. After draining excess water, spawn was added. In case of steam pasteurization, the pre-wetted chopped straw was packed in wooden trays and then kept in a pasteurization room at 60-80^{0}C for 2-3 hours. Substrate after cooling to room temperature was seeded with spawn. In solarization, the moistened straw was spread on cemented floor in thin layer and covered with clean polythene sheet and treated in sun-light from 10.00 AM to 4.00 PM in bright sunny days. In case of chemical pasteurization technique, in each 100l of water 7.5g bavistin and 125ml formaldehyde (40%) was mixed and chopped straw was wetted for the required period. Straw was pressed and covered with a polythene sheet. Straw was taken out excess water was drained and spawned. Chopped straw soaked in clean and cold water served as control. Each treatment was replicated four times. Appropriate conditions were maintained in the cropping room for induction of fruit bodies. Observations on time taken for spawn run, days taken for pinhead formation, tome takes for 1st and 2nd flush, average fruiting body weight and yield of mushroom (biological efficiency) were recorded.

3.6 Effect of type of spawning on mushroom yield

Various spawning methods such as thorough spawning, top spawning and layer spawning were evaluated in terms of mushroom yield. In thorough spawning, spawn (20-30 days old) was mixed thoroughly with the pre wetted and pasteurized substrate at the rate of 10% of the dry weight of the substrate, put inside the polythene bag of 80cm × 40cm size, tied and incubated. In case of top spawning, spawn was distributed on the top of the pre-filled polythene bag with substrate, covered, tied and incubated. In case of conventional layer spawning, spawn was put in layers (preferably four), tied and incubated. Each treatment was replicated seven times. Observations on time taken for spawn run, time taken for first harvest, average fruit body weight and mushroom yield (biological efficiency) were recorded.

3.7 Effect of different organic additives on mushroom productivity

An attempt was made to evaluate the efficacy of nine organic supplements and control (no supplement) such as maize powder, ragi powder, bengal gram powder, mustard oil cake, chicken manure, boiled wheat, rice bran, tapioca and boiled maize as additives over control (without any additive) in yield improvement of *Pleurotus pulmonarius.*

The procedure of cultivation was adopted as mentioned earlier. Different supplements were used @ 200g per bag. The supplements were steamed under pressure in the autoclave for 20 minutes at 10 pounds and cooled to room temperature. Bags prepared without using any additive served as control.

The treatments were replicated thrice and the experiment was laid down in the farm house of CTMRT in favourable conditions. Observations on time taken for spawn run, time taken for first harvest, average fruiting body weight and mushroom yield (biological efficiency) were recorded.

3.8 Effect of bag dimension on mushroom productivity

In this investigation, an attempt was made to manipulate the bag size in order to maximise the biological efficiency.

Various quantities of dry substrates (500g, 1000, 1500g, 2000g, 2500g and 3000g) were used for preparation of bags out of the test species. Layer spawning was followed with the pre-wetted and pasteurized substrate at the rate of 10% of the dry weight in bag of variable sizes, tied and incubated.

Each treatment was replicated four times. The bag containing 1500g of dry substrate served as check. Appropriate light, temperature, humidity and substrate moisture was maintained in the cropping room.

Observations on time taken for spawn run, first harvest, average weight of fruit body and mushroom yield were recorded.

After the bags were colonized by the fungus, they were shifted to the cropping room for induction of fruiting.

3.9 Role of post-spawning practices on yield and productivity of *Pleurotus pulmonarius*

Post spawning practices such as maintaining the substrate within the polythene cover till the end of the cropping cycle and uncovering the substrate at the initiation of fruiting were evaluated for their yield and productivity.

Oyster mushroom bag of normal size were prepared using 1.5 kg dry substrate each following standard protocol and were incubated. However, after mycelia colonization half of the bags were maintained as such without uncovering the polythene bags, having two different hole sizes of 1.0cm and 0.5cm and remaining bags were uncovered as usual. Each treatment was replicated 7 times and bags were placed on racks. Appropriate temperature, humidity, substrate moisture, light and ventilation were maintained in the cropping room. Observations on time taken for spawn run, first harvest average weight of fruiting body and mushroom yield were recorded.

3.10 Influence of weather parameters on biological efficiency of *Pleurotus pulmonarius*

In an attempt to find out the appropriate time of cultivation of *Pleurotus pulmonarius* bags were raised at 15 days interval from November 1st to February 28th, 2015-16 covering the winter season. Bags were prepared following the standard protocol as described earlier. However, the cultivation was subjected to ambient conditions in the mushroom growing room to ascertain the appropriate time of cultivation. Triplicates were maintained for each treatment in Randomized Block Design.

Observation on time taken for spawn run, first harvest, average weight of fruiting body and mushroom yield were recorded.

EXPERIMENTAL RESULTS

Oyster mushroom is an edible mushroom of the tropics and subtropics. Odisha is the leading state in terms of Oyster mushroom production in India. *Pleurotus sajor-caju* and *Pleurotus florida* are the ruling species of the state because of their soft and fleshy texture and excellent flavour. Mild winter coupled with humid coastal agro-climatic situation with abundance of man power and agriculture waste has made it most suitable for cultivation of Oyster mushroom.

It is often cultivated outdoor under the coconut, arecanut, mango, jack fruit, cashew nut, bamboo and casurina trees in the districts of Cuttack, Jagatsinghpur, Kendrapara, Khurda, Puri and Ganjam. However, it is largely an indoor crop in the remaining districts. An attempt was made to document the total mushroom production of the state vis-a-vis the production of oyster mushroom during the month of March, 2016. It was estimated that the total mushroom production of the state comprising of oyster, paddy straw and button was 15,986 tonnes in the year 2015-16, out of which the contribution of oyster mushroom was 6,310 tonnes accounting for 39 percent of total production. The four leading districts namely, Puri, Ganjam, Khurda and Dhenkanal are combinely contributing to 58 per cent of total oyster production of the state. However, it was observed that all the 30 districts of the state are engaged in commercial oyster mushroom cultivation employing the conventional cultivation procedure. The above exercise has established that oyster mushroom has been fairly popular one in the entire state of Odisha and assumed the shape of the cottage industry being livelihood option of a large section of the society. Since 2014, *Pleurotus pulmonarius,* commonly known as the Indian oyster has been introduced in the state with astounding success. The pale white soft textured mushroom with acceptable aroma and high yield is fast assuming popularity (Fig. 1 to 12).

4.1 Growth pattern and fruit body characteristics of *Pleurotus pulmonarius*

Among all the cultivated mushrooms, *Pleurotus* is appreciated for its culinary properties and broad adaptability under varied agro-climatic conditions. Simple cultivation technique, highest productivity, choice of species/variety, ability to degrade a large number of substrates and after all, longer shelf life has made this mushroom the preferred one among the tropics and sub-tropical ones.

The test species was grown aseptically in the petri plates containing potato dextrose agar medium for observation on the growth pattern. The data on pileus size, stipe length, stipe thickness, along with few morphological characters are presented in Table 1 and Fig. 13 to 15.

Table 1 Growth pattern and fruit body characteristics of *Pleurotus pulmonarius*

Sl. No.	Growth pattern on PGA			Characteristics of fruit bodies on paddy straw substrate				
	Mycelia density	Type of growth	Colony colour	Pileus size (cm)	Stipe length (cm)	Stipe thickness (cm)	colour	Margin
1	Dense	Dense strandy	Pale white	7.4	4.0	0.9	Pale white	Wavy
2	Dense	Dense strandy	Pale white	8.5	4.0	1.0	Pale white	Wavy
3	Dense	Dense strandy	Pale white	6.5	3.0	1.2	Pale white	Wavy
4	Dense	Dense strandy	Pale white	6.7	3.8	1.0	Pale white	Wavy
5	Dense	Dense strandy	Pale white	16.0	4.2	2.1	Pale white	Wavy
6	Dense	Dense strandy	Pale white	6.3	4.3	1.2	Pale white	Wavy
7	Dense	Dense strandy	Pale white	5.2	5.5	2.3	Pale white	Wavy
8	Dense	Dense strandy	Pale white	6.5	2.5	0.9	Pale white	Wavy
9	Dense	Dense strandy	Pale white	7.8	3.4	1.4	Pale white	Wavy
10	Dense	Dense strandy	Pale white	6.8	4.2	1.2	Pale white	Wavy
Mean	-	-	-	7.17	3.89	1.32	-	-

The observation on morphological characteristics of the test species raised in the month of December indicated that the mycelium of *Pleurotus pulmonarius* was dense strandy on PDA medium with pale with cottony colour. The pileus diameter, stipe length and stipe thickness varied between 5.2-10.0, 2.5-5.5 and 0.9-2.3 cm respectively. In all the 10 fruit bodies observed, the margin was found to be wavy with pale white pileus colour.

Production process of *Pleurotus pulmonarius*

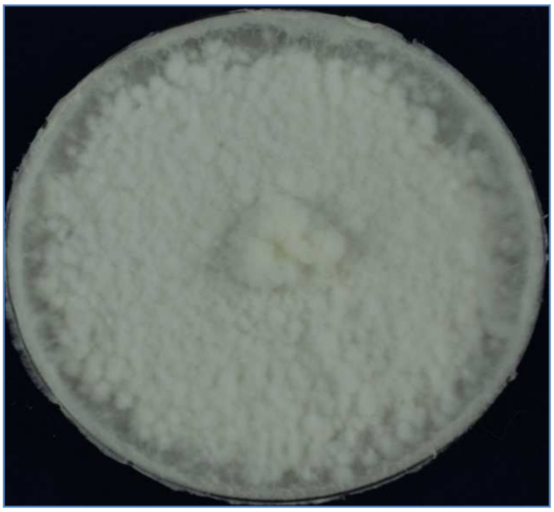


Fig: 1 Culture of *Pleurotus pulmonarius*

Fig: 2 Fully grown mushroom fruit bodies

Fig: 3 Mushroom spawn bottles

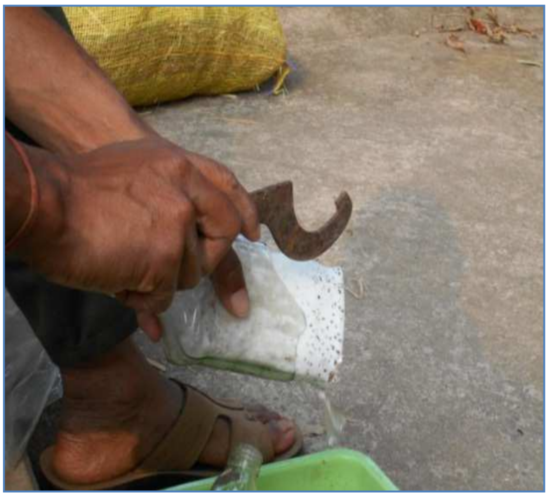


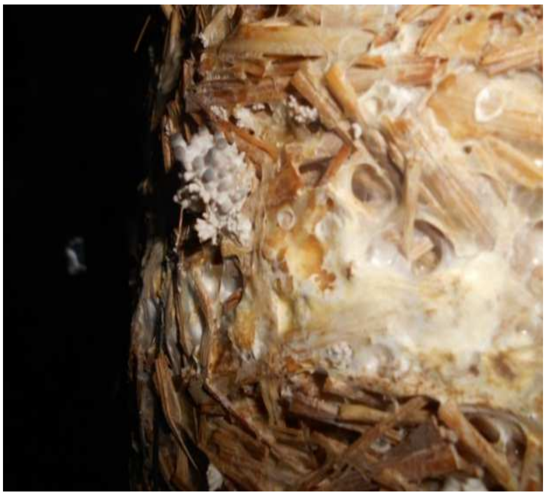
Fig: 4 Breaking of spawn bottles

Fig: 5 Spawn of ***Pleurotus pulmonarius***

Fig: 6 Spawn ready bagging

Fig: 7 Bag preparation

Fig: 8 Oyster bags



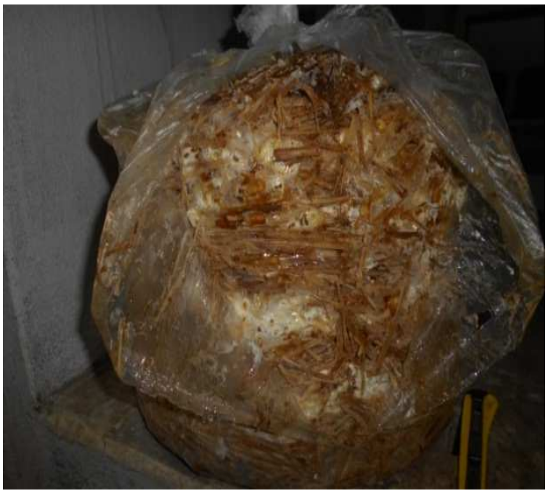


Fig: 9 Mycelia colonization

Fig: 10 Removing cover on bag

Fig: 11 Placing of bags in growing room

Fig: 12 Fruiting bodies of *Pleurotus*

Growth pattern and fruit body characteristics

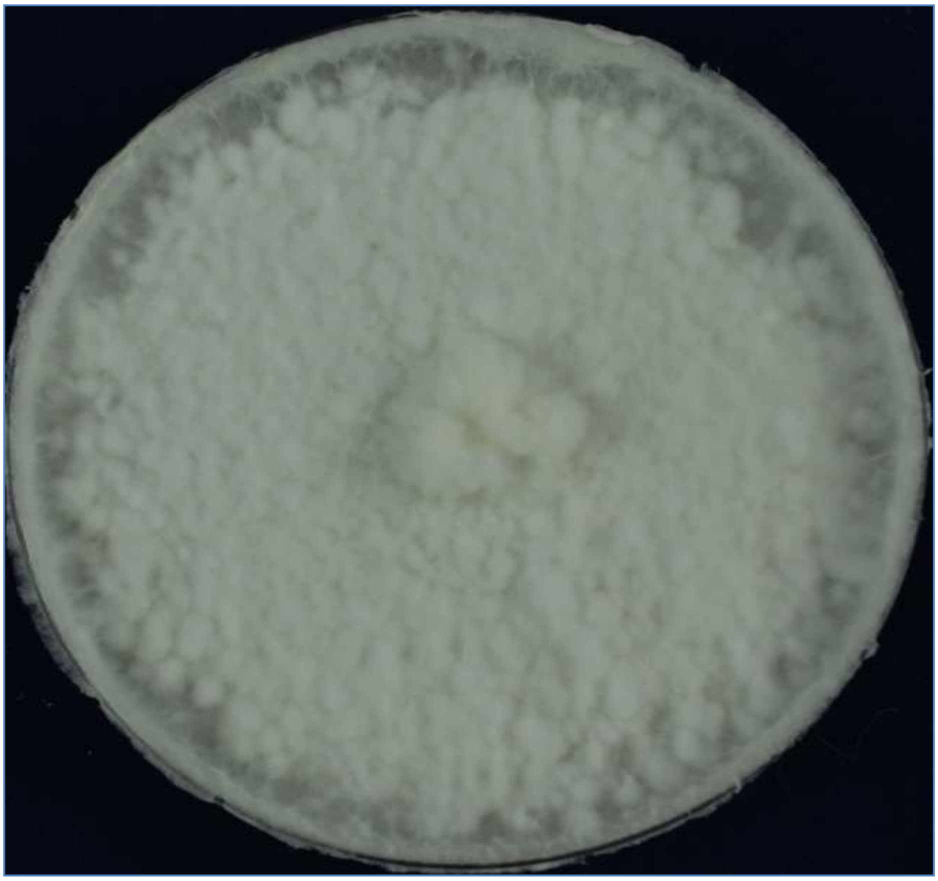


Fig.13 Culture of *Pleurotus pulmonarius*

Fig. 14 Detached fruit bodies of *Pleurotus pulmonarius*

Fig. 15 Fruit bodies of *Pleurotus pulmonarius*

4.2 Influence of substrates on yield and yield attributing parameters of *Pleurotus pulmonarius*

The experiment was conducted to find out the appropriate substrate in terms of days taken for spawn run, days to harvest, average weight (G) of fruiting bodies and biological efficiency. Nine different ligno-cellulosic substrates such as maize stalk, maize cob, sugarcane bagasse, coconut coir, rice bran, paddy husk, pulse stick , saw dust, paddy straw(check) were tried in the investigation to evaluate their potential on yield and yield attributing parameters. Observations recorded on days to spawn run, days of first harvest, average fruit body weight (g) and biological efficiency (%) are presented in Table 2 and Fig. 16.

Table 2. Effect of substrates methods on biological efficiency of *Pleurotus pulmonarius*

Sl. No.	Treatment (pasteurization method)	Days to spawn run	Days to first harvest	Average weight of fruit bodies (g)	Biological efficiency (%)
1	Maize stalk	17.33	24.33	9.73	92.25
2	Maize cob	17.67	24.67	9.84	91.93
3	Sugarcane bagasse	22.00	23.33	6.66	61.07
4	Coconut coir	21.00	29.33	5.99	64.37
5	Rice bran	19.67	25.67	7.41	70.20
6	Paddy husk	17.33	23.00	8.41	80.27
7	Green gram stick	17.00	24.00	10.05	88.27
8	Saw dust	20.33	27.33	8.39	77.40
9	Paddy straw (check)	16.33	21.33	10.71	99.30
	CD (0.05)	1.22	1.10	0.80	3.63
	CV (%)	3.76	2.51	5.42	2.60

Analysis of data showed significant difference among the substrates in terms of days to spawn run. Superiority of paddy straw substrates was observed with lowest days to spawn run (16.33 d0 among all the substrates. However, this was statistically at par with green gram stick (17d), maize stalk (17.33d) and paddy husk (17.33d). The incubation period varied in the range of 16.33-22.00d in the experimentally. Crop duration was also found significantly (21.33 d) in conventionally used paddy straw substrate. Further, the average weight (g) of fruit body was numerically highest (10.15g) in the paddy straw substrate which was followed by green gram stick (10.05g). Biological efficiency varied within 61.07% on sugarcane bagasse to 99.30% on paddy straw.

Hence, the results clearly indicated the superiority of paddy straw over other substrate in the terms of yield and yield attributing parameters in *Pleurotus pulmonarius.*

Influence of substrates on biological efficiency

Fig. 16 ***Pleurotus pulmonarius*** **raised on paddy straw**

4.3 Effect of substrate pasteurization methods on mushroom productivity

Data recorded on days to spawn run, days to first harvest, average fruit body weight (g) and biological efficiency (%) are presented in Table 3 and Fig. 17 to 21.

Table 3. Effect of substrate pasteurization methods on biological efficiency of *Pleurotus pulmonarius*

SL. No.	Treatment (pasteurization method)	Days to spawn run	Days to first harvest	Average weight of fruit bodies (g)	Biological efficiency (%)
1	Boiled water treatment	16.00	24.00	7.88	91.80
2	Steam treatment	14.50	21.75	8.45	98.90
3	Substrate solarisation	16.50	24.25	8.80	82.95
4	Chemical treatment	17.50	24.25	9.15	96.63
5	Control (No treatment)	16.50	22.50	7.10	79.28
	CD (0.05)	1.07	1.12	1.25	6.29
	CV (%)	7.48	3.10	9.83	4.54

Steam treatment of substrate at 60-80^{0}C for one hour was found superior in terms of days to spawn run (14.50 d) days to first harvest (21.75d) and biological efficiency (98.90%). Treatment of the substrate with the chemicals was association with longer incubation period (17.50d) and crop duration (24.25d). However, the average fruit body weight (9.15 g) was better than the steam treated substrates were 98.90 and 96.63% respectively and they were statistically at with each other. The untreated substrate produced the lowest yield (79.28 %). The steam treatment thus, proved its superiority over others in terms of yield of mushroom.

Effect of substrates pasteurization methods on biological efficiency

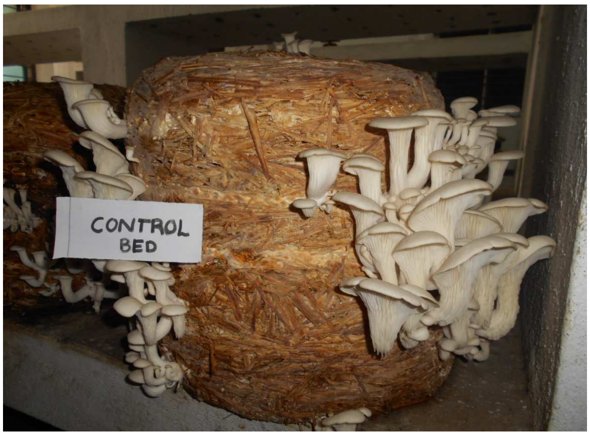


Fig.17 Fruiting on the untreated substrate

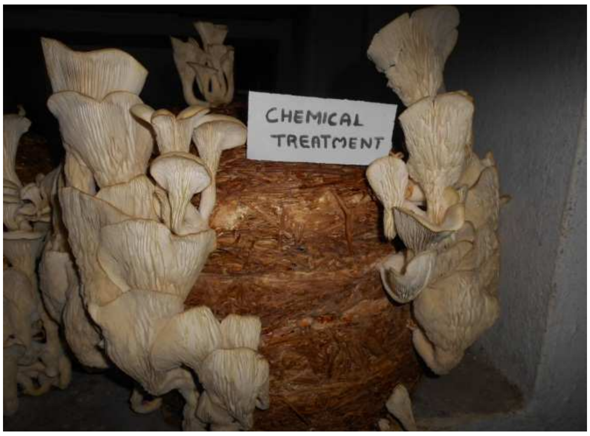


Fig.18 Fruiting on the chemical treated substrate

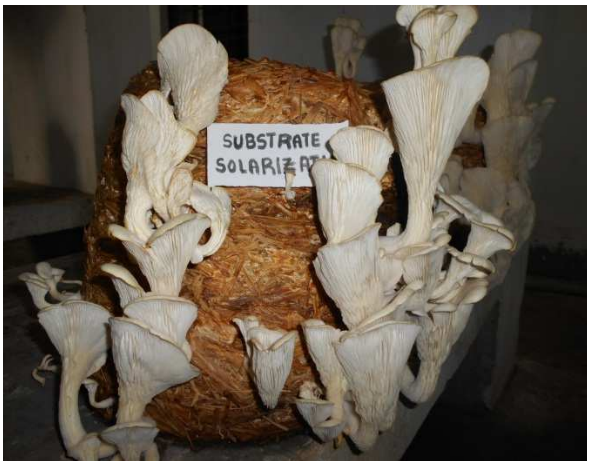


Fig.19 Fruiting on the solarized treated substrate

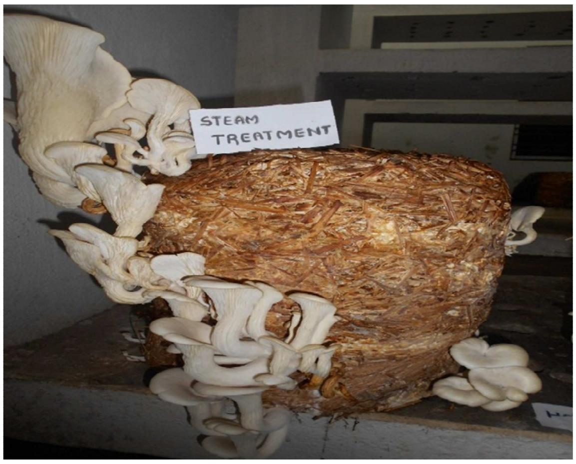


Fig.20 Fruiting on the steam treated substrate

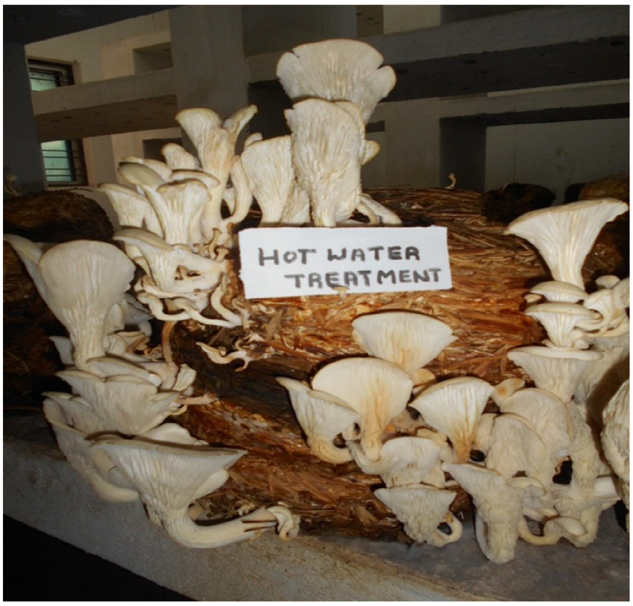


Fig.21 Fruiting on the hot water treated substrate

4.4 Effect of bag dimension on biological efficiency of *Pleurotus pulmonarius*

An attempt was made to manipulate the bag dimension in order to improve the productivity of *Pleurotus pulmonarius*. Six diverse bag types such as 20 x 15 cm, 25 x 20 cm, 30 x 25 cm, 40 x 25 cm, 50 x 25 cm and 60 x 25 cm accommodating 500, 1000, 100, 2000, 2500 and 3000g at substrates respectively were used to assess their yield performance. Analysed data on days to spawn run, days to first harvest, average fruit body weight (g) and biological efficiency (%) are presented in Table 4 and Fig. 22 to 24.

Table 4. Effect of bag dimension on biological efficiency of *Pleurotus pulmonarius*

Sl. No.	Treatment (substrate quantity in gram)	Days to spawn run	Days to first harvest	Average weight of fruit bodies (g)	Biological efficiency (%)
1	500 (20x15cm)	15.50	21.75	7.13	95.60
2	1000 (25x20cm)	15.50	23.00	7.40	98.40
3	1500 (30 x 25 cm)	17.00	24.50	10.23	101.13
4	2000 (40 x 25cm)	18.00	26.00	10.43	90.05
5	2500 (50x25)	18.50	26.75	10.93	87.40
6	3000 (60x25)	20.00	27.25	10.30	87.75
	CD (0.05)	1.36	1.32	1.04	2.12
	CV (%)	5.17	3.51	7.34	1.50

Significant difference was observed among treatments in respect of all the parameters recorded. An increase in the incubation period (15.50-20.00) and the crop duration (21.75-27.25) was observed with the increase in the quantity of substrate used from 500-3000g per bag. However, superiority of 30 x 25 cm bag accommodating 1500g substrate was recorded in respect of biological efficiency (101.18%) among all the six treatments evaluated, average fruit body weight (g) was numerically highest in 50 x 25 cm bag containing 2500g substrate. The variation in wield was in the range of 87.40-101.18 kg/100kg substrate in the investigation. Thus, it was found that neither the smaller nor the larger bags were beneficial for improvement of productivity in *Pleurotus pulmonarius.*

Effect of bag dimension on biological efficiency

Figure 22. Fruiting in beds having 30 × 25 cm

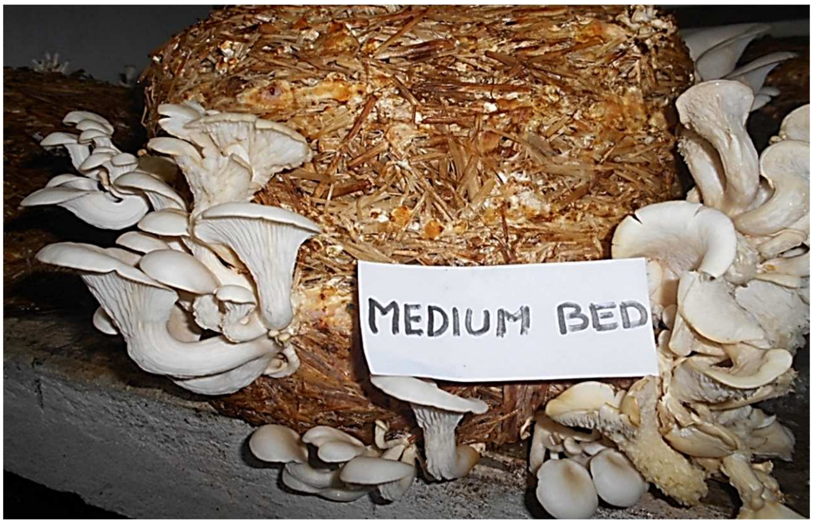


Fig.23 Fruiting in beds having 25 x 20 cm

Fig.24 Fruiting in beds having 20 x 15 cm

4.5 Influence of methods of spawning on biological efficiency of *Pleurotus pulmonarius*

Three spawning methods such as layer spawning, thorough spawning and top spawning were evaluated to assess their yield potential in *Pleurotus pulmonarius*. Analysed data on days to spawn run, days to first harvest, average fruit body weight (g) and biological efficiency (5) are presented in Table 5 and Fig.25 to 27.

Table 5. Influence of method of spawning practices on biological efficiency of *Pleurotus pulmonarius*

Sl. No.	Treatment (Method of Spawning)	Days to spawn run	Days to first harvest	Average weight of fruit bodies (g)	Biological efficiency (%)
1	Layer spawning	16.33	23.00	8.38	97.66
2	Thorough mixing	16.33	23.33	8.15	96.87
3	Top spawning	21.17	27.67	9.55	78.37
	CD (0.05)	1.36	1.59	1.07	5.90
	CV (%)	5.90	5.02	9.61	5.05

Data indicated that both the layer spawning and thorough mixing of spawn along with the substrate wee statistically at par in respect of all the four parameters recorded. However, the highest yield of 97.66 percent was recorded from bags where in thorough spawning method was adopted. It was statistically equal to the yield (96.87%) obtained from the treatment with thorough mixing of both spawn and substrate, the biological efficiency was significantly lowest (78.37%) in case of top spawning. Therefore, it was once again ascertained that top spawning procedure was not viable for commercial cultivation of oyster mushroom. In view of the increased labour requirement in case of layer spawning, thorough spawning could be a viable proposition for commercial farms.

Biological efficiency as influenced by method of spawning

Fig.25 Layer spawning

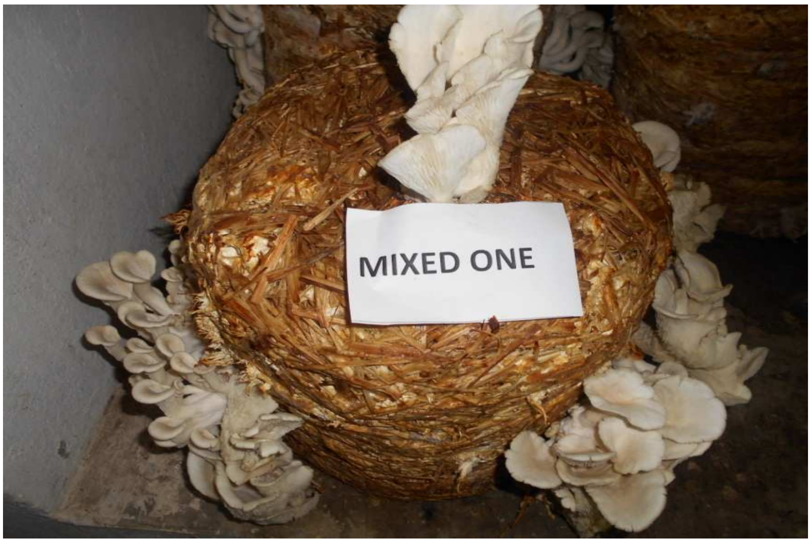


Fig.26 Thorough mixed spawning

Fig.27 Top spawning

4.6 Influence of organic supplements on biological efficiency of *Pleurotus pulmonarius*

This experiments was designed to evaluate as number of organic additives such as maize meal, ragi powder, bengal gram powder, mustard oil cake, chicken manure, boiled wheat, rice bran, tapioca pearls and boiled maize for their role in improving mushroom productivity over the untreated check (control). Data recorded on time taken for spawn run (d), first harvest (d), average weight of fruit bodies (g) and biological efficiency (%) are presented in Table 6 and Fig. 28 to 34.

Table 6. Effect of Organic supplements on biological efficiency of *Pleurotus pulmonarius*

Sl. No.	Treatment (Organic supplements)	Days to spawn run	Days to first harvest	Average weight of fruit bodies (g)	Biological efficiency (%)
1	Maize meal	17.67	24.33	10.33	91.00
2	Ragi powder	18.67	26.67	7.37	78.37
3	Bengal gram powder	17.33	23.33	10.20	90.99
4	Mustard oil cake	19.00	25.67	7.17	79.00
5	Chicken manure	18.33	25.00	10.93	93.07
6	Boiled wheat	17.67	24.33	11.00	93.37
7	Rice bran	17.33	24.67	10.94	92.33
8	Tapioca pearls	18.00	25.67	7.08	80.40
9	Boiled maize	17.00	23.33	9.02	92.53
10	Control (No supplement)	17.33	23.33	10.01	91.07
	CD (0.05)	1.29	2.01	0.98	5.42
	CV (%)	4.22	4.76	6.05	3.58

Influence of organic supplements on biological efficiency

Fig.28 Boiled maize as the supplement

Fig.29 Boiled wheat as the supplement

Fig.30 Bengal gram powder as the supplement

Fig.31 Rice bran as the supplement

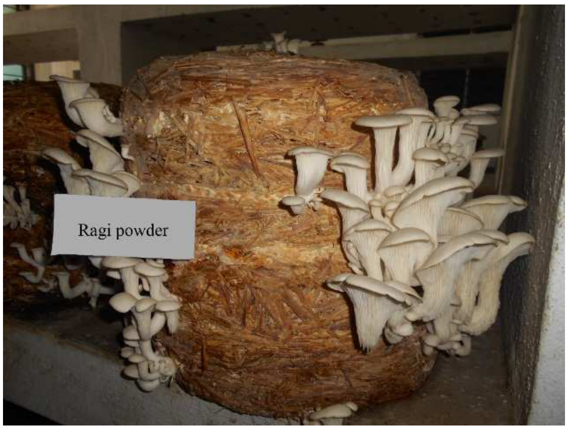


Fig.32 Ragi powder as the supplement

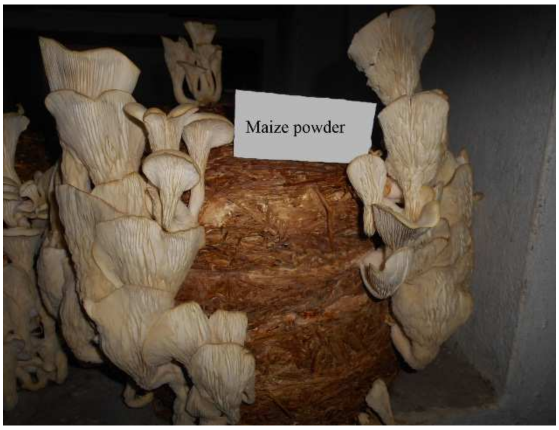


Fig.33 Maize powder as the supplement

Fig.34 Fruiting without supplement

Analysis of data indicated as close variation among the incubation period of the test species under the influence of nine additives. It ranged from 17.00 d in boiled maize to 19.00 d in mustard oil cake. The treatment that received no supplement was having an incubation period from 23.33 d to 26.67 d in the investigation. The crop duration was lower (23.33 d) in case of Bengal gram powder, boiled maize and control treatments. The average fruit body weight (g) was higher in treatment that required boiled wheat as supplement (11.00 g) which was statistically at par with rice bran (10.94 g), chicken manure (10.20 g), maize meal (10.33g) and bengal gram powder (10.20g). The biological efficiency was recorded numerically highest (93.37%) in boiled wheat treatment which was at par with the yields realized from chicken manure (93.07%), boiled maize (92.53 %), rice bran (92.33%), untreated control (91.07%), maize meal (91.00%) and Bengal gram powder (90.99%). The biological efficiency varied in the range of 78.37% in ragi powder to 93.37% in boiled wheat in the investigation.

4.7 Influence of post-spawning practices on biological efficiency of *Pleurotus pulmonarius*

Diverse post-spawning practices such as uncovering the oyster bags just after mycelia colonization or maintaining the bags throughout the crop period either with small holes (0.5 cm) or big holes 91.0cm) are in operation among the growers. Hence, an attempt was made to assess their yield performance of farm house situation during the winter season of 2015-16. Data recorded on days to spawn run and first harvest, number of fruit bodies per bag, average fruit body weight (g) and biological efficiency (%) were analysed and are presented in Table 7 and Fig.35 to 37.

Table 7. Influence of Post-Spawning practices on biological efficiency of *Pleurotus pulmonarius*

Sl. No.	Treatment (Post-Spawning practices)	Days to spawn run	Days to first harvest	No. of fruit bodies / kg	Average weight of fruit bodies (g)	Biological efficiency (%)
1	Bags with 1.0 cm hole	15.83	24.17	146.83	9.98	96.47
2	Bags with 0.5 cm hole	16.00	24.00	144.00	9.73	91.68
3	Bags uncovered	15.67	21.50	183.83	7.58	97.34
	CD (0.05)	1.17	1.36	6.98	1.18	4.07
	CV (%)	5.77	4.56	3.43	10.06	3.33

Role of post-spawning practices on productivity

Fig.35 Bag with big holes (1 cm)

Fig.36 Bag with small holes (0.5 cm)

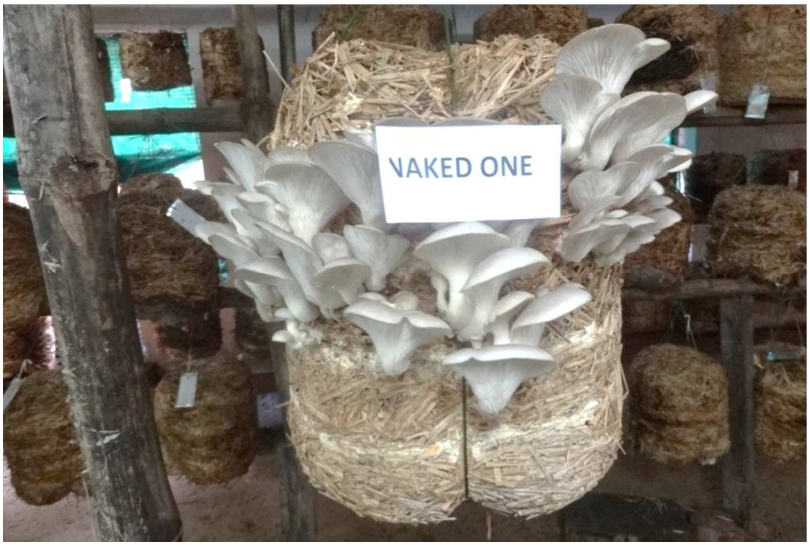


Fig.37 Bag without holes

Data indicated that the days to spawn run was statistically equal in all the three treatments ranging from 15.67-16.00 days. However, the days to first harvest (crop duration) was significantly lowest (21.50) in the uncovered bags. Similarly, the number of fruit bodies was significantly more (183.33) in the uncovered bags. Average fruit body weight was recorded lowest (7.58 g) in uncovered bags. However, they are recorded at 9.98g and 9.73g in bags with 1.0cm and 0.5cm holes respectively, which were statistically at par with each other. The biological efficiency was found highest in uncovered bags (97.34%) which were at par with nags with 1.0cm holes (96.47%). Bags with smaller holes were associated with a biological efficiency of 91.68 percent. Hence, it was ascertained that bags with either big holes of 1.0cm size or uncovered ones were almost parallel in their yield performance as recorded in the investigation.

4.8 Effect of period of spawning on biological efficiency of *Pleurotus pulmonarius*

Traditionally, *Pleurotus spp.* is growing during winter months (November – February) in the state of Odisha. However in an attempt to find out the influence of spawning period on yield and associated parameters, a trail was formulated by growing this newly introduced species from November – February at 15 days interval in the ambient situation. The data recorded on yield and yield attributing parameters were analysed and are presented in a Table 8.

Table 8. Effect of period of spawning on biological efficiency of *Pleurotus pulmonarius*

Sl. No.	Treatment (Date of Spawning)	Days to spawn run	Days to first harvest	Average weight of fruit bodies (g)	Biological efficiency (%)
1	1.11.2015	20.00	27.33	7.90	88.93
2	16.11.2015	20.67	27.00	8.04	88.00
3	1.12.2015	17.00	23.33	10.67	99.15
4	16.12.2015	15.67	22.67	10.64	99.87
5	1.01.2016	16.00	22.67	10.42	100.17
6	16.01.2016	16.67	24.67	8.53	80.43
7	1.02.2016	17.00	25.67	8.17	80.33
8	16.02.2016	20.67	29.00	7.67	77.37
	CD (0.05)	1.72	0.98	0.98	3.72
	CV (%)	5.48	2.22	6.18	2.38

Analysis of data showed significant difference among the eight dates of spawning. It was observed that spawning from 1st December, 2015 till 1st February, 2016 (fire date) were it par in respect of days of spawn were in the range of 15.67-17.00 days. The incubation period was lengthened as the spawning period was either preponed or postponed. The same trend was also noticed in respect of days to first harvest (crop duration). Average weight of fruit bodies were recorded maximum on its 1st December, 2015 spawning which as at par with spawning at 16th December, 2015 and 1st January, 2016. The biological efficiency was numerically highest (150.17%) on 1st January, 2016 spawning which was statistically equal to 1st and 16th December, 2015 spawning (99.15 and 99.86%). The results revealed that December and January spawning were appropriate in realizing superior yields out of *Pleurotus pulmonarius.*

DISCUSSION

Pleurotus is/ relatively new to the mushroom industry but has gained popularity at a tremendous pace because of the simple cultivation techniques, highest productivity among the cultivated mushroom, choice of species, adoptability to a variety of substrate and above all, longer shelf life. Out of 38 species describe under the genus, about 25 species are commercially cultivated in different parts of the world. In Odisha, two species namely, *Pleurotus sajor- caju* and *Pleurotus florida* are popular among the farmers owing to their high yield potential and acceptable taste and flavor. However, efforts are being taken to popularize few more promising species or varieties and different agro-climatic zones of Odisha. For the, nutritive value of Oyster mushroom is as good as other edible mushrooms.

Pleurotus mushrooms are rich in vitamin B complex and C, besides the presence of most of the mineral salts required by human beings. Moreover, a polycyclic aromatic compound pleurotin isolated from *Pleurotus spp.* possesses antibiotic properties. In view of the aforesaid importance associated with *Pleurotus* mushroom, a new species or variety, *Pleurotus palmonarius*, commonly known as the Indian oyster, Italian oyster, Phoenix mushroom or the lung oyster having similarity to *Pleurotus ostreatus*, the pearl oyster, has recently been introduced in the state of Odisha with success. This species or variety has got high yield potential, besides having excellent aroma and good shelf life. An attempt has by made to develop the package of practices for commercial cultivation of *Pleurotus palmonarius* in this investigation.

The study and growth pattern and fruit body characteristics of *Pleurotus palmonarius* raised during the month of December indicated that on the potato dextrose agar medium, the growth pattern of the species was dense strandy in behavior. Further, the colour of the fruit bodies was pale white at large. Margins of the fruit bodies were entire when young, wavy at harvest. The pileus diameter, stipe length and stipe thickness varied between 5.2 to 10.0, 2.5 to 5.5 and 0.9 to 2.3 cm respectively. Singh *et al.* (2010) established the fact that among all the edible mushrooms, wide variation in shape, color, margins of the fruit body and growth pattern on nutrient media existed in *Pleurotus*. Shukla and Jaitly (2011) characterized different species of *Pleurotus* morphologically which was conformity to the findings of this investigation.

A large number of agricultural, forest and agro industries by products rich in cellulose and hemicelluloses and lignin are useful for growing oyster mushroom. However, yield of oyster mushroom largely depends on the nutrition and nature of the substrate. Fresh, dry and properly stored substrates free from mould infestation are appropriate for the purpose. *Pleurotus* can utilized a large number of agro wastes including cereal straw, stalk and leaves of maize, jowar and bajra, sugarcane bagasse, jute and cotton waste, peanut shell and haulm, sunflower and niger stalk, discarded waste paper, dried grasses, used tea leaves waste etc. It can also be cultivated on industrial wastes. In the eastern parts of the country, *Pleurotus* is commonly cultivated on paddy straw substrate which is available in abundance at cheaper rates. However, in certain areas of Odisha, niger stick, groundnut haulm and coir pith in combination with paddy straw are successfully utilized as substrates for oyster mushroom cultivation. Over the years, various research works have been trying to find out the appropriate substrates for the purpose.

In the present study, nine agro wastes including the paddy straw were evaluated for their performance in terms of yield. Varying performance was observed among the substrates in respect of days to spawn run and fruit harvest, average weight of sporophores and biological efficiency. However, the paddy straw was found superior among the substrate in terms of fruit yield (99.30%). Coconut coir, rice bran and saw dust were proved to be the poor yielders with 64.37, 70.20 and 77.40% biological efficiency. Realization of superior yields through utilization of paddy straw was in corroboration with the findings of Park *et al.* (1975), Singh (1981), Ramzan (1982), Mathew *et al.* (1996) and Ingale and Ramteke (2010). *Pleurotus* comes up well in cellulose rich un-composted substrates having relatively higher C : N ratio (40-60) and the cellulose helps in more enzyme production which is correlated with good yields. Paddy straw substrate being rich in cellulose could probably facilitate higher production through the action of the lignocellulolytic *Pleurotus* species.

The mycelium of *Pleurotus* is saprophytic in nature and it does not require selective substrates for its growth. The mycelia growth can takes place on simple water treated straw but there are number of other competitor moulds sometimes restrict the growth of *Pleurotus* mycelium due to secretion of toxic substances or metabolites. Hence, four different substrate pasteurization methods along with untreated done (control) were evaluated for their yield potential superiority of steam treatment of substrate in terms of realization of mushroom yield (98.90%) was established in the investigation. However, yield obtained (96.63%) out of the treatment pasteurized with chemical was statistically at par with the steam treatment, through it was associated with longer incubation period (17.50 d) and crop duration (24.25 d). The fact is that steam was got greater penetrating power and hence, has been able to pasteurize the substrate well for manifesting better productivity (Siqueira *et al.,* 2012). Besides, the chemical treatment leaves residues in the substrate which probably lengthened the incubation period and the crop duration initially. The performance of chemical treatment was in agreement with the findings of Vijay and Sohi, 1987. The untreated substrate (control) produced the lowest yield (79.28%), which explained the abilities of the competitor moulds to reduce yield substantially.

Bag dimension plays a vital role in maintaining moisture and temperature of the and there by influencing the sporophore yield to a great extent. The investigation on the effect of bag dimension on mushroom productivity indicated the superiority of 30x25 cm bag accommodating 1500g substrate in recording. The significantly highest yield of 101.18 percent among all the six experiments evaluated. It was closely followed by the bag dimension of 25x20 cm accommodating 1000g substrate which recorded 98.40% biological efficiency. It was observed that the large sized bags (40x25 cm, 50x25 cm and 60x25 cm) were poor yielders (87.40-90.05%). The bag size of 20x15 cm accommodating substrate could give 95.60% biological efficiency which would probably be the best option for small growers. Suman and Sharma (2007) advocated use of 60x45 cm bags of 125-150 gauze thickness for oyster mushroom cultivation, which accommodated 3.0 kg of dry substrate. However, this investigation ascertained that medium sized bags (30x25 cm) with 1500g straw could be the best option for commercial growers.

The oyster mushroom growers employ different spawning methods such as layer spawning, top spawning and mixed spawning in different localities. However, to assess their yield potential, an investigation was undertaken during the winter season of 2015- 16. It was observed that both layer spawning and mixed spawning were statistically at par in respect of yield as well as the associated parameters recorded in the trail having given the yields of 97.66 and 96.87% respectively. However, the top spawning was poor yielder with a yield of 78.37% the findings of this investigation was in conformity with the findings of Sanger *et al.* (2006) and Ram (2007). A single focal point only at the top of the bag might be responsible in giving poor yields in top spawning in comparison to others which have large number of foci to initiate fruits.

The nitrogen contents in most of the substrates range between 0.5-0.8% and hence, addition of organic nitrogen in the substrate helps in obtaining higher yields. Some of the common supplements in use are wheat bran, rice bran, cotton seed mill, mustard oil cake, groundnut cake, soybean cake, boiled wheat grain, chicken manure, maize powder etc. they are used at 5-10% on dry weight basis of the substrate for yield improvement in oyster mushroom.

In the investigation on the effect of different organic additives on mushroom yield, nine additives along with control (no additives) were evaluated for their yield potential. Analysis of data indicated a close variation among the incubation period of the test species under the influence of nine additives. If ranged from 17.0 d in boiled maize to 19.0 d in mustard oilcake the untreated control was having an incubation period of 17.33d. The crop duration was lower (23.33d) in Bengal gram powder, boiled maize and untreated control.

The biological efficiency was reported numerically highest (93.37%) in boiled wheat which was at par with the yields realized from chicken manure (93.07%), boiled maize (92.53%), rice bran (92.33%), untreated control (91.07%), maize meal (91.10%) and bengal gram powder (90.99%). Various workers have evaluated diverse groups of supplements for yield promotions of *Pleurotus.* Upadhyay *et al.* (1991) indicated that rice bran at 5% level was the appropriate substrate for *Pleurotus.* He also used wheat bran and powdery manure as supplements but yields obtained were lower than that of rice bran. Veena *et al.* (1994) evaluated supplements in combination and indicated that *Pleurotus florida* performed well with a combination of rice bran and soys dal powder. *Pleurotus* mushroom have an appreciable degree of competitive saprophytic ability and therefore, the bags receiving no additive also produced statistically equal yield with the boiled wheat supplementation.

An attempt was made to evaluate few post-spawning procedures such as uncovering the mycelia bags after colonization. Maintaining the bags with either small (0.5cm) or big holes (1.0 cm) for their yield potential. It was observed that the yields obtained out of both uncovered bags (97.34%) and the bags with 1.0 cm holes (96.47%) were statistically at par, bags with smaller holes are comparatively poor yielders (91.68%). This might be attributed to the difficulty in fruit body emergence in case of small holes and/or availability of fresh air for fruit body induction.

The earlier workers suggested the opening of the bags after complete colonization in order to get more yield (Suman and Sharma, 2007, Pal *et al*., 2015). However, the present investigation ascertained that even without uncovering the bags, good yields could be recovered out of the bags. This finding may well facilitate saving of labour for large scale producers.

Pleurotus mushroom, being a sub-tropical mushroom, comes up well in winter season in the East and South-Eastern coastal plain zone of Odisha. In order to find out the appropriate time of spawning, eight dates of spawning starting from 1st November, 2015 to 16th February, 2016 were evaluated for their yield potential. Analysis of data showed significant difference among the eight dates of spawning in respect of yield and associated parameters. The biological efficiency was numerically highest (100.17 %) in 1st January, 2016 spawning which was statistically equal to 1st and 16th December, 2015 spawning (99.15 and 99.87 %). It was thus ascertained that for coastal situation of the state, December and January spawning were appropriate in realization of higher yields. Unlike other cultivated mushrooms, species of *Pleurotus* exhibit much diversity in their adaptability to varying agro-climatic conditions. Various workers have shown to recover good yields out of oyster mushroom raised during rainy/winter seasons at a temperature of 17.2-35^{o}C and relative humidity 70-90 % (Bano and Rajarathnam, 1982; Shanmugam, 1986 and Sanger *et al*., 2006).

SUMMARY AND CONCLUSION

Among the edible mushrooms grown in industrial scale in India, oyster mushroom (*Pleurotus* spp.) enjoys a special status owing to its cultivation on a variety of substrates, availability of a large number of species suitability to varied agro-climatic conditions of the country, simple and easy cultivation procedure, longer shelf life and above all, highest productivity. It is the 3rd largest cultivated mushroom of the world contributing to 15 % of the total world production of mushrooms after *Agaricus bisporus* and *Lentinula edodes*. In Odisha, this mushroom occupies second place next only to straw mushroom in terms of popularity. It is cultivated largely on paddy straw substrate with or without supplementation with a biological efficiency ranging from 80-110 per cent. Among the edible species, *P. sajor-caju* and *P. florida* are commercially cultivated because of their higher biological efficiency, attractive colour and excellent taste and flavour. During the present course of investigation, a series of experiments were conducted to evaluate the newly introduced species *P. pulmonarius* of oyster mushroom in the East and South-Eastern coastal plain zone of the state in terms of its productivity and associated attributes.

Studies on growth pattern and fruit body characteristics revealed that the mycelium *P. pulmonarius* was dense strandy on PDA medium with pale white colony colour. The pileus diameter, stipe length and stipe thickness varied between 5.2-10.0, 2.5-5.5 and 0.9-2.3 cm respectively. The margin of the fruit body was found to be wavy with pale white pileus colour.

Among the nine substrates evaluated for their yield potential, superiority of paddy straw was established in terms of realization of biological efficiency (99.30 %). Sugarcane bagasse, rice bran and saw dust were found inferior with biological efficiency of 61.07, 70.20 and 77.40 % respectively. The biological efficiency varied within 61.07 % on sugarcane bagasse to 99.30 % on paddy straw.

Among the pasteurization methods of substrate evaluated for cultivation of *P. pulmonarius*, biological efficiency of steam as well as chemical treated substrates 98.90 and 96.63 % respectively and statistically at par with one another. Solarization and boiled water treatment of substrate were associated with the biological efficiency of 86.0 and 85.5 % respectively. The non-pasteurized substrate (control) was a poor yielder with the biological efficiency of 76.0 %.

Six diverse bag types such as 20 x 15, 25 x 20, 30 x 25, 40 x 25, 50 x 25 and 60 x 25 cm accommodating 500, 1000, 1500, 2000, 2500 and 3000 g of straw were tested for their yield performance. It was revealed that the bag size of 30 x 25 cm accommodating 1500 g substrate was associated with the highest biological efficiency (101.18 %). Irrespective of bag size, the biological efficiency was quite appreciable in all the bag types (87.40-101.18 %).

Method of spawning in oyster mushroom has been the area of controversy over years. The highest yield of 97.66 per cent was recorded from bags wherein through spawning method was employed. However, it was statistically equal to the yield (96.87 %) obtained from the treatment with thorough mixing of both spawn and substrate. The biological efficiency was significantly lowest (78.37 %) in case of top spawning.

The study on the influence of supplements on yield of *P. pulmonarius* indicated that build wheat (93.37 %), chicken manure (98.07 %), boiled maize (92.53 %), rice bran (92.33 %), substrate without supplement (91.07 %), maize powder (91.0 %) and Bengal gram powder (90.99 %) were statistically at par in respect of yield performance. Ragi powder, mustard oil cake and tapioca pearls with productivity of 78.37, 79.00 and 80.40 % produced even lower yields (91.07 %) than control treatment. The finding ascertained that oyster mushrooms could successfully be grown in the absence of organic supplements as it has got highest degree of competitive saprophytic ability among all edible mushrooms.

Among the post-spawning practices, the biological efficiency was found highest (97.34 %) in uncovered bags which was statistically at par with bags with 1.0 cm holes surrounding the bags (96.47 %). Bags with smaller holes (0.5 cm) were associated with a biological efficiency of 91.68 %.

The biological efficiency was numerically highest (100.17 %) on the 1st January, 2016 spawning which was statistically equal to 1st and 16th December, 2015 spawning (99.15 and 99.87 %) among the eight spawning dates evaluated during the winter season at 15 days interval each. The results thus revealed that December and January spawning were appropriate in realizing superior yields out of *P. pulmonarius*.

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