STUDIES ON CULTIVATION OF GOLDEN OYSTER MUSHROOM (*PLEUROTUS CITRINOPILEATUS* SINGER)

THESIS

By

PRERNA THAKUR
(A-2017-30-073)

Submitted to

CHAUDHARY SARWAN KUMAR
HIMACHAL PRADESH KRISHI VISHVAVIDYALAYA
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Dr. Arun Kumar Sud
PES
(Plant Pathology)

Department of Plant Pathology,
College of Agriculture,
CSK Himachal Pradesh Krishi Vishvavidyalaya,
Palampur - 176 062 (H.P.) India

CERTIFICATE – I

This is to certify that the thesis entitled, **"Studies on cultivation of golden oyster mushroom (*Pleurotus citrinopileatus* Singer)"** submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Prerna Thakur** daughter of Smt. Gema Thakur and Shri Bhinder Thakur under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Place: Palampur
Dated: 20 June, 2019

(Dr.Arun Kumar Sud)
Major Advisor

CERTIFICATE- II

This is to certify that the thesis entitled, **"Studies on cultivation of golden oyster mushroom (*Pleurotus citrinopileatus* Singer)"** submitted by **Prerna Thakur (Admission No. A-2017-30-073)** daughter of **Shri Bhinder Thakur** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in the discipline of **Plant Pathology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

(Dr. Arun Kumar Sud) Chairperson Advisory Committee	( ) External Examiner
(Dr. Pardeep Kumar) Member	(Dr. R K Mittal) Member cum Dean's nominee

_____
Head of the Department

_____
Dean, Postgraduate Studies

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"Great works are performed not by strength, but by perseverance"

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Place: Palampur
Date: 20 June 2019 ***(Prerna Thakur)***

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LIST OF ABBREVIATIONS USED

S. No.	Abbreviation	Meaning
1	%	Per cent
2	&	And
3	/	Per
4	@	At the rate
5	°C	Degree centigrade
6	CD	Critical Difference
7	cm	Centimeter (s)
8	mg	Milli gram
9	e.g.	Example gratia (for example)
10	et al.	Etalli (and others)
11	etc.	etc etera
12	ml	Milli litre
13	i.e.	idest (that is)
14	hrs	Hours
15	kg	Kilogram
16	PDA	Potato Dextrose Agar
17	MEA	Malt Extract Agar
18	YPDA	Yeast Potato Dextrose Agar
19	pH	Pussance de hydrogen
20	P	Pertaining page (s)
21	mm	Milli meter
22	*viz.*,	Videlicent (namely)
23	µg	Micro gram
24	g	Gram
25	µm	Micro meter
26	sp., spp.	Species (Singular or Plural)
27	No.	Number

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Department of Plant Pathology
COA, CSK Himachal Pradesh Krishi Vishvavidyalaya
Palampur – 176 062 (HP)

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ABSTRACT

The present investigations entitled "Studies on cultivation of golden oyster mushroom (*Pleurotus citrinopileatus* Singer) was undertaken to evaluate various cultural requirements, grain substrate for spawn production and different agro-based substrates used for sporophore production of the test fungus. Out of seven solid media tested, Potato Dextrose Agar was found to be the best for mycelial growth of the test fungus and also took minimum days to full the Petri plate. Out of nine spawn substrates, the maximum mycelial growth rate was observed on sorghum grains (1.12cm/day) and minimum on pigeonpea (0.65cm/day). The minimum (27.33 days) time for spawn run was observed on sorghum grains spawn and maximum yield was observed in wheat grain spawn i.e. 893.33g/kg dry substrate. The minimum cost of production was observed on wheat grain spawn (Rs. 63.25) with maximum profit of Rs. 86.75. Out of nineteen agro-based substrates, minimum time for spawn run and primordia formation was taken by wheat straw i.e. 29 days and 34.67 days, respectively and maximum 5.00 number of fruit bodies was observed on soybean straw substrate. The soybean+maize combination took minimum days for spawn run (28.67 days) and primordia formation (34.67 days) and maximum number of primordia (4.33). So far as morphological characters of *P.citrinopileatus* in different substrates was concerned, largest pileus size (7.22x6.39cm) was observed on oat straw with highest average sporophore weight (11g). Among different agro-based substrate combinations, soybean+maize+oat produced largest pileus size (9.00x7.24cm) and maximum sporophore weight (12.33g). Maximum yield of 893.33g/kg dry substrate was observed on wheat straw substrate with maximum number of flushes (3) followed by soybean i.e. 860g/kg. Biological efficiency of different substrates, ranged between 16.67 to 89.33 per cent, with highest on wheat straw (89.33%). In different agro-based substrate combinations, soybean+maize+wheat performed quite well with biological efficiency of 87.33 per cent which is at par with the wheat straw.

(Prerna Thakur)
Student
Date: 20 June, 2019

(Dr.Arun Kumar Sud)
Major Advisor
Date: 20 June, 2019

Head of the Department

1. INTRODUCTION

Mushroom is a large macroscopic fruiting body of edible fungi which belongs to division Basidiomycotina. It may be either epigeal or hypogeal. The vegetative parts of mushroom mainly consist of thread like long thin mycelium and form fruiting bodies under suitable condition. Mushrooms, constitute low caloric and high nutritious food and are also useful in nutrient recycling. Mushroom cultivation is a lucrative activity and is becoming increasingly popular in the world. Mushrooms have several medicinal properties and are important source of protein. The cultivation of mushroom serves as the most efficient and economically viable biotechnology for the conservation of lignocelluloses waste material to high quality of food. This leads to open up new job opportunities especially in rural areas. Mushroom cultivation is followed due to their delicious flavor and low calorific value. It has been determined that more than 3000 mushroom species are edible, but only 10 of those are on an industrial scale (Chang and Miles 2004). The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Pleurotus* spp. that constitutes about 27% of the world's cultivated mushrooms.

The *Pleurotus* species, commonly known as Oyster mushrooms, are edible fungi cultivated worldwide especially in India, Europe, Africa and South East Asia. The name *Pleurotus* has its origin from Greek word, 'Pleuro' that means 'formed laterally' or 'lateral position of the stalk or stem'. *Pleurotus* belongs to the family *Pleurotaceae* of the order Agaricales, class Agaricomycetes, sub-phylum Agaricomycotina in the Phylum Basidiomycota and subkingdom Dikarya of Kingdom Fungi (Kirk et al. 2008).The genus is known with 40 species most of which are edible and widely cultivated around all corners of the world. Many species of *Pleurotus* possess pleasant flavor and are considered as delicacy by the connoisseurs of food. *Pleurotus* spp. contains various types of vitamins and amino acids, high content of fiber and protein and low fat content. Besides nutritional attributes of *Pleurotus* spp. the health benefitting effects like anticancer, antihyperlipidemic, antioxidant, hepatoprotective, antiflammatory, and antimicrobial activities makes them a health food (Atila 2017).

The golden oyster mushroom (*Pleurotus citrinopileatus*) is an edible gilled fungus and is one of the most popular wild edible mushrooms. It is native to eastern Russia, northern China, and Japan. The golden oyster mushroom is very closely related to *P. cornucopiae* of Europe. In far eastern Russia, *P. citrinopileatus* is one of the most popular wild edible mushrooms. *Pleurotus citrinopileatus* Singer, an edible mushroom was first time collected in India from dead wood by Sivaprakasam et al. (1986) from Tamilnadu. Golden oyster mushrooms (*Pleurotus citrinopileatus*), one of the most beautiful oyster species, are really quite stunning in clusters. They resemble a bouquet of beautiful yellow flowers, smell like fresh watermelon. The mushroom is spicy and bitter if cooked lightly, when cooked crispy they take on a strong nutty flavor that is quite delicious and taste similar like cashews. This mushroom also thrives in higher temperatures as compared to most other oyster mushrooms. Also when more light is introduced, the caps will become more yellow-golden in color.

Pleurotus citrinopileatus grows wild in the forests of hilly areas and is cultivated in temperate and subtropical regions of the world. It can be grown on agricultural and industrial waste. This mushroom grows on a wide range of substrates including straws of wheat, rye, oat, paddy, barley, maize, also on coffee grounds, banana waste, cotton stalks, most hardwoods and hardwood by-products like sawdust, paper and compost etc. This mushroom is becoming more popular by chefs for its beautiful appearance and bold nutty flavor. So, other sources of substrates need to be identified for mushroom cultivation in this region which are locally available. This mushroom is a good source of proteins, sugar, fats, vitamins and minerals. Golden oyster mushroom is having highest total phenolic content (Rodrigues et al. 2015). *P. citrinopileatus* mushrooms are a source of antioxidants. All essential elements are present in adequate amounts, as are K, P, Fe, Ca, Na, Mg and Mn. The heavy metals, Cu and Zn, are present but in very low concentration, much below the tolerance limit of the human beings. Extracts from *P.citrinopileatus* have been studied for their anti-hyperglycemic properties, decreasing blood sugar levels in diabetic rats. This mushroom also possesses antioxidant, anti-inflammatory and antitumor activities (Ravikumar et al. 2018).

The main obstacles for increased mushroom production in Himachal Pradesh are poor quality of the basic material, poor cultivation techniques and the frequent contamination of the mushroom growing beds with competitor moulds, diseases, insects and nematodes. The first record of the occurrence of *Pleurotus citrinopileatus* Singer on host in Kenya was reported by Musieba et al. (2011). However, its rarity and broad range of flavors make this species uniquely marketable and pleasurable to grow. Since there is need to develop mushroom cultivation techniques for the adaptation by local farmers and the technology of growing oyster mushroom is much easier than for button mushrooms, there is a good scope of expanding the cultivation of this mushroom because of having tremendous commercial and biological potential. As this mushroom is highly edible and has considerable social dietary importance so it is important to have study on yield and quality parameters. Since no such study on the yield and quality parameters have been made so far in CSK HPKV, Palampur. Consequently, the present study is undertaken to find out the suitable substrates giving higher yield, biological efficiency and longer shelf life for the cultivation of *P.citrinopileatus* and also to assess the cultural and morphological characters.

Keeping in view the obvious potentialities which would probably make *Pleurotus citrinopileatus* commercial feasibility and an article of food for the common man, the present investigation were undertaken to achieve the following objectives:

1. To study cultural characteristics on different media,
2. To study the yield and quality parameters.

2. REVIEW OF LITERATURE

Among the mushrooms, the genus *Pleurotus* having 40 species is noted for its cosmopolitan distribution (Deepalakshmi and Mirunalini 2014). Most of them have high edible status. *Pleurotus citrinopileatus*, bright yellow oyster mushroom, one of the species of genus *Pleurotus* which belongs to the phylum Basidiomycota. This species produces light-yellow fruit bodies with an edible stem centrally located in relation to the pileus. The fruit bodies are characterized by high taste values. *Pleurotus citrinopileatus* could be suggested as a new potential source of natural therapeutic use in many remedies. Further, *P.citrinopileatus* possesses nephroprotective, antioxidant and anticancer properties (Kim et al. 2009). The literature on various aspects of *Pleurotus citrinopileatus* has been reviewed under following headings:

2.1 Cultural studies

2.2 Spawn preparation

2.3 Cultivation Studies

2.3.1 Incubation period for spawn run

2.3.2 Time required for pinhead formation

2.4 Yield parameters

2.4.1 Number of Flushes

2.4.2 Comparative yield performance

2.4.3 Number of fruits per kg dry substrate

2.4.4 Average mature fruit body weight

2.4.5 Biological efficiency

2.4.6 Morphological characteristics

2.1 Cultural studies

Kligman (1943) reported two types of colonies as “appressed”, a grayish white colony with little aerial hyphae and “fluffy” which produced an abundance of extensive aerial growth giving the appearance of cotton wool. However, many intermediate variations were encountered from appressed growth to fluffy and vice-versa.

Bahukhandi and Munjal (1989) observed that *P. sajor-caju*, *P. florida* and *P. cornucopiae* had irregular, entire and dentate margins, respectively. Margin of *Pleurotus* spp. was found irregular, entire, dentate, incurved and lobed at maturity.

Suharban and Nair (1994) studied the effect of different media on the growth of different *Pleurotus* spp. and reported that Oat Meal Agar, Potato Dextrose Agar and Malt Dextrose Agar were the most suitable for the radial growth of *Pleurotus* species.

Peksen et al. (1999) tested various media and found that Malt Agar Media supported best growth of *Pleurotus* species.

Rafique et al. (1999) reported that natural media produce better mycelial growth than synthetic media. Among natural media, Potato Dextrose Agar was found to support maximum mycelial growth of all *Pleurotus* species and Malt Extract Agar was found to be the second best medium for the growth of most of the species.

Obodai and Vowotor (2002) examined that *P. citrinopileatus* strain PCB produced very poor and patchy growth and the mycelium did not fully colonize the substrate than the other stains of *Pleurotus* species.

Eswaran and Ramabadran (2000), Fallal et al. (2003) and Adenipekun and Gbolagade (2006) observed that the best solid medium for the growth of *Pleurotus* sp. is Potato Dextrose Agar (PDA).

Khandakar et al. (2008) studied that the corn extract agar was suitable medium for the mycelial growth of *Pleurotus citrinopileatus.*

Zagrean et al. (2009) studied the growth of five isolate of *P. ostreatus* on Malt Extract Agar (MEA), Potato Dextrose Agar (PDA) and Wheat Extract Agar (WEA) and found that PDA supported highest mycelial growth.

Mishra et al. (2015) examined that in case of *P. citrinopileatus*, the pattern of mycelial growth on Potato Dextrose Agar in Petri plate was highly fluffy.

2.2 Spawn preparation

The first modern advancement in commercial spawn making was germinating spores in bottles containing sterilized, washed horse manure referred to also as "the pure culture bottle spawn". The standard procedure for grain spawn preparation was first time patented by Sinden (1934) when Sinden proved that mycelium of *Agaricus bisporus* grew vigorously on cereal grains. Kligman (1943) defined spawn as merely the vegetative mycelium from a selected mushroom grown on a convenient medium.

Terrier (1945) used wheat grains as the basal medium for spawn production. Later on, perlite spawn was developed by Lemke (1972) using perlite- 1450 g, wheat bran- 1650 g, gypsum- 200 g, calcium carbonate- 50 g and tap water- 6650 ml.

Antonio and Hwang (1971) observed that cereal grains were the common substrates for mushroom mycelium production, whereas Biserka (1972) used rye, wheat and smaller grains for commercial spawn production.

Diwakar et al. (1989) reported the use of wheat grain as basal medium for spawn production of *P. sajor-caju, P. sapidus, P. ostreatus* and *P. florida*.

Abourouh (1995) reported that the maize grain was used as a basal medium for spawn making of *P. ostreatus*.

Rafique (1998) used different grain substrates as a basal medium for spawn production of *Pleurotus* species and reported thuli spawn was best, producing the most rapid mycelial growth and highest yield in all *Pleurotus* species.

Sangeetha et al. (2008) evaluated different grains for spawn production *viz*. proso millet, pearl millet, chaffy paddy grain, horse gram, maize, sorghum, barnyard millet, wheat, finger millet, kodo millet and foxtail millet and found that sorghum grain was the best medium for spawn running followed by wheat grains for *P. citrinopileatus.*

Dhancholia and Thakur (2008) used different grain substrates viz., pea, rajmash, kathu, phoolan as basal medium for spawn production of *P. sajor caju* and Kathu was most suitable among others.

Sathyaprabha et al. (2011) compared spawn production on different substrates viz., sorghum grain, paddy grain and maize grain and reported that paddy grain as best spawn substrate for *P. platypus* and *P. eous*.

Musieba et al. (2012) reported that inoculated wheat grains with *Pleurotus citrinopileatus* were incubated in a dark room at 25 ± 2^0C until full spawn run.

Shevale and Deshmukh (2016) reported that the entire wheat grains were covered with the whitish mycelial growth of *Pleurotus citrinopileatus* within 10-12 days.

2.3 Cultivation studies

2.3.1 Incubation period for spawn run

Sangeetha and Theradimani (2007) recorded that paddy straw showed the shortest spawn run of 9.7 days and the corn cob and hariyali leaves showed the longest spawn run of 16.3 and 19.3 days respectively.

Liang et al. (2009) reported that the mycelium of the fungus totally colonized the substrates with in the period of 28 days of the spawn run. They also studied that the shortest time that the fungus took to colonize the substrate (30 *Zea mays*+60 sawdust) was 22.1 days followed by the substrate (60 *Zea mays*+ 30 sawdust) i.e. 22.4 days and substrate (60 *Panicum repens* + 30 sawdust) i.e. 27.2 days.

Museiba et al. (2012) reported that the shortest period of colonization of *P. citrinopileatus* was on bean straw of 8 days and the other substrate like rice straw, sugarcane bagasse and wheat straw took 13 days compared to banana leaves, maize cobs and sawdust which took 21 days.

Kulshreshtha et al. (2013) reported that the time required for spawn run was 12 days in case of 100% wheat straw, which was used as a control. On the other side handmade paper sludge alone and cardboard industrial sludge had the spawn running time of 7.4 days and 6 days respectively.

Owaid et al. (2016) studied that sawdust and date palm fiber substrates exhibited long mycelium completion time of yellow oyster mushroom *Pleurotus cornucopiae* var. *citrinopileatus* i.e. 23 and 22 days respectively, compared with the control (wheat straw) 18 days.

Atila (2017) evaluated that *Pleurotus citrinopileatus* needed 20.4 days to colonize safflower hay while colonization period was 24.2 days on oak sawdust.

2.3.2 Time required for pinhead formation

Geetha and Sivaprakasam (1994) while working on *P. citrinopileatus* and *P. djamor*, observed that paddy straw substrate require minimum time for appearance of crop followed by its combination (1:1) with other substrates *viz*., soybean haulm and sugarcane bagasse.

Ragunathan et al. (1996) observed that in *Pleurotus citrinopileatus,* the number of days for primordial initiation was minimum i.e. 27 days in case sugarcane bagasse and coir pith followed by maize stover (29 days) and maximum in case of paddy straw (30 days).

Ragunathan and Swaminathan (2003) recorded that cotton stalk took minimum days for primordia formation i.e. 25 days.

Muhammad et al. (2005) revealed that time required for pinhead formation of *Pleurotus* species wheat straw (46.3 days), paddy straw (45.3 days) and 32 days required on cotton waste.

Sangeetha and Theradimani (2007) showed that the days for initial appearance of sporophore was comparatively less in paddy straw (10.7 days) followed by sugarcane trash (13.7 days), sugarcane bagasse (15.7 days) , broken groundnut shell (17 days), corn cob (20.7 days) and hariyali leaves it was 22 days.

Owaid et al. (2016) observed that date palm substrate exhibited higher primordial formation time 36 days, followed by sawdust substrate 27 days, whereas in wheat straw recorded the lowest time about 18 days.

Atila (2017) reported that the time of appearance of primordia in the different substrates varies from 24.4 days to 29.8 days in *Pleurotus citrinopileatus.*

2.4 Yield parameters

2.4.1 Number of flushes

Owaid et al. (2016) reported that yellow oyster mushroom had three flushes of total yield during 30 days on wheat straw, while date palm fiber exhibited 2 flushes followed by sawdust having only one flush.

Atila (2017) concluded that in *Pleurotus citrinopileatus* the maximum yield (221.6g/kg) on dry weight basis was obtained from oak sawdust substrate, which was distributed in two flushes with maximum yield in first flush. He recorded two flushes on bean straw, safflower hay and sunflower head residue and only one flush from oak sawdust substrate.

2.4.2 Comparative yield performance

Jadhav (1996) studied the yield performance of different *Pleurotus* spp. throughout the year. *P. sajor-caju*, *P. florida*, *P. citrinopileatus* and *P.ostreatus* were tested using wheat straw. *P. florida* and *P. ostreatus* gave the highest yield i.e. 721g/kg and 434g/kg, respectively. Highest yield was recorded in case of *P. florida* among all other species. In another experiment different agricultural wastes namely wheat straw, paddy straw, cotton stalk and leaves, groundnut creepers, jowar stalk and leaves, soybean stalk and leaves were tested for the productivity of the different *Pleurotus* spp. Cotton stalks and leaves recorded the highest yield (819g/kg) followed by wheat straw (734g/kg) by maintaining the temperature and relative humidity at 21-27^{0}C and 80-90% respectively.

Ragunathan et al. (1996) studied the yield of *Pleurotus citrinopileatus* on various agro-residues namely paddy straw, maize stover, sugarcane bagasse, coir pith and further reported that the maximum yield was on sugarcane bagasse (334g/kg) followed by paddy straw (301g/kg).

Ragunathan and Swaminathan (2003) recorded that *Pleurotus citrinopileatus* yielded maximum on cotton stalk (326g/kg), sorghum stover yielded 321g/kg of fruit bodies, whereas mixed bed and coir fibre yielded 315 and 236g/kg fruit bodies respectively.

Sangeetha and Theradimani (2007) reported that the yield of *Pleurotus citrinopileatus* on paddy straw was significantly higher (590g) followed by broken groundnut shell (546g), sugarcane bagasse (507g),corn cob (498g) and sugarcane trash (486g) and low yield was obtained from hariyali leaves (211.7g). They also reported that zero yield was recorded in banana leaves

Kumuthakalavalli and Kuzhali (2008) recorded the performance of sawdust namely padak, silver oak, pine and coconut by using them in combination with paddy straw in 1:1 ratio and maximum yield was obtained in case of silver oak.

Sangeetha et al. (2011) studied that the yield obtained by using nitrogen fixing bacteria was significantly higher with *Azospirillum lipoferum* (458.50g), followed by *Rhizobium japonicum* (450g) and paddy straw (389g).

Musieba et al. (2012) reported that the maximum yield of *Pleurotus citrinopileatus* was obtained from bean straw (397.71g/kg) followed by rice straw (213.62g/kg), wheat straw (109.59g/kg) and also reported that no harvestable produce was reported from sawdust.

Srivastava et al. (2012) evaluated locally suitable substrate namely paddy straw, wheat straw, sorghum straw, maize straw, sugarcane dry leaves and sugarcane bagasse for the production of *P. citrinopileatus* and reported that the maximum yield was obtained from the paddy straw (408.5g/kg) and minimum in case of sorghum straw i.e. 213.20g/kg.

Mishra et al. (2015) studied that the *Pleurotus citrinopileatus* gave the yield of 685.2g/kg substrate on wheat straw.

Owaid et al. (2016) reported that sawdust substrate had been affected on the yield negatively at average 0.7g/1.5kg, while date palm fibers exhibited 14.1g/1.5kg, compared to wheat straw i.e.102g/1.5kg.

2.4.3 Number of fruits per kg dry substrate

Sangeetha and Theradimani (2007) reported that in the cultivation of *Pleurotus citrinopileatus* among the different substrates, paddy straw produced higher number of sporophore per bed (75.6) followed by broken groundnut shell (70.7).

Mishra et al. (2015) studied the performance of 6 *Pleurotus* spp. and recorded maximum fruit bodies in *Pleurotus florida* (96), followed by *P. citrinopileatus* (88), *P. ostreatus* (86), *P. djamor* (48), *P. sajor-caju* (46) and *P. flabellatus* (17).

2.4.4 Average mature fruit body weight

Sharma and Jandaik (1984) in their studies with *Pleurotus* spp. recorded fruit body weight of 5.0 to 5.8 g.

Shukla and Jaitly (2011) in their studies with *Pleurotus* spp. recorded maximum average fruit body weight of 51g in case of *P. florida,* followed by *P. citrinopileatus* (44g), *P. djamor*(38g) and *P. sajor-caju*(34.5g).

Musieba et al. (2012) reported that highest mushroom weight was in the first flush for all the substrates, except for sugarcane bagasse and banana leaves.

Atila (2017) recorded that in *Pleurotus citrinopileatus* mushroom size was generally bigger for oak sawdust and sunflower head residue i.e. 4.84g and 4.54g respectively as compared to safflower hay and bean straw i.e. 3.36g and 3.46g respectively.

2.4.5 Biological efficiency

Upadhyay and Vijay (1991) evaluated 5 selected *Pleurotus* spp. i.e. *P. ostreatus, P. florida, P. fossulatus, P. eryngii and P. corrnucopiae* for yield. Highest yield of 94% biological efficiency was obtained with *P. florida* followed by 32% biological efficiency for *P. ostreatus* on wheat straw at low temperature ranging 12-16ºC and relative humidity 65-75%.

Ragunathan et al. (1996) reported that the maximum biological efficiency was on sugarcane bagasse (38.63%) followed by paddy straw (35.42%).

Ragunathan and Swaminathan (2003) showed that the maximum biological efficiency was observed on cotton stalk i.e. 32.69%.

Sangeetha and Theradimani (2007) reported that highest yield was obtained in paddy straw with bio-efficiency of 118% followed by broken groundnut shell (109.2%), sugarcane bagasse(101.5%), corn cobs(99.7%), sugarcane trash (97.3%) and hariyali leaves (42.3%).

Liang et al. (2009) reported that the total biological efficiencies of all the substrates namely 30% *Panicum repens* stalk (PRS) +60% sawdust, 45% *P.repens* stalk (PRS) +45% sawdust, 60% *P.repens* stalk (PRS) +30% sawdust, 30% *Pennisetum purpureum* stalk (PPS)+ 60% sawdust, 45% *Pennisetum purpureum* stalk (PPS)+ 45% sawdust, 60% *Pennisetum purpureum* stalk (PPS)+ 30% sawdust, 30% *Zea mays* stalk (ZMS) + 60% sawdust, 45% *Zea mays* stalk (ZMS) + 45% sawdust and 60% *Zea mays* stalk (ZMS) + 30% sawdust were higher than that of sawdust (S).

The most suitable substrate for high biological efficiency was 45 ZMS+ 45 S i.e. 65.40% , followed by 45 PRS + 45 S (57.58%), 60ZMS + 30 S (57.23%), 60 PRS + 30 S (56.85%), and 30 PPS + 60 S (53.58%).

Singh et al. (2009) studied the yields of *P.cornucopiae* grown on eleven waste materials (rice straw, sugarcane bagasse, maize stalk, mustard stick, pea haulms, coconut coir, waste paper, pine needles, pseudo stem, rice husk and sawdust) and reported the highest biological efficiency on rice straw i.e. 66.7%.

Musieba et al. (2012) reported that rice straw had highest biological efficiency of 98% followed by sugarcane bagasse(78%), wheat straw(41%), banana leaves (16%) and maize cobs (5%).

Kulshreshtha et al. (2013) evaluated the biological efficiency of wheat straw (58.2%), handmade paper (3.3%) and cardboard industrial sludge (5.2%).

Mishra et al. (2015) revealed that *Pleurotus citrinopileatus* had the biological efficiency of 68.52% on wheat straw substrate.

Shevale and Deshmukh (2016) showed that biological efficiency of *Pleurotus citrinopileatus* on 70% paddy straw+30% other agrowastes were significantly superior than 80% paddy straw+20% agrowastes combination. They also reported that paddy straw and vegetable wastes combination gave better result in terms of biological efficiency than paddy straw alone.

Atila (2017) evaluated that for *Pleurotus citrinopileatus,* the maximum biological efficiency was 73.9% obtained from oak sawdust substrate. The other substrates i.e. bean straw, safflower hay and sunflower head residue had the biological efficiencies of 43%, 42.5% and 54.1% respectively.

2.4.6 Morphological characteristics

Owaid et al. (2016) reported that the best diameter of pileus 54.5mm was on S_3 substrate (50% wheat straw, 30% sawdust and 20% datepalm fiber) while sawdust alone produced a smallest diameter of 28.5mm compared with the wheat straw at average of 43.6mm.

The past studies clearly show the performance with respect to different quality parameters of *Pleurotus citrinopileatus* reported in India and abroad. However, there are few studies on this aspect in the state of Himachal Pradesh which is certainty has rich potential for commercialization of *Pleurotus citrinopileatus* because of its unique flavor, aromatic properties and it also possessed fatigue resistance, immunity enhancing, delay aging, antigenotoxicity and antioxidant activity (Liang et al. 2005). It is significantly appeared that this mushroom has potential for researchers to develop its application on food and medicinal use. Therefore, this study has been undertaken to examine in detail the yield and quality parameters for this species.

3. MATERIALS AND METHODS

The present investigation entitled "**Studies on cultivation of golden oyster mushroom (*Pleurotus citrinopileatus* Singer)**" was carried out at Department of Plant Pathology of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. During the present study, culture of *Pleurotus citrinopileatus* was procured from Directorate of Mushroom Research (DMR), Solan, Himachal Pradesh. The culture was maintained on Potato Dextrose Agar (PDA) medium by regular subculturing.

The details of material and methodology followed during the course of study are given here under:

3.1 Materials

3.1.1 Collection of pure cultures

The pure cultures of *Pleurotus citrinopileatus* was procured from Directorate of Mushroom Research (DMR), Solan, Himachal Pradesh.

3.1.2 Maintenance of pure culture

Culture of golden oyster mushroom was sub-cultured and maintained on Potato Dextrose Agar (PDA) medium by incubating at 25 ± 1^0C and then master culture was preserved in the refrigerator at 4^0C for further laboratory studies.

3.1.3 Glasswares

The borosil brand glassware viz. Petri plates (diameter of 90 mm), test tubes, conical flasks, measuring cylinder, etc. were used wherever, necessary**.**

3.1.4 Equipments

The common laboratory equipments were autoclave, hot air oven, Biological Oxygen Demand (BOD) incubator, laminar air flow cabinet, refrigerator, hot water bath, weighing balance etc. were used for laboratory studies.

3.1.5 Miscellaneous material

Inoculating needle, scalpel, spirit lamp, spirit, cork borer, mercuric chloride, polypropylene bags, rubber bands, thread, pins, racks, hangers, formalin, wheat grains, paddy grains, maize, sorghum, bajra, cowpea, pigeonpea, blackgram, mash, calcium carbonate, wheat straw, paddy straw, oat straw, soyabean straw, mash straw, pea pods, pea straw, banana leaves, marigold straw, cauliflower leaves, maize cobs, maize leaves, lantana, ageratum, eupatorium, compost, sugarcane bagasse, paper waste, pine needles, sawdust, *Streptomycine sulphate*, non-absorbent cotton, muslin cloth, plastic rings and aluminium foil etc. were used for investigation.

3.2 Cleaning and sterilization of glassware

Glasswares to be used during experiments were surface sterilized by dipping overnight in Potassium dichromate ($K_2Cr_2O_7$) solution (potassium dichromate 75g, distilled water 1000ml and concentrated HCl acid 500ml). These were then rinsed thrice in tap water and sterilized in hot air oven at 180^0C for 2hrs. The inoculation chamber and hands were sterilized with rectified spirit.

3.2.1 Sterilization of inoculation needle & cork borer

Clean inoculating needle and cork borer were sterilized by dipping in spirit and heating over the flame until red hot. It was repeated 2-3 times. Inoculating needle was used for transferring mycelium from one culture tube to another and also in Petri plates. Cork borer was used for making 5 mm fungal disc (measured amount) for cultural studies, purification of the isolates and other laboratory studies.

3.3 Methods

3.3.1 Media preparation and disinfection

Potato Dextrose Agar is the most commonly used medium for growing of *Pleurotus* spp. of mushroom and prepared as follows.

Two hundred grams of peeled and sliced potatoes were boiled in 500 ml of distilled water till the tissues were softened. Then the extract was filtered through a clean muslin cloth. 20g of agar was boiled in 500 ml of distilled water till it melted completely. Both the solutions were mixed subsequently. 20g of dextrose was added and the volume was made up to 1000 ml. Before sterilization aliquots of 10 ml of the

medium were poured in culture tubes of 40 ml capacity for preparation of agar slants. Media to be poured into Petri plates were taken in Erlenmeyer conical flasks. The culture tubes and conical flasks were plugged with non-absorbent cotton and autoclaved at 121^0C for 20 minutes. In order to suppress bacterial contamination *Streptomycin sulphate* @ 250 mg per 1000 ml was added to the media after sterilization just before the pouring of media in Petri plates. These tubes and Petri plates were subsequently stored in refrigerator at 5 to 8^0C for further use.

3.3.3 Mass multiplication of culture

Potato Dextrose Agar (PDA) slants were prepared and culture of *Pleurotus citrinopileatus* was inoculated aseptically. The inoculated slants were kept for incubation at 23 to 25°C till the complete growth of mycelium on the agar slants.

3.3.4 Maintenance of cultures

Cultures of *Pleurotus citrinopileatus* was maintained on Potato Dextrose Agar (PDA) slants at 4°C in a refrigerator.

3.4 Selection of best solid media for optimum mycelial growth

Seven different solid media namely Potato Dextrose Agar (PDA), Yeast Potato Dextrose Agar (YPDA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA), Sabouraud's Agar (SA), Potato Agar (PA) and Yam Dextrose Agar (YDA) were evaluated to find out suitable medium for mycelial growth. The compositions of media used are mentioned in Appendix-1. The media were prepared and autoclaved at 121^0C for 20 minutes. After sterilization, the media were poured into pre-sterilized Petri plates, aseptically and allowed to solidify. Culture bit of the size 5mm of one week old culture of fungus was cut out with the help of sterilized cork borer and transferred to the center of the Petri plates and incubated at 25 ± 1^0C. Each treatment was replicated four times and statistical analysis was carried out using Completely Randomized Design (CRD). Observations were recorded as number of days taken to cover full Petri plate and the diameter of the mycelia colony was recorded by taking the average linear growth in three different directions after seven days of incubation. The best results and type of growth were also recorded.

Plate 3.1: Isolation and maintenance of pure culture A. Isolation under laminar air flow B. Petriplates after isolation C. Full mycelia growth in petriplate D. Maintenance of pure cultures

3.5 Spawn preparation

The wheat grains were utilized for spawn production of *Pleurotus citrinopileatus* following the standard procedure as explained below**.**

3.5.1 Preparation of master spawn

Mushroom spawn has been defined as the vegetative mycelial growth of mushroom which is grown on cereal grains. The spawn comprises mycelium of the mushroom and a supporting medium, which provides nutrition to the fungus during its growth. Wheat grains were used as the base material for multiplying the mycelium in the spawn bottles and polypropylene bags.

Master spawn was prepared from the pure culture of *Pleurotus citrinopileatus.* Wheat grains were used as substrates for the preparation of master spawn. Grains were cleaned and broken, undersized grains were removed. The bold, healthy, uncrumbled and pesticide free grains were chosen for substrate purpose.

Well cleaned and whole one kg wheat grains were washed in tap water and then boiled for 30 minutes or until the grain become half cooked and care was taken to avoid splitting of grains. Excess water was drained off from the grains and the grains were allowed to dry on sieve. The boiled and drained grains were mixed in the ratio of 4:1 with gypsum (2%) and calcium carbonate (0.5%) on the basis of weight of dry grains to prevent the grains from sticking together and keeping pH optimum for mycelial growth. Prepared grains (300 g/bottle) were filled into 500 ml sterilized glass bottles and plugged with non-absorbent cotton plugs. These grain filled bottles were sterilized in an autoclave for 2 hours at 128^0C. After sterilization, the bottles were allowed to cool at room temperature and were shaken to avoid clumping of the grains. The next day, the bottles were inoculated under laminar air flow using bits of agar medium colonized with mycelium of pure culture (7-10 days old). The inoculated grain bottles were kept for incubation at 25^oC temperature in BOD incubator. After 7-10 days of inoculation, bottles were shaken vigorously so that mycelia threads were broken and become well mixed with the grains. After 15 to 21 days, master culture become white due to complete mycelium growth of fungus, these bottles were used as master spawn bottles for subsequent multiplication and ready for preparation of commercial spawning in polypropylene bags. The spawn prepared by this way was known as master spawn and used throughout the period of study.

Plate 3.2: Preparation of spawn A. Boiling of wheat grains B. Gypsum and chalk powder added to wheat grains C. Autoclaving for sterilization process D. Inoculation E. Master cultures F. Commercial spawn

3.5.2 Preparation of commercial spawn

For the preparation of commercial spawn firstly the wheat grains were washed for twice to remove the suspended dust particles or foreign materials. Then wheat grains were boiled in the container with water till they soften. Care was taken that the starch should not ooze out of the grains. The boiled grains were spread as thin layer on a sieve under shade for draining out excess water from grain surface to avoid greasiness. The boiled and drained grains were mixed in the ratio of 4:1 with gypsum (2%) and calcium carbonate (0.5%) on the basis of weight of dry grains for maintenance of proper pH (6.5 to 7.0) in the spawn substrate. The grains were filled up to $3/4^{th}$ in the polypropylene bags of 100 gauge thickness. Then polypropylene bags were plugged with non-absorbent cotton after putting a plastic (PVC) ring at the neck and sterilized in autoclave at 128^0C for 2 hrs. After sterilization, bags were allow to cool overnight and then transferred to the inoculation chamber. The inoculation chamber was sterilized by spraying four percent formalin solution. Sterilized bags were inoculated in an inoculation chamber. The inoculating needle and forceps were properly sterilized by flame and transfer of the culture was made close to the flame of spirit lamp. Sterilized bags were inoculated with master culture which was 15 days old and were incubated at 25 ± 1°C for 15 days and in between the polypropylene bags were shaked to disturb the mycelial thread for good spawn run. The mycelium covered the grains in 15 to 20 days and the spawn was ready for growing mushroom on large scale.

Precautions taken during spawn preparation:

1. The inoculation was done under aseptic condition.
2. In case there was fungal or bacterial contamination in the spawn bags, the entire content of the bottles or bags were destroyed.
3. 15 to 20 days old spawn was used for spawning in wheat straw filled bags.
4. Culture and spawn was stored at 4^0C in refrigerator.

3.5.3 Selection of suitable grain substrate for spawn production

Following grain substrates were evaluated for their suitability to support fastest mycelium spread of the mushroom studied:-

i) Wheat

ii) Paddy

iii) Maize

iv) Sorghum

v) Bajra

vi) Blackgram

vii) Mash

viii) Cowpea

ix) Pigeonpea

Various grains were used after boiling as given above procedure for commercial spawn production. Five replications of each treatment were kept and fresh master culture prepared was used for inoculation at the top of the substrate columns. The inoculated bottles were incubated at 25 ± 1^{0}C. The downward linear growth of mycelium was recorded at regular intervals. The growth was measured by taking average linear growth from four different places. Average of replications was used to compare the mycelial growth on different substrates. The cost of production of different spawn substrates was calculated and their days to spawn run and primordia formation and yield potential was also recorded on wheat straw.

3.5.4 Substrate preparation and sterilization

Various agricultural waste substrates viz. wheat straw, paddy straw, oat straw, soybean straw, mash straw, hulled maize cobs, maize straw, cauliflower leaves, banana leaves, *Lantana* straw, *Ageratum* straw, *Eupatorium* straw, pine needles, paper waste, sawdust, marigold straw, shelled pea pods, pea straw and compost and their combination were evaluated for sporophore production ability of the *Pleurotus citrinopileatus*. All the substrates were compared to wheat straw because it is most

commonly available substrates for *Pleurotus* cultivation. All the substrates were chopped into small pieces (4-5cm) in order to improve the water holding capacity of the substrates. The chopped one kg dry substrates were then filled in gunny bags and soaked in fresh water overnight to obtain 70-75% moisture content. The excess water was allowed to drain off. The pre-soaked wheat straw was pasteurized by immersing them in hot water at 80°C for 1 hr. After pasteurization the substrates were taken out and spread on plastic sheet treated with 2% formalin solution to drain out excess and allowed to cool. The appropriate moisture content of the substrate was determined by squeezing the substrate in the palm; about 67% moisture was maintained (Savalgi and Savalgi 1994).

3.5.5 Bag filling and spawning

The grain spawn was used for spawning @ 2 per cent in dry weight basis of the substrate containing 65-70% moisture. Spawning in layers was done in polypropylene bags of size 35×55 cm. After spawning the top of polypropylene bag was tied with thread and about 25 to 30 holes with sterilized pin were made per polypropylene bag for proper aeration. The spawned bags were then incubated at temperature 21- 25^0C and humidity of 80% for spawn run. The observations regarding spawn run were recorded.

3.5.6 Incubation and spawn run

After spawning, bags were kept for incubation in incubation room. Sufficient amount of light, proper ventilation, optimum temperature (20 to 25°C) and required humidity (70 to 85%) was maintained during entire cropping period.

Bags were kept until the complete spawn run take place and then transferred to growing room having controlled conditions as that of incubation room.

3.5.7 Cropping

The bags were cut opened by sharp and sterilized blade when different substrates were fully covered with whitish mycelium to expose the substratum surface for initiation of pinheads. The environmental conditions *viz*., temperature in between 20 to 25°C, relative humidity in between 70 to 80 per cent and diffused light during day time were maintained for primordial formation and fruit body development.

Ventilation of 2 to 3 hours per day was given for maintaining CO_2 level in the growing room and observations regarding days required for pinhead formation were taken at regular intervals. Light mist spray of water was given to bags twice in a day till the end of cropping seasons.

3.5.8 Harvesting

Fruiting bodies were harvested at full maturity. Mature fruiting bodies were those which started forming spores but the margins of pileus become wavy. The fruiting bodies were harvested before curling of fruit body and well formed gills, because over matured fruit body is fragile and difficult to handle. Harvesting was done by hand holding the stipes at the base and twisting lightly. After first harvest, bags were scrapped slightly to remove dead mycelial growth then second and third flushes were taken. Harvested fruiting bodies were packed into polypropylene bags and preserved by drying.

3.6 Microscopic studies

The macroscopic descriptions were based on studies of fresh and dried specimens. Microscopic preparations were mounted using dried material in 5% aqueous solution of potassium hydroxide, using a light microscope. The size of mature spores was measured on 30 spores in distilled water.

In particular we observed the hyphal system (monomitic or dimitic), the type of hyphal wall, the shape and the size of basidia and basidiospores.

3.7 Observations recorded

Following observations were recorded during entire research period

1. Growth performance on culture media,

a) Colony diameter

b) Type of growth

c) Number of days taken to full Petri plates

2. Growth performance on different spawn grain substrates,

a) Downward growth of mycelium

b) Cost of production

c) Days to spawn run and pin head formation

d) Total yield per kg of dry substrate

3. Growth observations on different substrates of golden oyster mushroom,

a) Days required for spawn run

b) Days required for primordia initiation

4. Fruit body observations of golden oyster mushroom,

a) Pileus diameter b) Stipe length and size c) Colour

e) Average mature fruit body weight

f) Average fresh yield per kg dry substrate

g) Number of flushes

5. Microscopic studies and camera lucida drawing of golden oyster mushroom,

a) Size of Basidiospore

b) Size of Basidia

c) Size of Cystidia

d) Width of Hyphae

3.8 Biological efficiency (B.E.)

The biological efficiency (%) was determined using the ratio of the total weight of fresh mushrooms to the absolute dry weight of the substrates.

Biological efficiency was calculated using the formula given by Chang and Miles (1989):

$$\text{Biological Efficiency} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

3.9 Statistical analysis

The data obtained from different observations were computed as per Completely Randomized Design (CRD) by using standard statistical methods as described by Panse and Sukhatme (1967) for its statistical significance. The data were presented in tabular form with suitable illustrations and figures at appropriate places. The significance of treatments was taken at 5 per cent level of significance.

4. RESULTS AND DISCUSSION

The results obtained from the present studies are presented under the following headings:

4.1 Maintenance of pure culture

4.2 Cultural characteristics

4.3 Spawn production

4.4 Cultivation

4.1 Maintenance of pure culture

Pure culture of *Pleurotus citrinopileatus* was maintained on Potato Dextrose Agar (PDA) and has been used in the present investigation.

4.2 Cultural characteristics

In order to get maximum growth of fungi, selection of basal medium is very essential. Various solid media viz., Potato Dextrose Agar (PDA), Yeast Potato Dextrose Agar (YPDA), Malt Extract Agar (MEA), Yeast Extract Agar (YEA), Sabouraud's Agar (SA), Potato Agar (PA) and Yam Dextrose Agar (YDA) were tested for the vegetative growth of mycelium. Each treatment was replicated four times and conducted twice. The data is presented in the table 4.1. Media were tested to obtain good mycelial growth. Mycelial growth performance was measured on the basis of colony diameter after seven days of inoculation, type of growth and number of days taken to colonize full Petri plate.

It is evident from the data that the different solid media supported mycelial growth of the mushroom. However, Potato Dextrose Agar (84.3mm) was found to be the most effective for supporting maximum mycelial growth of *Pleurotus citrinopileatus* followed by Malt Extract Agar (81.0mm), Sabouraud's Agar (58.0mm) , Yeast Potato Dextrose Agar(53.7mm), Yeast Extract Agar (47.7mm), Potato agar (41.2mm) and Yam Dextrose agar (34.3mm). It is also clear from the Table 4.1 that the minimum number of days taken to fill the Petri plate was 9.25 days in case of Potato Dextrose agar followed by Malt Extract Agar and Sabouraud's Agar and took 11.50 days and 13.50 days, respectively.

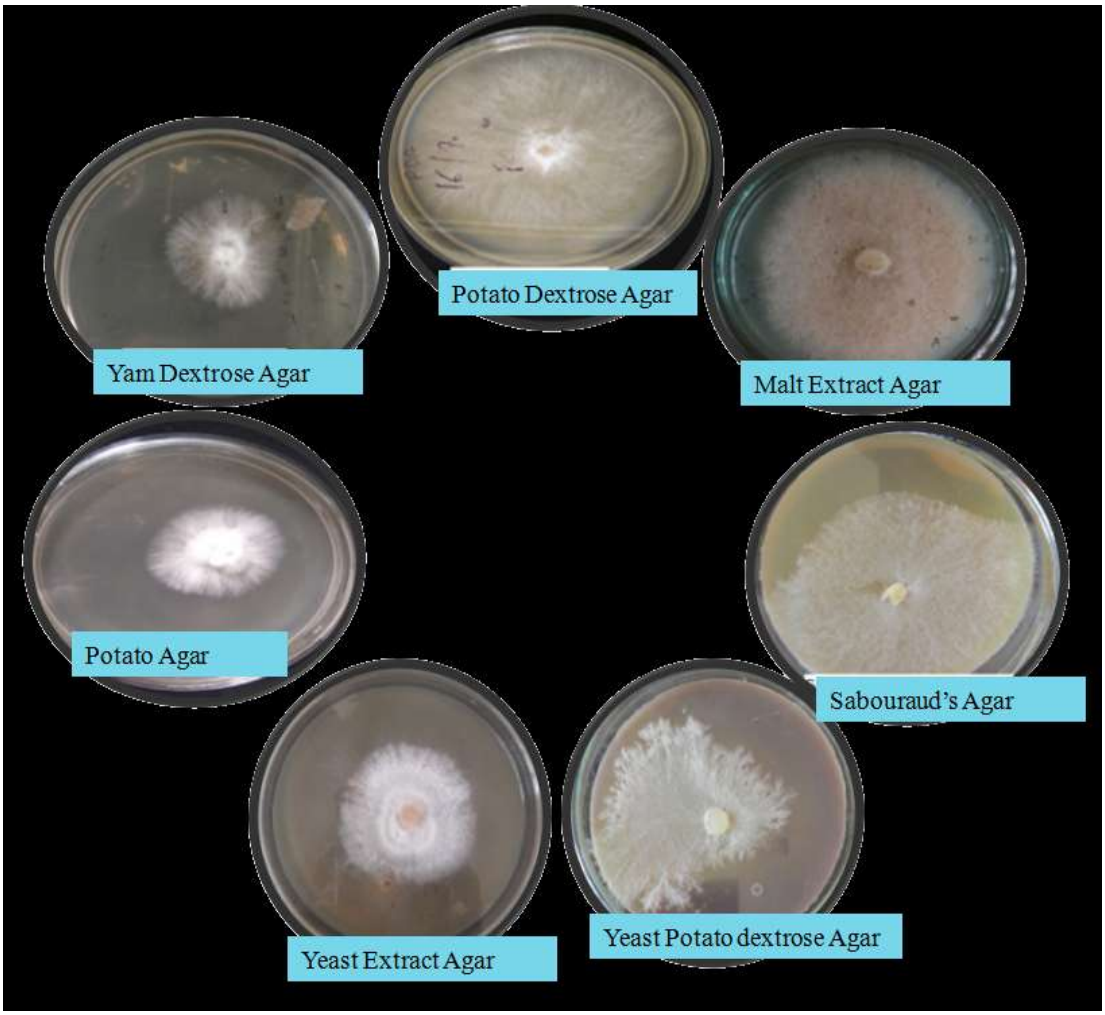

Plate 4.1 Mycelial growth of *Pleurotus citrinopileatus* on different solid media

Then it is clear from the above data that the Potato Dextrose Agar (PDA) appeared to be the best solid media for the mycelia growth of *Pleurotus citrinopileatus*. Since, Potato Dextrose Agar supported maximum mycelia growth of the species, so it was used for further studies.

P. sajor- caju gave best radial growth on PDA (Yadav 2003). The studies are at par with Rafique et al. (1999), Eswaran and Ramabadran (2000) and Fallal et al. (2003) that Potato Dextrose Agar was found to support maximum mycelial growth of all *Pleurotus* species. So, the present study on the solid media showed that Potato Dextrose Agar as the best media which is in agreement with earlier investigations.

Table 4.1 Evaluation of different solid media for the mycelial growth of *Pleurotus citrinopileatus*

Media	Mycelial Growth (mm)*	Days taken to full Petri plate*	Type of growth
Potato Dextrose Agar	84.3	9.25	Regular, highly fluffy, white in color
Malt Extract Agar	81.0	11.50	Regular, appressed, dull white in color
Sabouraud's Agar	58.0	13.50	Irregular, appressed, white in color
Yeast Potato Dextrose Agar	53.7	14.75	Irregular, appressed, white in color
Yam Dextrose Agar	34.5	17.75	Regular, cottony , snow white in color
Yeast Extract Agar	47.7	15.75	Irregular, appressed, white in color, fainted zonations
Potato Agar	41.2	15.50	Regular, appresssed, white in colour, fainted zonations
CD(p=0.05)	1.26	0.79	

*****Average of four replications

The mycelial characteristics of *P. citrinopileatus* on different solid media were studied. A total of seven substrates were inoculated with a bit of mycelium and the mycelial characteristics were recorded. Regular mycelial growth was seen in case of Potato Dextrose Agar and Malt Dextrose Agar and irregular and fainted zonation was seen in Yeast Potato Dextrose Agar and Sabouraud's Agar. The mycelial growth was fluffy in Potato Dextrose Agar and Yam Dextrose Agar whereas in case of Malt Extract Agar appressed mycelial growth was present.

Mishra et al. (2015) also reported that the pattern of mycelial growth on Potato Dextrose Agar in Petri plate was highly fluffy in case of *P. citrinopileatus*. Therefore, the results in present study are at par with previous study.

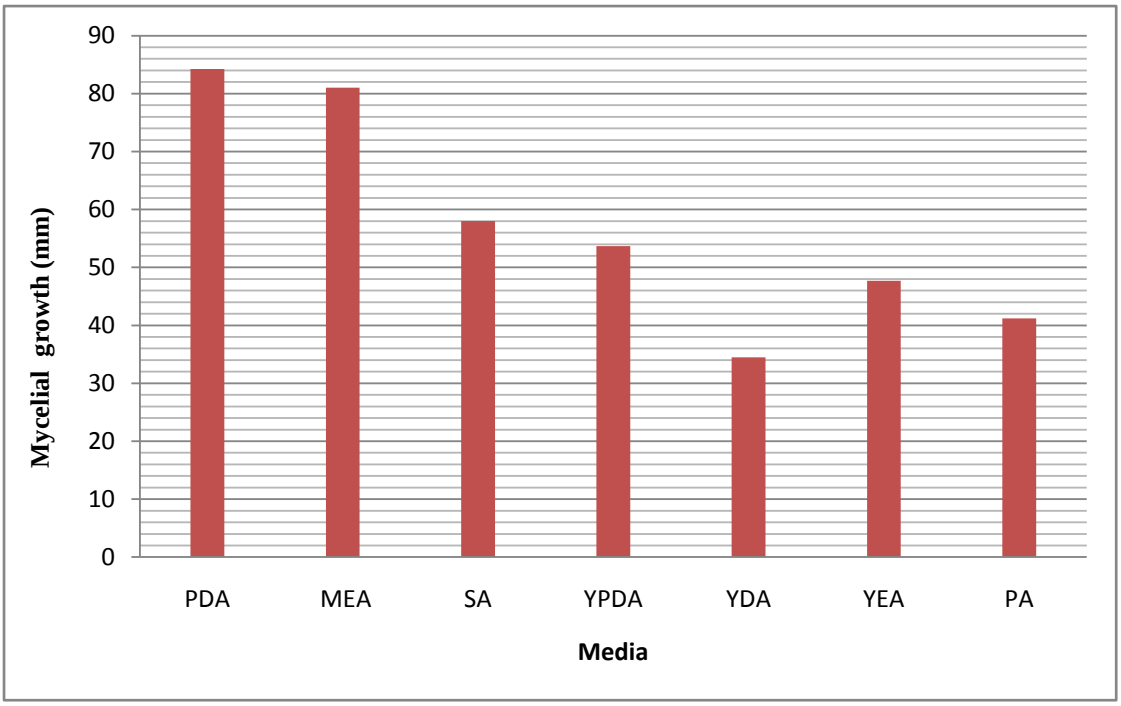

Fig.4.1 **Effect of different solid media on the mycelial growth of *Pleurotus citrinopileatus***

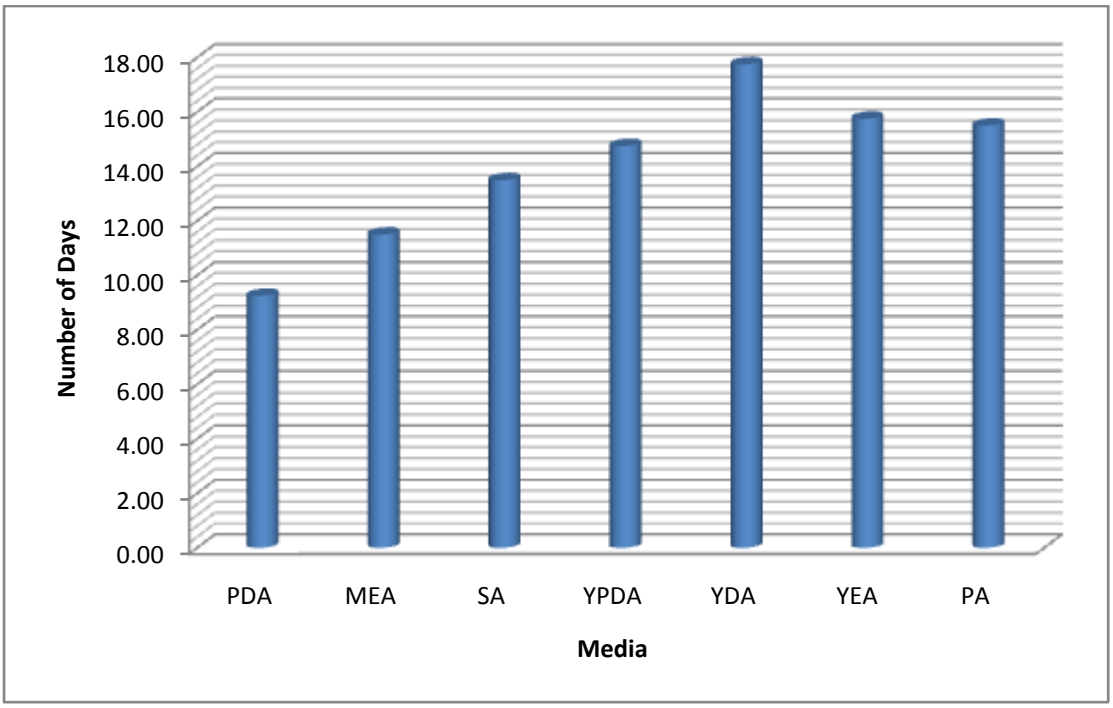

Fig. 4.2 **Effect of different solid media on number of days taken to fill Petri plate of *Pleurotus citrinopileatus***

4.3 Spawn production

4.3.1 Evaluation of different grain substrates

The mycelial growth rate of *Pleurotus citrinopileatus* was evaluated on nine different grain substrates namely wheat, paddy, maize, sorghum, bajra, pigeonpea, cowpea, blackgram and mash. The data were recorded on downward growth of mycelium on every alternate day till the 9^{th} day after inoculation. It revealed that the mycelial growth differed significantly on different substrates at all dates of observations. On the third day, the maximum downward growth was observed on sorghum (2.23cm) followed by bajra (1.85cm), wheat (1.78cm) and maize (1.38cm) whereas downward mycelial growth was least on pigeonpea (0.98cm).

On the fifth day, mycelial growth was slower on pigeon pea (1.63cm) and mash (1.85cm). But on the seventh day the growth was faster on sorghum (6.70cm) followed by black gram (6.62cm) and bajra (6.39cm). The mycelial run was least on pigeonpea till the ninth day (2.55cm). On the ninth day, the growth of the mycelium on different spawn grains differed significantly with highest mycelial growth on sorghum grains.

The table also reveals that the growth rate per unit per day (r) on sorghum was more favourable to the test fungus (1.12cm/day) followed by blackgram (1.07cm/day), bajra (1.05cm/day) and maize (0.97cm/day). The least mycelial growth rate was observed on pigeonpea (0.26cm/day).

The results are at par with the results of Sangeetha et al. (2008) that the sorghum grain was the best medium for spawn run followed by wheat grain for *Pleurotus citrinopileatus*. The maize grain was used as a basal medium for spawn making of *P. ostreatus* (Abourouh 1995). Maize grains showed maximum mycelial growth in case of *Pleurotus sapidus*, *Pleurotus florida* and *Pleurotus flabellatus* (Mishra et al. 2018). So, the present studies corroborate the results of previous studies.

Table 4.2 Evaluation of different grain substrates for spawn production of *Pleurotus citrinopileatus*

Substrates	Mycelial growth in cm (days after inoculation)*				
	3rd day	5th day	7th day	9th day	r
Wheat	1.78	3.54	5.49	6.61	0.80
Paddy	1.36	2.48	4.25	5.32	0.66
Maize	1.38	2.60	4.96	7.22	0.97
Sorghum	2.23	4.50	6.70	9.10	1.12
Bajra	1.85	3.29	6.39	8.18	1.05
Mash	1.22	1.85	2.50	2.91	0.28
Blackgram	1.23	3.54	6.62	7.65	1.07
Pigeonpea	0.98	1.63	2.18	2.55	0.26
Cowpea	1.39	3.48	4.34	5.31	0.65
CD (p=0.05)	0.20	0.21	0.23	0.29	

*average of four replications
r=growth rate (cm/day)

4.3.2 Cost of production

Cost of production of spawn preparation on different food grains was worked out to find out its economy. The cost of production and net profit is calculated and shown in table 4.3.

Variable expenditure

Cost of 1 kg of grain given in table 4.3

Fixed expenditure

Electricity used for boiling wetted gains until it became soft = Rs. 5

Electricity charge for autoclaving for 2 hour = Rs. 15

Other expenditures = Rs. 25

Fixed expenditure = Rs. 45

Cost of Production= Variable expenditure + Fixed expenditure

Net profit = Spawn prepared from 1 kg of grain x cost of 1 kg spawn (Rs.100) - cost of production

Table 4.3 Cost of production for spawn preparation on different grain substrates

Grain spawn	Cost of grains (Rs.)	Fixed expenditure (Rs.)	Cost of production (Rs.)	Spawn prepared (kg)	Net Profit (Rs.)
Wheat	18.25	45	63.25	1.5	86.75
Paddy	40	45	85	1.5	65
Maize	20	45	65	1.4	75
Sorghum	25	45	70	1.4	70
Bajra	40	45	85	1.5	65
Blackgram	60	45	105	1.4	35
Cowpea	55	45	100	1.5	50

The above table showed that the cost of production of spawn prepared from different grains and thereby the net profit was calculated. It showed that the net profit was highest in case of wheat (Rs. 86.75) followed by maize (Rs.75) and sorghum (Rs.70) and the lowest profit were in case of blackgram. The cost of production was minimum in wheat i.e. Rs.63.25 and maximum in case of blackgram Rs.105. So, it is concluded that the spawn prepared from the wheat grains is economically best among all other grains followed by maize and sorghum.

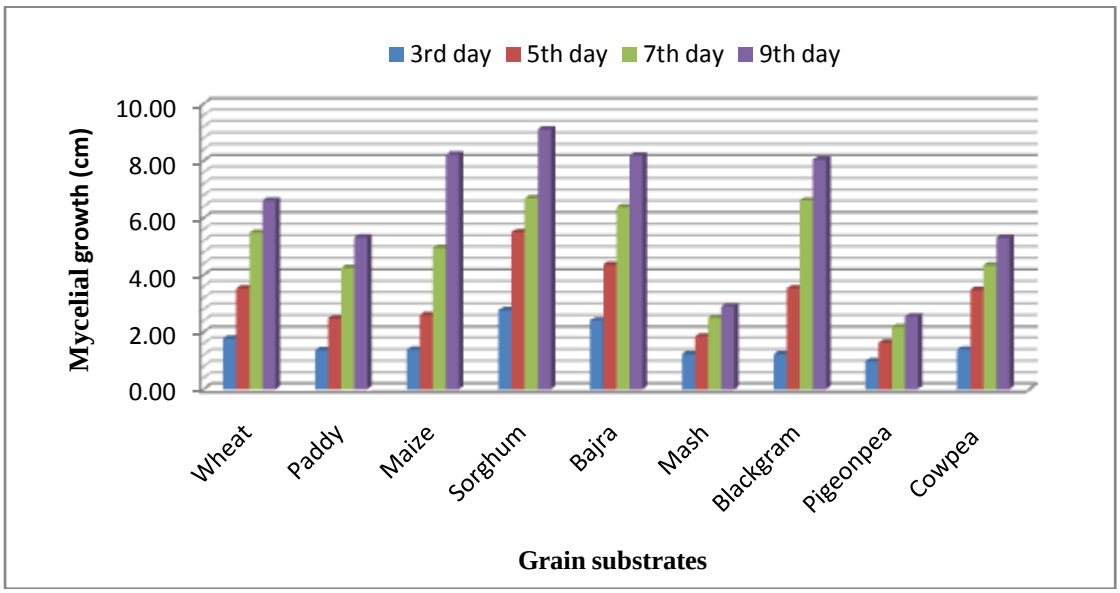

Fig.4.3 Downward mycelial growth of *Pleurotus citrinopileatus* on different grain substrates

Plate 4.2 Mycelial growth of *Pleurotus citrinopileatus* on various grain substrates

4.3.3 Cultivation studies of different grain substrates

The spawn prepared on different grain substrates were further tested on wheat straw substrate for spawn run, primordia initiation and yield potential of *Pleurotus citrinopileatus.*

The time taken for spawn run and primordial initiation of *Pleurotus citrinopileatus* was evaluated on wheat straw substrate spawned by six different grain spawn, and the data were recorded. Each treatment was replicated three times and observations are presented in Table 4.4.

It is observed that the days taken for spawn run was minimum in case of the sorghum i.e. 27.33 days followed by wheat grains (29 days) and maize (31.33 days). Cowpea took maximum time for spawn run i.e. 44 days after inoculation. Similarly, the earliest primordial initiation was on sorghum after 33.67 days of spawn inoculation with 2 primordia followed by wheat grains (34.67 days) with 4.33 primordia and maize (37.33 days) with 2.33 primordia.

Table 4.4 Performance of spawn produced on different grain substrates for yield parameters of *Pleurotus citrinopileatus* on wheat straw

Grain spawn	Spawn run (days)*	Primordia initiation (days)*	Primordia (no.)*	Average yield (g)*	B.E. (%)*
Wheat	29.00	34.67	4.33	893.33	89.33
Paddy	34.33	39.67	1.33	526.67	52.67
Maize	31.33	37.33	2.33	700.00	70.00
Sorghum	27.33	33.67	2.00	553.33	55.33
Bajra	37.00	43.33	1.00	513.33	51.33
Black gram	41.00	48.33	1.00	433.33	43.33
Cowpea	44.33	52.33	1.33	393.33	37.33
CD(p=0.05)	1.54	2.04	0.86	42.26	4.23

*Average of three replications

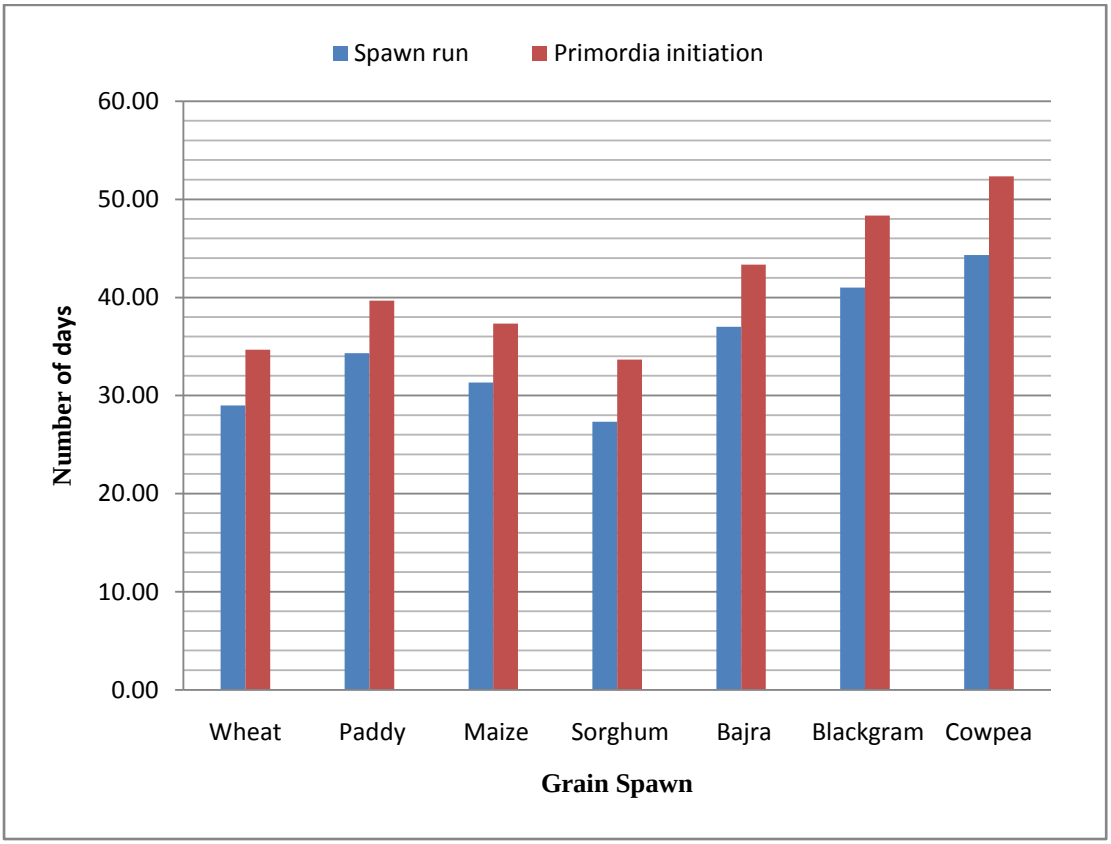

Fig. 4.4 Evaluation of grain substrates for spawn run and primordia initiation of *Pleurotus citrinopileatus*

Hence, the time taken for spawn run and primordia initiation of different spawn grains on wheat straw varied between 27 to 44 days and 33 to 52 days, respectively. All the treatments were significantly different from each other. These grain substrates were spawned in wheat filled polypropylene bags sorghum spawn showed the minimum time for spawn run and primordia initiation. So, considering the days to spawn run, primordial initiation and number of primordia, the wheat spawn was found to be superior to the others.

Table 4.4 reflects the yield potential of various grains spawn of *Pleurotus citrinopileatus* on the yield of golden oyster mushroom. The total yield of golden oyster mushroom was recorded and finally the biological efficiency was calculated. The table revealed that the maximum yield was recorded on polypropylene bags spawned with wheat grain which was 893.33g/kg of dry substrates having 89% biological efficiency followed by maize grain spawn (700g) with 70% biological efficiency. The least yield was obtained from cowpea spawned bags i.e. 393.33g with

minimum biological efficiency (43%). So, the wheat grains were considered to be the best spawn grains for further studies.

Zadrazil (1978) reported that more rapid growth of fungus might be theoretically useful since rapidity of growth along with high saprophytic colonization capacity facilitates speedy penetration of substrates which simplifies cultivation as a whole. In the present studies however, faster colonization of the sorghum, maize grains and black gram by *Pleurotus citrinopileatus* did not translate into higher yield. Moorthy and Mohanan (1991) also reported that faster mycelial spread need not necessarily lead to high yield. So, the present findings are in conformity with the previous studies.

4.4 Cultivation

Pleurotus species draws their nutritional requirement from a host substrate or from the agricultural wastes rich in lignin, cellulose and hemicelluloses. In the present study, different substrates were examined for the growth and yield of *Pleurotus citrinopileatus*. Mycelial growth is a preliminary step that creates suitable internal conditions for fruiting. Thus, outstanding growth of mycelium is a vital factor in oyster mushroom cultivation. Mycelium (vegetative phase) is important in the ecosystem because they are able to biodegrade the substratum and therefore, use the wastes of agricultural products.

A majority of the edible basidiomycetes readily grow in culture in mycelial form but the cultivation of their fruit bodies has been successful only in few cases. In present investigation, the cultivation of *Pleurotus citrinopileatus* was taken up.

4.4.1 Mycelial characteristics during spawn run

Mycelial characteristics of the mushroom during spawn run on different substrates were studied and the observations were recorded and the observations are presented in the table 4.5. A total of nineteen agro-based straw substrates were inoculated with golden oyster mushroom spawn and the mycelial characteristics and primordia formation were noted.

Dense mycelial growth was noticed on seven different substrates viz., Wheat straw, Paddy straw, Soybean straw, Mash straw, Marigold, Maize leaves and Oat

straw. A less dense or sparse mycelium could grow on Pine needles, Pea pods and Maize cobs and no spawn run was seen in case of Cauliflower leaves, Banana leaves, *Lantana, Ageratum, Eupatorium,* Sawdust. In case of Compost and Paper waste, the spawn run took place but this did not correspond with the appearance of primordia. The mycelial run in some of the above mentioned substrates viz., Cauliflower leaves, Banana leaves, *Lantana, Ageratum, Eupatorium*, Paper waste and Sawdust were sparse or could not spread, indicating thereby that mycelium of the *P.citrinopileatus* is unable to decompose the substrates and due to lack of available nutrients the mycelium could not spread or primordia did not develop. In the present investigation, *Pleurotus citrinopileatus* failed to grow when cultivated on Cauliflower leaves. The probable reason is, the vegetable wastes when processed before cultivation, they hold large amount of water. Hence, due to presence of excess water in the substrate and lack of proper aeration mycelia does not develop and spawn run fails to occur. As Banana leaves did not support the fruiting of *Pleurotus* which is in accordance with Sangeetha and Theradimani (2007). The result was at par with the investigation. The reason for this may be the absorption of more moisture by the substrate which will adversely affect the development of sporophore. The spawn run took place but there was no primordial initiation in case of paper waste and compost. The possible reason for no primordia initiation on paper waste is due to sticking properties of cellulose which result in over compaction of the bags. This lead to restriction of gaseous exchange between the void spaces in the bags required during primordia initiation. So, in the present study, therefore, paper waste was not found suitable for the growth of the golden oyster mushroom which is statistically at par with the observation of Kulshreshtha et al. (2013). Since the spawn run was scanty on these substrates, meaning thereby that the mycelium could not draw adequate amount of nutrition and further indicate that these substrates were unsuitable for the production of *Pleurotus citrinopileatus*.

Table 4.5 Mycelial characteristics and primordia formation of *Pleurotus citrinopileatus* on different substrates

Substrates	Mycelial characteristics	Primordia formation
Wheat straw	Spawn run complete , compact	+
Paddy straw	Colonization incomplete, thin, sparse growth	+
Oat straw	Spawn run complete, compact growth	+
Soybean straw	Spawn run complete, compact, cottony growth	+
Mash straw	Thick uniform and fluffy mycelium	+
Hulled Maize cobs	Compact, incomplete mycelium run	+
Maize straw	Thick uniform ,cottony mycelium	+
Cauliflower leaves	Spawn run nil	-
Banana leaves	Spawn run nil	-
Lantana straw	Spawn run nil	-
Ageratum straw	Spawn run nil	-
Eupatorium straw	Spawn run nil	-
Pine needles	Fast mycelium run but incomplete and compact	+
Paper waste	Compact and complete spawn run	-
Sawdust	Spawn run nil	-
Marigold straw	Fast and complete spawn run, thick cottony growth	+
Shelled Pea pods	Incomplete mycelium run, compact	+
Pea straw	Complete mycelium run , thick, cottony	+
Compost	Sparse spawn run	-

+ Sign= Primordia Formation

-Sign= No Primordia Formation

The mycelial characteristics on 17 selected substrate combinations (Table 4.6) were studied considering wheat straw as a traditional standard substrate. Mycelial colonization was thin and sparse on substrate combinations of wheat straw with oat, pea pods, pea straw, pine needles and sugarcane bagasse. However, colonization by the mycelium was incomplete on all the substrate combinations of wheat straw except oat and soybean straw.

Among the substrate combinations of paddy straw all the combinations had non uniform growth of mycelium. The combinations of paddy straw i.e. paddy+maize straw and paddy+soybean supported thin mycelial growth whereas paddy+mash showed thick mycelial growth. The mycelial growth was uniform, thick and compact in case of soybean+maize, soybean+maize+wheat and soybean+maize+oat.

Plate 4.3 Mycelial characteristics of *Pleurotus citrinopileatus* on different substrates

Table 4.6 Mycelial characteristics and primordia formation of *Pleurotus citrinopileatus* on different substrates in combinations

Substrates	Mycelial characteristics	Primordia Formation
Wheat +Paddy	Compact, thick, cottony, non uniform growth	+
Wheat + Oat	Uniform growth , compact, thin	+
Wheat +Maize straw	Quite thick , compact, non uniform growth	+
Wheat +Soybean	Thick, compact, slightly fluffy	+
Wheat + Mash	Less compact, moderately fluffy	+
Wheat + Marigold	Slightly thick, compact, non-uniform growth	+
Wheat +Maize cobs	Less compact, thin, non-uniform growth	+
Wheat +Pea pods	Thin, loose, non-uniform growth	+
Wheat +Pea straw	Thin, compact, fluffy, non-uniform growth	+
Wheat + Pine needles	Thin, loose, non-uniform growth	+
Wheat + Sugarcane bagasse	Thin, compact, non-uniform growth	+
Paddy+Maize straw	Thin, compact, non-uniform growth	+
Paddy+ Soybean	Thin, compact, non-uniform growth	+
Paddy+Mash	Thick, compact, non-uniform growth	+
Soybean +Maize	Thick, compact, uniform growth	+
Soybean + Maize +Oat	Thick, compact, uniform growth	+
Soybean +Maize +Wheat	Thick, compact, uniform growth	+

+ Sign= Primordia Formation

-Sign= No Primordia Formation

4.4.2 Spawn run and primordial initiation

Since the formation of the primordia on different substrates is likely to be a good indicator of their yield potential so the various agro-based substrates and their combinations were evaluated for days taken to spawn run and primordia initiation of *P.citrinopileatus.*

4.4.2.1 Spawn run and primordia initiation on agro-based different substrates

The time taken for spawn run, primordia initiation and number of primordia were recorded on various agro-based substrates and the observations are presented in Table 4.7.

It is evident from the data that the minimum time taken for spawn run was 29 days in case of wheat straw followed by pea pods (29.33 days) and soybean straw (30.33 days). The other substrates viz., pea straw, paddy straw and marigold straw took about 31 days for spawn run. Hulled maize cobs took maximum days for spawn run (36 days) which is significantly higher than that of wheat straw. The days to spawn run in different substrates varied from 29 to 36 days.

Table 4.7 Evaluation of different substrates for spawn run and primordia initiation of *Pleurotus citrinopileatus***

Substrates	Spawn run (days)	Primordia initiation (days)	Primordia (no.)
Pine needles	34.00	37.00	2.67
Soybean straw	30.33	35.67	5.00
Wheat straw	29.00	34.67	4.00
Maize straw	30.67	37.33	3.00
Oat straw	35.00	39.33	4.00
Paddy straw	31.67	35.67	4.33
Mash straw	35.00	38.67	2.33
Marigold straw	31.67	37.67	3.00
Hulled Maize cobs	36.00	42.33	1.67
Shelled Pea Pods	29.33	36.67	2.33
Pea Straw	31.33	38.33	2.00
CD (p=0.05)	1.63	1.19	1.33

*Average of three replications

Plate 4.4 Primordia formation of *Pleurotus citrinopileatus* on different substrates

It is observed that the earliest primordia initiation was on wheat straw i.e. after 34.67 days of spawn inoculation followed by paddy straw and soybean straw in which the primordia initiated after 35.67 days each. The maximum time for primordia initiation was taken in case of the hulled maize cobs where it was observed after 42.33 days of inoculation. In some cases the spawn run took place early like pea pods, pea straw and maize leaves but the primordia initiation was late comparative to other substrates. The time taken for primordia initiation varied in between 34 to 42 days after inoculation on the various substrates. The period was of significant difference so its impact can be seen in the early harvest of the crop.

On soybean and paddy straw, though primordia initiation occurred on the same day (35.67 days), but there were difference in the number of primordia i.e. 5 and 4, respectively. The minimum number of primordia was on hulled maize cobs (1.67).

The result of present study showed that the minimum time for spawn run and primordia initiation was 29 days and 34 days, respectively which are contradictory to the finding of Musieba et al. (2012) and Owaid et al. (2016). But these studies are at par with the findings of Liang et al. (2009). The days taken to spawn run and primordia formation of pea straw and maize straw were at par with the results of Pokhrel et al. (2013).

4.4.2.2 Spawn run and primordia initiation on substrate combinations

The data were recorded for the days to spawn run and primordia initiation and number of primordia of *Pleurotus citrinopileatus* on agro-based substrates in combinations and are presented in table 4.8.

The days to spawn run varied from 28 to 36 days on various agro-based substrate combinations. The minimum days to spawn run was recorded on soybean+maize (28.67 days) followed by wheat+soybean (29.67 days), soybean+maize+wheat (30.33 days) and soybean+maize+oat (30.67 days). The maximum time for spawn run was taken by the wheat+marigold (35.67 days) followed by wheat+pea straw (36 days). The spawn run on wheat straw substrate as check took 29 days which is statistically at par with the soybean+maize and wheat+soybean.

Among the various substrate combinations, the minimum days for primordia initiation were recorded on the soybean+maize (34.67 days) with 4.33 primordia followed by soybean+maize+oat (35.67 days) with 3.33 primordia, soybean+maize+ wheat (36.67 days) with 2.67 primordia and wheat+soybean (37.33 days) with 2.67 primordia. The maximum time for primordia initiation was recorded on wheat+pea straw and wheat+maize straw i.e. 42.33 days and 42.67 days, respectively with 1.67 primordia each.

Table 4.8 Evaluation of different substrates in combinations for spawn run and primordial initiation of *Pleurotus citrinopileatus**

Substrates	**Spawn run (days)**	**Primordia initiation (days)**	**Primordia (no.)**
Wheat+Paddy	34.67	40.00	2.67
Wheat+Oat	34.67	39.67	2.67
Wheat+Maize straw	31.67	38.00	2.33
Wheat+Soybean	29.67	37.33	2.67
Wheat+Mash	35.00	39.33	1.33
Wheat+Marigold	35.67	41.67	1.33
Wheat+Maize cobs	35.00	41.33	1.00
Wheat+Pea pods	33.00	38.00	2.67
Wheat+Pea straw	36.00	42.33	1.67
Wheat+Pine needles	35.33	41.33	1.67
Wheat+Sugarcane bagasse	34.33	40.33	1.33
Paddy+Maize straw	35.33	42.67	2.67
Paddy+ Soybean	33.67	38.67	2.00
Paddy+Mash	34.33	42.00	1.67
Soybean +Maize straw	28.67	34.67	4.33
Soybean+Maize +Oat	30.67	35.67	3.33
Soybean+Maize+Wheat	30.33	36.67	3.67
Wheat	29.00	34.67	4.00
CD(p=0.05)	1.26	1.15	1.19

*Average of three replications

It can be seen from the data that the combination of soybean+maize+oat and soybean+maize+wheat straw did not varied significantly with each other. The best combination in accordance with spawn run, primordia initiation and their number was of soybean+maize followed by combination of soybean+maize+oat and wheat+soybean.

Plate 4.5 Primordia formation of *Pleurotus citrinopileatus* on substrates in combinations

4.4.3 Yield Potential

4.4.3.1 Yield potential of different agro-based substrates

The data were taken to study the yield potential of *P.citrinopileatus* on various agro-based substrates. The yield of golden oyster mushroom was recorded and finally biological efficiency of each substrate was calculated. The table 4.9 depicts that the maximum number of flushes were obtained from the wheat straw (3 flushes) and only 1 flush form maize cobs. It showed that the maximum yield in the first flush was obtained from soybean and wheat i.e. 400g each while, the minimum yield was obtained from maize cobs (166g). The maximum yield of 300g and minimum of 46g was obtained on wheat straw and pea straw, respectively during the second flush. In the third flush, wheat straw gave the highest yield of 193g followed by soybean i.e. 166g and minimum was obtained from the marigold (60g). There were some substrates which did not give yield during the second and third flush. The present results are in conformity with Musieba et al. (2012) and Shevale and Deshmukh (2016) that the maximum yield was obtained from first flush among all the flushes. The results are at par with the study of Srivastava et al. (2012) that the yield obtained by wheat straw was significantly higher than maize straw. Das et al. (1987) found that best average yield of *P. flabellatus* and *P. sajor-caju* was from wheat straw which is at par with the present findings.

Plate 4.6 Cultivation of *P.citrinopileatus* on different agro-based substrates

On the basis of total yield, wheat straw gave the highest yield of 893g followed by soybean straw (860g) and oat (740g). Only 166g of golden oyster mushrooms were harvested from the maize cobs showing the least potential. The yield on soybean, oat and paddy straw substrates was more than 600g. The straw substrates viz., maize, mash and marigold gave sporophore yield of more than 400 g but it was below 360g in case of shelled pea pods, pine needles and pea straw and least (166g) in case of maize cobs. Similarly, highest biological efficiency was obtained from wheat straw (89.3%) followed by soybean straw (86%), oat straw (74%) and paddy straw (63.07%) and lowest from hulled maize cobs i.e. 16.67%.

In present investigation, wheat straw performed well among all the substrates producing yield of 893.33g which are contradictory to the finding of Srivastava et al. (2012), Mishra et al. (2015). Maize cobs performed least amongst the substrates tested which is at par with the study of Musieba et al. (2012). The present study showed that the wheat straw produced highest biological efficiency followed by soybean straw which is at par with the findings of Kumar (2017).

Table 4.9 Yield potential of *Pleurotus citrinopileatus* on different agro-based substrates*

Substrates	No. of flushes	First flush (g)	Second Flush (g)	Third Flush (g)	Total yield (g)	B.E. (%)
Pine needles	2.00	233.33	106.67 (10.37)**	0.00 (1.00)	340.00	34.00
Soybean straw	2.67	400.00	293.33 (17.15)	166.67 (12.94)	860.00	86.00
Wheat straw	3.00	400.00	300 (17.34)	193.33 (13.93)	893.33	89.33
Maize leaves	1.67	286.67	113.33 (9.05)	0.00 (1.00)	400.00	40.00
Oat straw	2.67	366.67	260 (16.15)	113.33 (9.05)	740.00	74.00
Paddy straw	2.00	360.00	270.67 (16.15)	0.00 (1.00)	630.67	63.07
Mash straw	2.00	326.67	230.67 (15.22)	0.00 (1.00)	557.33	55.73
Marigold straw	2.67	253.33	168 (12.90)	60.00 (6.68)	481.33	48.13
Hulled Maize cobs	1.00	166.67	0.00 (1.00)	0.00 (1.00)	166.67	16.67
Shelled Pea pods	1.67	253.33	104 (8.69)	0.00 (1.00)	357.33	35.73
Pea straw	1.33	253.33	46.67 (4.62)	0.00 (1.00)	300.00	30.00
CD(p=0.05)	0.73	26.53	82.49	58.13	100.29	10.03

* Average of three replications

**The figure in parentheses is square root transformed values

4.4.4.2 Yield potential on different agro-based substrates in combinations

The yield potential of different combinations of agro-based substrates was studied and consequently their biological efficiency (B.E.) was calculated. The data of the yield upto third flush with total yield and B.E. are presented in table 4.10. The yield on wheat straw substrate and its B.E. were kept as standard checks.

Among the substrates combinations of wheat straw, the total yield was maximum in case of wheat+oat i.e. 806.67g per kg of dry substrate with biological efficiency of 80.67% followed by wheat+maize straw (633.33g) with B.E. of 63.33%. The minimum yield (166.6g) was produced by wheat+pea straw with B.E. of 16%. Whereas, substrates of wheat+maize and wheat+mash showed higher yield potential as compared to the other combinations of wheat straw. The maximum number of flushes was observed from the combinations of soybean straw (3) and minimum from wheat+pea straw. In the first flush, highest yield was obtained from soybean+maize+wheat (506.67g), soybean+maize+oat (486.67g) and soybean+maize (466.67g) which was statistically superior to the wheat straw i.e. 400g. There were combinations which did not produce yield during second or third flush i.e. wheat+pea straw, wheat+sugarcane bagasse, wheat+mash, wheat+marigold, wheat+maize cobs, paddy+mash and paddy+maize straw.

The total yield (873.33g) of soybean+maize+wheat was highest among all other combinations with biological efficiency (87.33%). The table also reveals that the total yield of soybean+maize+wheat (873.33g), soybean+maize (866.67g), soybean+maize+oat (820g) and wheat+oat (633.33g) were statistically at par with the performance of wheat straw.

Dehariya and Vyas (2013) reported that the maximum yield of *Pleurotus sajor caju* was obtained from the combination of soybean+wheat straw which is in conformity with the present study, where soybean+maize+wheat performed quite well.

Plate 4.7 Cultivation of *Pleurotus citrinopileatus* on different combinations of agro-based substrates

Table 4.10 Yield potential of *Pleurotus citrinopileatus* on different combination of agro-based substrates

Substrates	No. of flushes	1st Flush (g)*	2nd Flush (g)*	3rd flush (g)*	Total yield (g)*	B.E. (%)*
Wheat+Paddy	2.33	233.33	106.67 (10.37)**	33.33 (4.02)	373.33	37.33
Wheat+Oat	2.66	400.00	293.33 (17.15)	113.33 (9.05)	806.67	80.67
Wheat+Maize straw	2.33	306.67	193.33 (13.94)	133.33 (9.79)	633.33	63.33
Wheat+Soybean	2.66	286.67	166.67 (12.94)	113.33 (9.05)	566.67	56.67
Wheat+Mash	1.66	366.67	153.33 (10.46)	0.00 (1.00)	520.00	52.00
Wheat+Marigold	1.33	360.00	73.33 (5.62)	0.00 (1.00)	433.33	43.33
Wheat+Maize cobs	1.33	326.67	77.33 (5.76)	0.00 (1.00)	404.00	40.40
Wheat+Pea pods	1.66	169.67	73.33 (7.35)	26.67 (3.67)	269.67	26.97
Wheat+Pea straw	1.00	168.67	0.00 (1.00)	0.00 (1.00)	168.67	16.87
Wheat+Pine needles	1.66	253.33	104.00 (8.69)	0.00 (1.00)	357.33	35.73
Wheat+Sugarcane bagasse	1.33	253.33	46.67 (4.63)	0.00 (1.00)	300.00	30.00
Paddy+Maize straw	1.66	297.33	120.00 (9.30)	0.00 (1.00)	417.33	41.73
Paddy+ Soybean	2.33	373.33	220.00 (14.87)	40.00 (4.33)	633.33	63.33
Paddy+Mash	1.66	253.33	100.00 (8.52)	0.00 (1.00)	353.33	35.33
Soybean +Maize straw	3.00	466.67	253.33 (15.95)	146.67 (12.15)	866.67	86.67
Soybean+Maize +Oat	3.00	486.67	233.33 (15.31)	100.00 (10.02)	820.00	82.00
Soybean+Maize+ Wheat	3.00	506.67	246.67 (15.74)	120.00 (10.98)	873.33	87.33
Wheat	3.00	400.00	300.00 (17.34)	193.33 (13.94)	893.33	89.33
CD(p=0.05)	0.816	25.18	117.79	82.33	148.30	14.40

* Average of three replications

**The figure in parentheses is square root transformed values

4.4.5 Morphological variation of sporophores

4.4.5.1 Comparative sizes of sporophores on different agro-based substrates

The data on comparative size of fruit bodies of *P.citrinopileatus* on various agro-based substrates were recorded. It shows that the maximum pileus size was on oat straw (7.22x6.39cm) followed by wheat straw (6.53x5.68cm) and soybean straw (5.57x4.10cm). The minimum size of pileus was obtained from pine needles and maize cobs. The average pileus size of sporophore was more than 5cm in case of wheat straw, soybean straw and paddy straw. Similarly, the maximum stipe length and width was obtained from oat straw i.e. 4.21x0.97cm followed by wheat straw (4.1x0.8cm) and the minimum stipe size was obtained from the pine needles (2.66x0.68cm).

On the basis of average mature sporophore weight of the golden oyster mushroom, the maximum weight was obtained by oat straw (11g) which is statistically at par with the mash straw. The minimum average sporophore weight was obtained by maize cobs. The present study is at par with the study of Shukla and Jaitly (2011) and Mishra et al. (2015) that *P. citrinopileatus* recorded average pileus diameter length of 5.0-8.5 cm.

Table 4.11 Comparative size of fruit bodies of *Pleurotus citrinopileatus* on different agro-based substrates*

Substrates	Pileus (cm)		Stipe(cm)		Lamellae Width(cm)	Sporophore Weight(g)
	Length	Width	Length	Width		
Pine needles	4.13	3.95	2.66	0.68	0.32	4.33
Soybean straw	5.57	4.70	3.72	0.87	0.69	8.50
Wheat straw	6.53	5.68	4.10	0.90	0.56	10.00
Maize straw	4.65	4.17	3.76	0.75	0.50	5.67
Oat straw	7.22	6.39	4.21	0.97	0.68	11.00
Paddy straw	5.55	5.13	3.85	0.80	0.55	8.67
Mash straw	5.56	5.15	3.08	0.80	0.62	9.33
Marigold straw	4.58	4.11	2.98	0.78	0.46	5.67
Hulled Maize cobs	4.13	3.45	3.21	0.82	0.46	4.00
Shelled Pea pods	4.57	3.72	3.99	0.78	0.42	5.17
Pea straw	4.22	3.57	3.81	0.85	0.48	5.03
CD(p=0.05)	0.63	0.44	0.19	0.15	0.03	1.14

*Average of three replications

Plate 4.8 **Sporophores of *Pleurotus citrinopileatus* on different agro-based substrates**

4.4.5.2 Comparative size of sporophores on different combinations`of agro-based substrates

The data on comparative size of fruit bodies of *P.citrinopileatus* on various substrate combinations were recorded and compared with those on wheat straw.

It was also remarkable to notice that the combinations of soybean+maize+oat produced maximum sized sporophore with pileus size (9x7.24cm) and stipe size (4.14x1.19cm) among all the substrate combinations followed by soybean+maize+wheat, wheat+soybean and soybean+maize. The table reveals that among the wheat straw combinations, the largest sporophore was produced on wheat+soybean having average pileus size (7.22x6.38cm) with stipe size (3.77x0.97 cm). It is also evident that the combinations of paddy straw produced smaller sized sporophore than the wheat straw combinations. The sporophore of paddy+soybean was of pileus size (4.61x3.63cm) and stipe size (3.54x0.78cm) which is greater than other paddy straw combinations. The smallest pileus was found on the wheat+maize cobs with dimensions 3.52x2.68cm.

Similarly, it showed that the morphological size reflects the average mature sporophore weight of the golden oyster mushroom. The average sporophore weight was maximum in case soybean+maize+oat (12.33g) followed by soybean+maize+wheat (10.83g) and soybean+maize (10.33g). The average sporophore weight of soybean+maize+oat was statistically superior to the wheat straw. The combination of wheat+soybean (10.00g) and soybean+maize (10.33g) were statistically at par with the wheat straw. The minimum mature sporophore weight was obtained from the combination of wheat+maize cobs i.e. 3.68g. So, it is evident that the combination of soybean performed best among all other substrates in combinations.

The present study shows that the best pileus diameter of 9.00cm was obtained from the combination of soybean+maize+oat which differs from the findings of Owaid et al. (2016). As they reported that the best diameter of 5.45cm was obtained from 50% wheat straw +30% sawdust+ 20% datepalm fibre. Dehariya and Vyas (2013) reported that the stipe length of 3.1cm and cap diameter(8.06cm) of *Pleurotus sajor caju* on soybean+wheat straw which is at par with the present investigations.

Table 4.12 Comparative size of fruit bodies of *Pleurotus citrinopileatus* on different combinations of agro-based substrates*

Substrates combinations	Pileus (cm)		Stipe (cm)		Lamellae Width (cm)	Sporophore Weight (g)
	Length	Width	Length	Width		
Wheat+Paddy	4.90	4.14	2.86	0.87	0.33	7.67
Wheat+Oat	5.70	4.87	2.70	1.00	0.68	9.23
Wheat+Maize straw	5.56	5.15	3.20	0.80	0.62	9.13
Wheat+Soybean	7.22	6.39	3.77	0.97	0.68	10.00
Wheat+Mash	4.20	3.24	2.38	0.82	0.42	4.83
Wheat+Marigold	5.10	4.13	2.47	0.80	0.45	8.83
Wheat+Maize cobs	3.52	2.68	2.40	0.97	0.44	3.60
Wheat+Pea pods	4.20	3.11	2.33	0.68	0.40	4.77
Wheat+Pea straw	4.10	3.57	2.49	0.82	0.46	4.80
Wheat+Pine needles	4.57	3.72	2.99	0.78	0.42	5.50
Wheat+Sugarcane bagasse	4.22	3.45	2.78	0.85	0.48	4.67
Paddy+Maize straw	3.95	3.18	2.29	0.77	0.41	4.33
Paddy+ Soybean	4.61	3.63	3.54	0.78	0.32	6.40
Paddy+Mash	3.76	3.06	2.81	0.64	0.40	4.00
Soybean +Maize straw	6.50	6.05	4.12	1.23	0.46	10.33
Soybean+Maize +Oat	9.00	7.24	4.14	1.19	0.81	12.33
Soybean+Maize+Wheat	7.28	5.46	3.35	0.74	0.62	10.83
Wheat	5.56	5.15	3.72	0.80	0.62	9.50
CD(p=0.05)	0.49	0.31	0.24	0.10	0.03	0.85

*Average of three replications

4.4.5 Morphological studies

Macroscopic studies like colour and texture of pileus and stipe and nature of gills and microscopic studies of hyphae and spore were done.

Plate 4.9 Mature sporophore and spore print of *Pleurotus citrinopileatus*

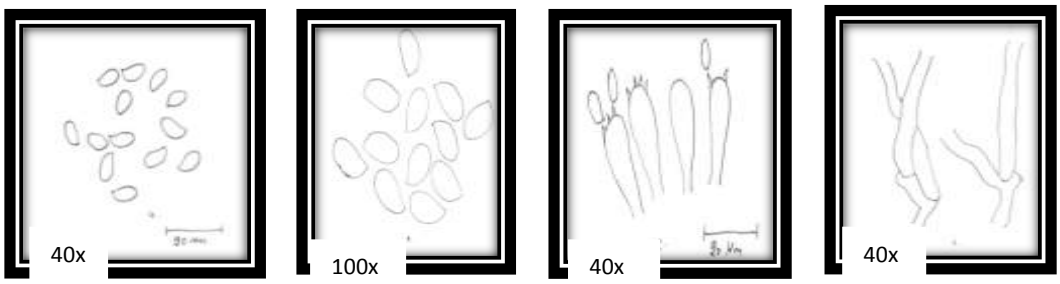

Plate 4.10 Drawing of basidiospores at 40x and 100x, basidium and hyphae

Sporocarps: Medium to large in size having yellow or white colour in the pinhead stage became golden yellow on maturity.

Pileus: 3-9 cm diameter, funnel shape, golden yellow on maturity, convex, depressed towards the base.

Stipe: 2-4 cm long, thick, solid, cylindrical, smooth, eccentric or lateral, yellowish white coloured.

Lamellae: Attached to stem and running down, decurrent, creamy white in colour, 0.3 to 1cm in width.

Spores: 2.30-3.94, 0.77-1.54 µm in size, thin-walled, smooth and cylindrical to sub-cylindrical in shape, smooth, hyaline.

Hyphae: 1.5 to 3 µm width, monomitic, clamp connections present

Basidium: Club shaped

Cystidia: Absent

Spore print: White in colour

5. SUMMARY AND CONCLUSIONS

The present studies on "Studies on cultivation of golden oyster mushroom (*Pleurotus citrinopileatus* Singer)*"* were conducted knowing that, the species was new and so far no cultural and cultivation studies were conducted on it. Hence present study was undertaken on its cultural, mycelial, fruiting characteristics and yield parameters. All the observations according to the objectives were recorded and statistically analyzed. The results were discussed, reviewed with reference to literature. The salient findings which emerged out of this study are summarized here in.

The cultural studies of *Pleurotus citrinopileatus* was performed on different solid media and revealed that Potato Dextrose Agar supported regular and fluffy colony growth and maximum radial growth of fungus (84.3mm) and took minimum days (9.25 days) to fill Petri plate followed by Malt Extract Agar (11.50 days).

Among the nine different grains, wheat grains were found to support best mycelial growth and subsequent spawn production and also produced highest yield when spawned on wheat straw substrate. It was also the most economic grain substrate for spawn production.

As oyster mushroom is a primary decomposer i.e. it breaks down the lignin and cellulose in wood, straw and other plant matter, that's why straws of various substrates were used in the present study. Nineteen substrates and seventeen substrate combinations in ratio 1:1 were evaluated during the period of study. Spawn run was found complete on Wheat, Oat, Soybean, Pea straw, Marigold substrate and Mash and incomplete on Paddy straw, Pine needles, Maize leaves; while on, Maize Cobs, Compost, Paper waste *Lantana, Eupatorium* and *Ageratum* the mycelium did not colonize and were found unsuitable.

Wheat straw was found to be the best substrate for mushroom yield (893.33g/kg of dry substrate) with biological efficiency of 89.33% and it took minimum days for spawn run (29 days) and primordia initiation (34.67 days).The new

substrates for the production of *Pleurotus citrinopileatus* which were not used earlier are pine needles and marigold straw. Among the combination of substrates soybean+maize+wheat and soybean+maize were found to be superior to other substrate combinations as the highest yield was obtained i.e. 873.33g/kg of dry substrate and 866.67g/kg of dry substrate, respectively with biological efficiency of 87.33% and 86.67%, respectively.

Among the different substrates, oat straw produced largest sized fruiting body of pileus size 7.22x6.39cm and stipe size of 4.21x0.97cm with highest sporophore weight of 11g. The best pileus size i.e. 9.00x7.24cm was obtained from the combination of soybean+maize+oat with sporophore weight of 12.33g.

Conclusion

- Potato Dextrose Agar supported maximum mycelial growth of fungus and took minimum days to fill Petri plate.
- Wheat grains were found to support best mycelial growth and most economic among others.
- Wheat straw took minimum days for spawn run and primordia initiation.
- Among the different substrates, oat straw produced largest size fruiting body with highest sporophore weight.
- The best pileus size was obtained from the combination of soybean+maize+oat followed by soybean+maize+wheat.
- Wheat straw was found to be the best substrate for yield of golden oyster mushroom.
- Among the combination of substrates soybean+maize+wheat and soybean+maize were found to be superior to other substrate combinations.

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APPENDIX

Composition of media

Potato dextrose agar (PDA)

Peeled potato	200 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml

Yeast potato Dextrose agar

Peeled potato	200 g
Yeast extract	1 g
Dextrose	20 g
Agar	20 g
Distilled water	1000 ml

Malt Extract Agar

Malt extract	20 g
Agar	20 g
Distilled water	1000 ml

Sabouraud's Agar

Dextrose	40 g
Peptone	10 g
Agar	20 g
Distilled water	1000 ml

Potato Agar

Peeled potato	200 g
Agar	20 g
Distilled water	1000 ml

Yam Dextrose Agar

Yam	200 g
Dextrose	20g
Agar	20 g
Distilled water	1000 ml

Yeast Extract Agar

Yeast	20 g
Agar	20 g
Distilled water	1000 ml

Brief Biodata of student

Name : Prerna Thakur

Father's Name : Sh. Bhinder Thakur

Mother's Name : Smt. Gema Thakur

Date of Birth : 5th October, 1994

Permanent Address : Vill- Sanyardi PO- Talyard Teh- Sadar Distt Mandi (H.P.) PC 175001

Academic Qualifications:

Examination passed	Year	School/Board/ University	Marks (%)	Division	Major Subjects
10th	2010	Jawahar Navodaya Vidhayalaya ,Pandoh Mandi (Central Board of Secondary Education)	81.7	First	English, Hindi, Maths, Science, Social Studies
10+2	2012	Jawahar Navodaya Vidhayalaya ,Pandoh Mandi (Central Board of Secondary Education)	84.2	First	English, Biology, Physics, Chemistry, and Computer sciences
B.Sc. hons.(Horticulture)	2017	COH, YSPUHF, Nauni Solan (H.P.)	85.7	First	All Horticultural Subjects with Commercial Horticulture as a elective
M.Sc. (Agri.) (Plant Pathology)	2019	COA, CSKHPKV, Palampur (H.P.)	85.6	First	Major Discipline: Plant Pathology Minor Discipline: Genetics and Plant Breeding

Fellowships/Scholarships: University Merit Scholarship

Publications:

Total: Nil

Research papers (in peered journals): - Nil

Others: Nil