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Comparative effect of different culture media on mycelial growth performance of *Pleurotus sapidus*

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Abstract
The mycelium growth performance of *Pleurotus sapidus* on six different culture media was investigated. The present study evaluates the growth performance of *Pleurotus sapidus* on different culture media such as Potato Dextrose Agar Medium (PDA), Malt Extract Agar Medium (MEA), Glucose Peptone Agar Medium (GPA), Yeast Malt Agar Medium (YMA), Saboraud's Dextrose Agar Medium (SDA) and Czapek Dox Agar (CDA). The studies revealed that MEA, PDA and YMA medium have very abundant mycelia growth rate and density, followed by GPA, SDA and CDA medium. This Study Concludes that PDA and MEA are the best culture media for the effective mycelial growth of *Pleurotus sapidus.*

Keywords: *Pleurotus sapidus*, mycelial growth, culture media, mycelial density, colony diameter

1. Introduction
Pleurotus species, commonly known as oyster mushrooms, are edible fungi cultivated worldwide especially in south East Asia, India, Europe and Africa [1]. Mushrooms, a basidiomycetous fungus, are becoming more popular nowadays for remediation purposes because it is not only a bioremediation tool but also provide mycelium or fruiting bodies as a source of protein [2].
Mushroom are abundantly available throughout the world, and are mainly focused than ever before because they have the capability of producing many benefits indeed to mankind especially in the line of medicine. Mushrooms are rich in protein and dietary fiber; and they also contain vitamins and minerals such as vitamin B, vitamin D, potassium and magnesium [3, 4]. The efficiency of mushroom species in producing food protein in the form of biomass or fruiting bodies from different wastes lies in their ability to degrade waste via secretion of a variety of hydrolyzing and oxidizing enzymes [5, 6].
Many surveys from different area of the world confirmed that the *Pleurotus* mushroom having highly nutrition and also contains various bioactive compounds including terpenoids, steroids, phenols, alkaloids, lectins and nucleotides, which have been isolated and identified from the fruit body, mycelium and culture broth of mushrooms are shown to have promising biological effects [7].
Dietary mushrooms provide a wide variety of medicinal properties and they are effective against certain life-threatening diseases. Major medicinal properties attributes to mushrooms include anticancer, antibiotic, antiviral activities, immunity and blood lipid lowering effects [8, 9]. The availability of good quality spawn is the limiting factor for mushroom cultivation in many developing countries. Spawn is prepared on different media depending upon the mushroom type and it is further multiplied on grains. Grains are preferred because they are very nutritious for fungi [10]. Mushroom mycelium is used for medicinal and therapeutic purposes; mycelial biomass powder can be used to formulate various types of health tablets and capsules [11].
The identification of suitable agar media is essential to obtain maximum yield and quality of mushroom spawn. Therefore, the present investigation was based on the evaluation of mycelial growth performance of *Pleurotus sapidus* on six different culture media.

2. Material and Methods
Collection and Maintenance of *Pleurotus sapidus* Culture
Viable mycelial culture of *Pleurotus sapidus* was obtained from the Directorate of Mushroom Research (DMR), Chambaghat, Solan, Himachal Pradesh procured on July 2017.

Preparation of culture media
The culture media preparation as follows:

Table 1: Composition of media used for the growth of *Pleurotus sapidus*

Contents of media	PDA	MEA	GPA	YMA	SDA	CDA
Potato dextrose agar	39 g	…	…	…	…	…
Malt extract	…	30 g	…	20 g	…	…
Agar-Agar	…	15 g	…	15 g	15 g	15 g
Peptone	…	…	20 g	…	10 g	…
Dextrose	…	…	10 g	…	40 g	…
Nacl	…	…	5 g	…	…	…
Yeast	…	…	…	2 g	…	…
Sucrose	…	…	…	…	…	30 g
Sodium Nitrate	…	…	…	…	…	2 g
Dipotassium Phosphate	…	…	…	…	…	1 g
Magnesium Sulphate	…	…	…	…	…	0.5g
Ferrous Sulphate	…	…	…	…	…	0.01 g
Distilled water	1 L	1 L	1 L	1 L	1 L	1 L

All Sixculture media were prepared according to above mentioned composition.

Whereas, PDA - Potato Dextrose Agar, MEA - Malt Extract Agar, GPA - Glucose Peptone Agar, YMA - Yeast Malt Agar, SDA - Saboraud's Dextrose Agar , CDA – Czapex Dox Agar, g - Gram, L - Litre.

Sterilization of Medium

The flasks having media were sterilized in the autoclave at 15 pound square inch (psi) pressure for one hour and then poured in 90 mm Petri dishes under the laminar flow hood to avoid contamination. Media were cooled to 37^0C. The joint, stalk and veil of the fresh mushrooms were inoculated on culture media. Radial growth of mycelium of different portions was observed until the Petri dishes were filled with it. The experiment was repeated for 5 times. The plates was incubated at 37^0C and observed for 10 days during which the mycelial vegetative growth and mycelial density of *Pleurotus sapidus* were recorded.

Mycelial Density

The mycelial density was rated as described as follows[12].

+ = Very Scanty mycelial density
2+ = Scanty mycelial density
3+ = Moderate mycelial density
4+ = Abundant mycelial density
5+ = Very abundant mycelial density

Mycelial growth

Mycelial growth was determined using a ruler across the Petri-dish horizontally.

The growth rate calculation is given by the formula below:

Growth rate = Colony diameter on the last day (cm) /Number of day's measurement was taken after inoculation.

3. Results and Discussion

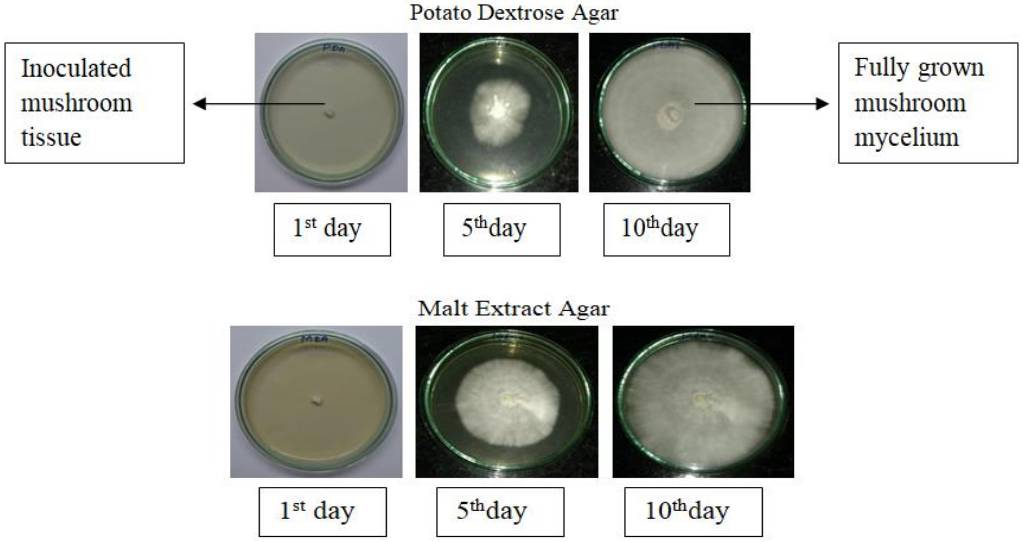
The results presented in Table 2 and 4 revealed that the studies of different culture media on mycelial growth of *Pleurotus sapidus* mushroom tissue. Six types of culture media were tested for the mycelial growth and measured for 10 days. The result showed the mycelial growth of *Pleurotus sapidus* on the 10^{th} day had the highest mycelial diameter 8.97 ± 0.05cm, density 5 + and growth rate 0.9cm/day on PDA media followed by MEA media with 8.97 ± 0.05cm, 5 + and 0.9cm/day being colony diameter, mycelial density and growth rate respectively. YMA media colony diameter was 8.97 ± 0.05cm, density 5 + and growth rate was 0.9cm/day.

On CDA media colony diameter was 3.55 ± 0.08cm, density 4 + and growth rate was 0.5cm/day. Moreover, the growth on GPA media had 3.4 ± 0.21cm as colony diameter, 3+ as mycelia density and 0.3cm/day as the growth rate. The tiniest growth was recorded on SDA media was poor with 3.48 ± 0.18cm as colony diameter, 3+ as mycelial density and 0.3 cm/day as the growth rate.

Table 2: Effect of different culture media on the mycelium growth of mushroom tissue–*Pleurotus sapidus*

S. No	Culture medium	Mycelium growth diameter (cm)				
		DAY 2	DAY 4	DAY 6	DAY 8	DAY 10
1.	Potato Dextrose Agar	0.90 ± 0.13	1.82 ± 0.30	3.87 ± 0.53	6.37 ± 0.24	8.97 ± 0.05
2.	Malt Extract Agar	0.83 ± 0.10	2.42 ± 0.37	4.22 ± 0.37	7.38 ± 0.36	8.97 ± 0.05
3.	Glucose Peptone Agar	0.52 ± 0.08	1.38 ± 0.33	2.15 ± 0.23	3.10 ± 0.24	3.4 ± 0.21
4.	Yeast Malt Agar	1.47 ± 0.28	3.22 ± 0.20	5.32 ± 0.18	7.72 ± 0.16	8.97 ± 0.05
5.	Saboraud's Dextrose Agar	0.53 ± 0.12	1.00 ± 0.09	1.97 ±0.16	2.83 ± 0.18	3.48 ± 0.18
6.	Czapek Dox Agar	0.58 ± 0.12	1.85 ± 0.16	2.62 ± 0.16	2.93 ± 0.14	3.55 ± 0.08

Each value is expressed as mean ± SD (n=6).



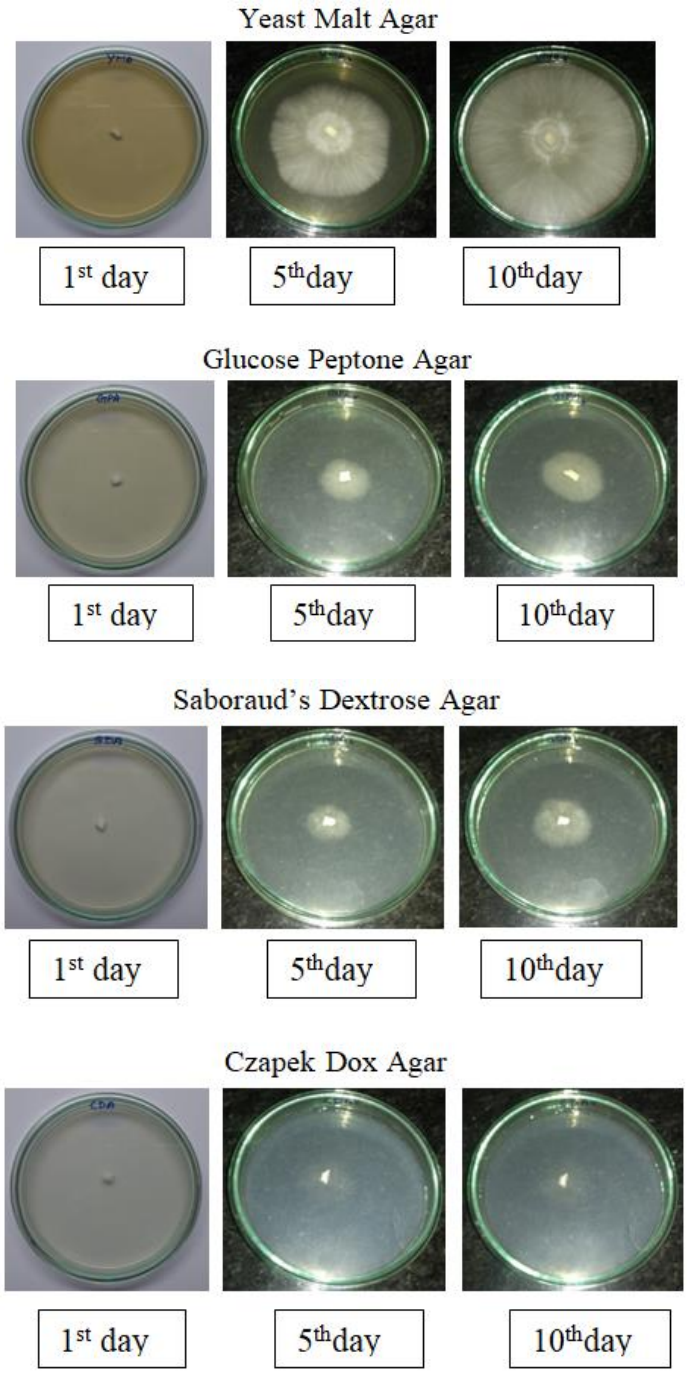


Fig 1: Effect of different culture media on the mycelium growth of mushroom tissue culture –*Pleurotus sapidus*

Table 3: Effect of different culture media on the mycelium growth of mushroom culture –*Pleurotus sapidus*

S. No	Culture medium	Mycelium growth diameter (cm)			
		DAY 2	DAY 4	DAY 6	DAY 8
1.	Potato Dextrose Agar	1.18 ± 0.04	3.07 ± 0.27	5.13 ± 0.54	8.98 ± 0.04
2.	Malt Extract Agar	1.40 ± 0.09	3.10 ± 0.14	5.38 ± 0.31	8.97 ± 0.05
3.	Glucose Peptone Agar	1.13 ± 0.10	2.82 ± 0.10	4.52 ± 0.28	5.70 ± 0.15
4.	Yeast Malt Agar	1.85 ± 0.16	4.62 ± 0.22	7.65 ± 0.23	9.00 ± 0.01
5.	Saboraud's Dextrose Agar	1.22 ± 0.08	2.25 ± 0.16	3.73 ± 0.26	5.75 ± 0.29
6.	Czapek Dox Agar	1.15 ± 0.16	2.15 ± 0.10	3.12 ± 1.10	4.15 ± 0.19

Each value is expressed as mean ± SD (n=6).

The results presented in Table 3 and 4revealed that the studies of different culture media on mycelial growth of *Pleurotus sapidus* mushroom culture. Six types of culture media were tested for the mycelial growth and measured for 8 days. The result showed the mycelial growth of *Pleurotus sapidus* on the 8th day had the highest mycelial diameter 9.00 ± 0.01cm, density 5 + and growth rate 1.1 cm/day on YMA media followed by PDA media with 8.98 ± 0.04cm, 5 + and 1.1 cm/day being colony diameter, mycelial density and growth rate respectively. MEA media colony diameter was 8.97 ± 0.05cm, density 5 + and growth rate was 1.1 cm/day.

On SDA media colony diameter was 5.75 ± 0.29cm, density 4 + and growth rate was 0.7cm/day. Moreover, the growth on GPA media had 5.70 ± 0.15cm as colony diameter, 4+ as mycelia density and 0.7cm/day as the growth rate. The tiniest growth was recorded on CDA media was poor with 4.15 ± 0.19cm as colony diameter, 3+ as mycelial density and 0.5 cm/day as the growth rate.

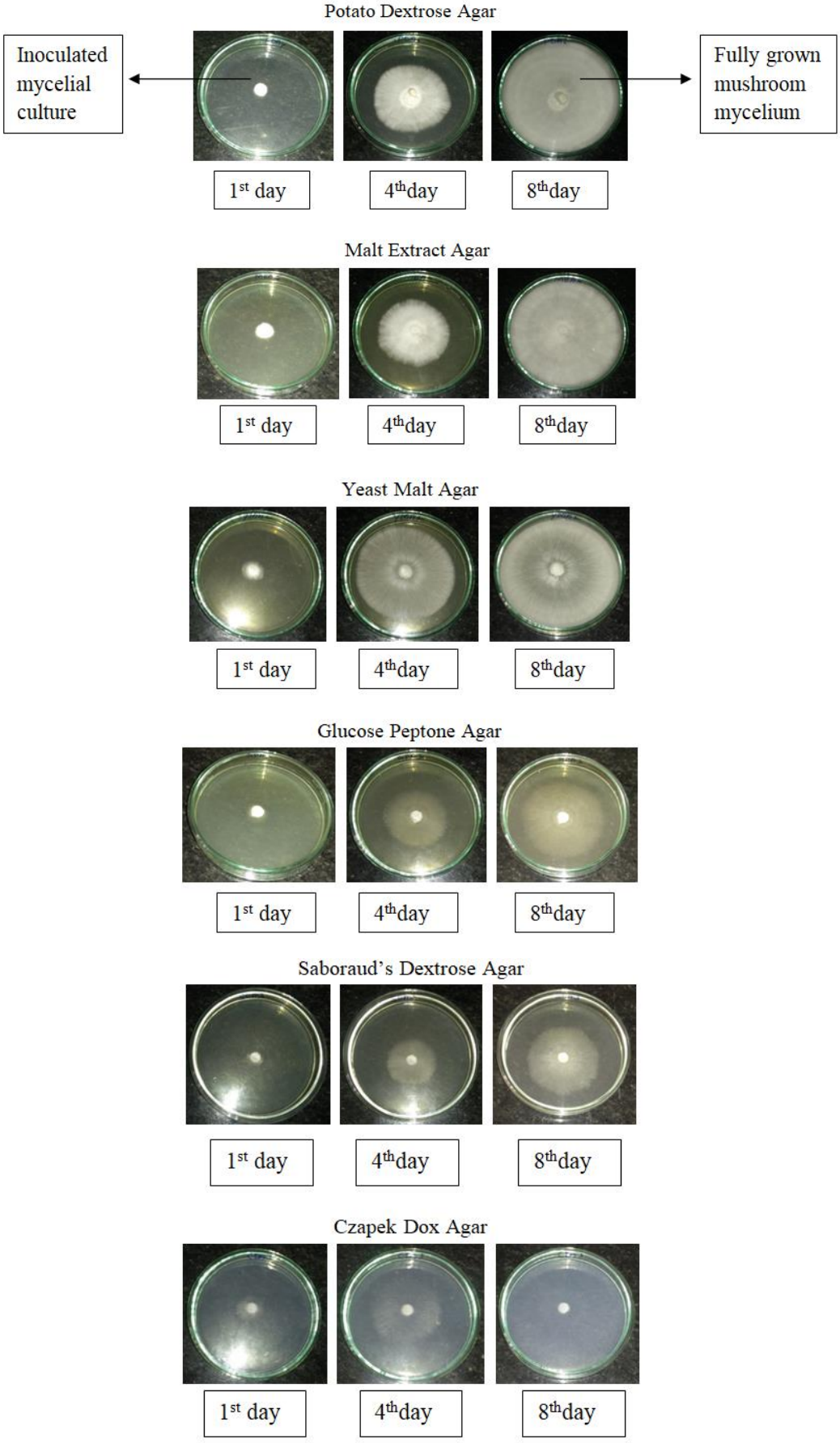


Fig 2: Effect of different culture media on the mycelium growth of mushroom mycelial culture –*Pleurotus sapidus*

Table 4: Mycelial colony diameter, growth rate and density of *Pleurotus sapidus* on various culture media of both mushroom tissue and mycelium

S. No	Culture medium	Colony diameter (cm)		Mycelial growth rate		Mycelial density	
		Tissue	Mycelium	Tissue	Mycelium	Tissue	Mycelium
1.	Potato Dextrose Agar	9.0	9.0	0.9	1.1	5 +	5 +
2.	Malt Extract Agar	9.0	9.0	0.9	1.1	5 +	5 +
3.	Glucose Peptone Agar	3.4	5.7	0.3	0.7	3 +	4 +
4.	Yeast Malt Agar	9.0	9.0	0.9	1.1	5 +	5 +
5.	Saboraud's Dextrose Agar	3.4	5.7	0.3	0.7	3 +	4 +
6.	Czapek Dox Agar	3.5	4.1	0.5	0.5	4 +	3 +

Whereas 5+ = Very abundant, 4+ = Abundant, 3+ = Moderate.

Each value is expressed as mean ± SD (n=6).

The figure 1 and 2 showed that significant ($P \leq 0.05$) differences regarding mycelial growth on different agar media i.e. PDA, MEA, GPA, YMA, SPA and CDA. Among these growing agar media, on an average mycelial growth of PDA, MEA and YMA. Followed by lowest mycelial growth of CDA, SPA and GPA.

Mycelial density, colony diameter and growth rate are various incidices for measuring the growth of mushroom mycelia on culture media. Mycelial density of mushroom tissue were very abundant (5+) on PDA, MEA and YMA. On CDA it was abundant (4+) whereas on SDA and GPA it was moderate (3+). Mycelial density of mushroom culture were very abundant (5+) on PDA, MEA and YMA. On GPA and SDA it was abundant (4+) whereas on CDA it was moderate (3+). Growth rate was high on PDA, MEA and YMA media in slightly desending order respectively, but was lowest on GPA, SDAand CDA.

4. Conclusion

The composition of solid growth media determines the extent and effectiveness of growth of the culture inoculated. In the present study Malt Extract Agar Medium (MEA), Potato Dextrose Agar Medium (PDA) and Yeast Malt Agar Medium (YMA) were found to stimulate luxuriant mycelial growth rate and whereas comparatively less mycelial growth were recorded on Czapek Dox Agar Medium (CDA), Glucose Peptone Agar Medium (GPA) and Saboraud's Dextrose Agar Medium (SDA). Mushroom mycelium is the most excellent tool for categorize necessary nutrients for the production of fruiting bodies.

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