



## CULTURAL STUDIES ON MYCELIA OF *PLEUROTUS PULMONARIUS* (OYSTER MUSHROOM) IN SELECTED CULTURE MEDIA

<sup>1</sup>H.O. Stanley, and <sup>2</sup>C.U. Nyenke

<sup>1</sup>Department of microbiology, University of Port Harcourt, Nigeria.

<sup>2</sup>School of Medical laboratory Science, College of Health Science and Technology Port Harcourt, Nigeria.

### ABSTRACT

The influence of various culture media on the mycelial growth of *pleurotus pulmonarius* was investigated on six solid culture media. Malt extract Agar (MEA), Corn cob, Extract Agar (CEEA) and cassava peelings Extract Agar (CPEA) media supported an excellent mycelial growth rate and density of *P. pulmonarius* while potato Dextrose Agar (P.D.A) and plantain peelings Extract Agar (PPEA) media were observed to support poor mycelial growth. The use of organic wastes for the production of good quality mycelial should be encouraged and sustained as it will help to check poor waste handling in our major towns/cities in Nigeria and also reduce production cost in the mushroom industry.

**KEYWORDS:** Culture media, Mycelial growth, *pleurotus pulmonarius*, mycelial density, colony diameter.

### INTRODUCTION

Mushrooms have been universally recognized now as food and are grown on commercial scale in many parts of the world. Cultivation of mushroom is widely practical in Europe, North America, Taiwan and China. The Oyster mushrooms (*Pleurotus spp.*) have been cultivated in large quantities in Japan for several years. Commercial production and consumption of mushroom has increased dramatically during the past few years in Europe, Asia and United States (Edwards, 1977; Lelley, 1982).

In Nigeria, the industry of mushroom is newly borne and is progressing slowly as compared to other countries of the world. The reasons for the slow progress in mushroom industry may be due to lack of interest on the part of Government and private investors, shortage of technically trained people, absence of mushroom research laboratories and ignorance of the people to advantages and importance of mushroom. Mushroom has a lot of production potential and due to its rapid growth it gives so large amount of crop which could not be compared with any other crop (Robinson and Davidson, 1959). There is need for cultivation of mushrooms on a large scale because of enormous increase in Nigeria's population (in excess of 140M people) with concomitant nutritional deficiency in the diets of average Nigerians, since increase in food production does not match population increase. It would provide additional source of good quality protein, vitamins etc., to the people and create job opportunities for the unemployed people in the country.

This study investigated into the performance of *pleurotus pulmonarius* on selected growth media in the Laboratory. Inefficiency, inability and lack of technical know-how to culture edible mushrooms have prevented interested persons into the business of mushroom production. The techniques employed in this study will definitely assist and encourage farmers of all categories to develop interest in mushroom cultivation. It is an environmental and economic driven research especially meant to encourage rural farmers to invest in mushroom cultivation. The production of active mycelium, which this study focuses

on, is one of the most sensitive and critical stage in mushroom cultivation, which has prevented a lot of interested and would be mushroom growers from getting involved. Stanley, (2010) described spawn (active mycelium) production as the inevitable bedrock for the development of the mushroom industry and also the limiting factor to mushroom cultivation or production all over the world. In Europe, North America, Japan, China, South East Asia and Australia where adequate technology and available mushrooms are cultivated for export trade and local consumption.

Ugandan mushroom growers for instance are currently selling 44 tones per year to Japan, 40 tones to U.S.A. and 1 tones to the Democratic Republic of Congo (Spore, 2006). Today China is the major producer of mushroom in the world (Qei, 1992). In the present study, stock culture of vigorous growing mycelia of *pleurotus pulmonarius* were cultivated on different media i.e. MEA, PDA, SDA, CCEA, CPEA and PPEA and their mycelial growth rate was determined. The purpose of the present study was to determine the most suitable media, for the growth of the mushroom.

### MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, Faculty of Science, University of Port Harcourt Nigeria located at (4° 54' 26" N, 6° 55' 13" E).

#### Sources of materials

Viable mycelial culture was obtained from tissue culture of *pleurotus pulmonarius* picked from a farm in Choba town, Rivers State. Organic wastes (corn cobs, cassava and plantain peelings) used for the experimental work were obtained from Choba town while MEA, PDA, and SDA were purchased from a scientific shop in Port Harcourt.

#### Media preparation

- Malt Extract Agar (MEA). MEA medium was prepared by adding 33.6g of malt extract agar powder, 1Litre of distilled water and autoclaved at 121°C for 15minutes.

## Cultural studies of oyster mushroom in selected culture media

After cooling to about 45<sup>0</sup>C. It was then dispensed into plastic Petri-dishes of 8.5cm in diameter.

- b. Potato Dextrose Agar (PDA): The same process as in (a) above was adopted in obtaining PDA medium by adding 39g of PDA powder to 1Litre of distilled water.
- c. Sabouraud Dextrose Agar (SDA): The same process as in (a) above was also adopted in obtaining SDA by adding 23g of the powder to 1 litre of distilled water. MEA, PDA, and SDA media were prepared as described by Chang and Hayes (1978).
- d. Corn cob Extract Agar (CCEA): CCEA medium was prepared using 150g of corn cob waste, 1 litre of distilled water added to the waste in a 500ml glass beaker. This was placed in a steaming water bath for 2hours. It was allowed to cool to room temperature; filtered with a piece of muslin cloth and the supernatant retrieved. 7g of pure agar (Agar technical) was added to the corn cob extract (300ml) to give approximately 20g/L, boiled to dissolve and autoclaved at 121<sup>0</sup>C for 15min. CCEA medium was then Dispensed into plastic petri- dishes (8.5cm). The same process was adopted in obtaining cassava plantain peelings extract agar culture media

Two perpendicular diameters were drawn on the bottom of the Petri-dishes (Plates) containing the various culture media used for the study, so that they intercept at the centre of the plates. In each case, the inoculum was placed at the centre of the medium (i.e. loopful of active growing mycelial per plate). This was evenly spread in a circular manner within the centre of the medium (about 3mm diameter).

The plates was incubated at 30<sup>0</sup>C and observed for nine (9) days during which the mycelial vegetative growth and mycelial density of *pleurotus pulmonarius* were recorded.

The mycelial density was rated as described by Kadiri (1998) as follows:

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

The growth rate is given by the formular below:

Growth rate =  $\frac{\text{Colony diameter on the last day(cm)}}{\text{Number of days measurement was taken after inoculation}}$

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

### RESULTS

The result in (table 1) on the 9<sup>th</sup> day showed that *p. pulmonarius* had the highest mycelial colony diameter (7.5cm), density (5+) and growth rate (2.5cm/day) on MEA media followed by CCEA media with 7.1cm, 5<sup>+</sup> and 2.4cm/day being colony diameter, mycelial density and growth rate respectively.

On CPE media colony diameter was 6.8cm, mycelial density 5<sup>+</sup> and growth rate was 2.3cm/day. Furthermore, the growth on SDA media had 6.2 cm as colony diameter, 5<sup>+</sup> as mycelial density and 2.1cm/day as the growth rate. Growth on PDA media was poor with 4.4cm as colony diameter, 4<sup>+</sup> as mycelial density and 1.5 cm/day as growth rate. The least growth was recorded on PPEA media with 3.2cm as colony diameter, 3<sup>+</sup> as mycelial density and 1.1cm/ day as growth rate.

**TABLE-1**

Growth media	Average daily mycelial growth of colony diameter of 3 replicates (cm)									Average mycelial density on the 9 <sup>th</sup> day of incubation	
	DAY1	DAY2	DAY3	DAY4	DAY5	DAY6	DAY7	DAY8	DAY9	GROWTH RATE cm/day	
MEA	0.7	1.2	1.9	2.6	3.5	4.5	5.3	6.3	7.5	5+	2.50
PDA	0.4	0.8	1.4	1.7	2.0	2.5	3.2	3.8	4.4	4 <sup>+</sup>	1.5
SDA	0.5	0.9	1.6	2.3	3.0	3.8	4.7	5.5	6.2	5+	2.1
CCEA	0.6	1.0	1.8	2.5	3.4	4.2	5.2	6.1	7.1	5+	2.4
CPEA	0.4	0.8	1.3	2.0	3.0	3.9	4.8	5.9	6.8	5+	2.3
PPEA	0.2	0.4	0.7	1.2	1.6	2.0	2.4	2.9	3.2	3+	1.1

3+	=	Moderate mycelial density
4+	=	Abundant mycelial density
5+	=	Very abundant mycelial density
MEA	=	Malt Extract Agar
SDA	=	Sabouraud Dextrose Agar
PDA	=	Potato Dextrose Agar
CCEA	=	Corn cob Extract Agar
CPEA	=	Cassava peelings Extract Agar
PPEA:	=	Plantain peeling Extract Agar

### DISCUSSION

All the supernatant culture media (composed from agricultural (organic) wastes) stimulated and sustained high mycelial growth except the PPEA media.

MEA media (synthetic agriculture media) and CCEA media (supernatant culture media) proved to be the most

suitable culture media for the mycelial growth of *p. pulmonarius*, CPEA media (supernatant culture media) supported the mycelial growth of the mushroom specie studied better than PDA and SDA (synthetic agar culture media).

The finding in this study is in line with Nasim et al 2001 who found that MEA provided faster. *P. ostreatus* mycelial growth rates than did murashige and skoog's (MS) medium and PDA. They also found that, the slowest growth was observed on PDA medium.

Colony diameter, mycelial density and growth rate are various indices for measuring the growth of mushroom mycelia on culture media. Mycelial density were very abundant (5<sup>+</sup>) on MEA, SDA, CCEA and CPEA. On PDA it was abundant (4<sup>+</sup>) whereas on PPEA it was moderate (3<sup>+</sup>). Growth rate was high on MEA, CCEA and CPEA media in slightly descending order respectively, but was lowest on PPEA.

These results compare favourably with the findings of Jenison (1948) and Kaul (1981) who obtained excellent mycelial growth due to incorporation of agar in legumes, molasses, gluten, meals of grains, corn steep, extracts of orange, banana, celery, alfalfa, parsnip etc. This result is further supported by Quimio (1981) who obtained accelerated mycelia growth of *Auricularia Spp.* Due to incorporation of malt and rice bran extracts in agar. The stimulatory substances suspected to be present in the culture media were amino acids, vitamins, and essential nutrients, which combined to influence the growth of mycelia on these culture media. This view is also supported by Kadiri and Kehinde (1990) who investigated the effect of amino acids and aspartic acids and their corresponding keto acids and malt amides on mycelia growth of *Tricholoma species* and found all the compounds to accelerate mycelia growth.

## CONCLUSION

Malt Extract Agar (MEA), Corn cob Extract Agar (CCEA) and cassava peelings Extract Agar (CPEA) media were found to stimulate luxuriant mycelial growth rate and extension whereas poor mycelial growth were recorded on potato Dextrose Agar and plantain peelings Extract Agar media.

Materials and methods or processes adopted for production of excellent quantity and quality of active mycelia should be encouraged as it can said to be the power house for the sustainable development of the mushroom industry all over the world. The use of organic waste materials for the production of active mycelia (spawn) is a good development as it could provide a new approach in waste management. The spent substrate can also be reintegrated into farmlands as organic fertilizer to supplement or replace chemical fertilizers which are scarce and expensive among rural farmers in Nigeria.

Combination or supplementation therapy may be adopted to improve on the results obtained on PDA and PPEA.

## REFERENCES

Chang, S.T., Hayes, W.A. (1978) Nutrition, substrate and principles of culture media. In biology and cultivation of edible mushrooms, Ed, S.T., Chang and W.A. Hyes. Pp219-237 Academic Press, New York.

Edwards, R.L. (1977) A look at mushroom growing in France and Italy. *Mushroom J.*, 49:11-14

Jenison, W.M. (1948) The growth of wood-rotting fungi in submerged culture. *Am. J. Bot.*, 35: 801-804.

Kadiri, M. (1998) Spawn and fruit body production of *Pleurotus sajor-caju* in Abeokuta , Nigeria, *Niger. J. Bot* 11: 125 -131.

Kadiri, M. and I.A. Kehinde (1990) Production of grain mother and planting spawns of *Lentinus suhnudus*. *Nigerian J. Rot.* 12: 37 - 44.

Kaul, T.N., 1981. *Cultural Studies on Movelis. Mushroom Sci.* xi, 781 -788.

Lelley, J. (1982) The economic importance of macromycetes; the actual situation and different liquid media. *Orissa J. Agric. Res.*, 7: 66-68.

Nasim, G; Malik, S.H; Bajwa; R; Afzal, M and Mianm S.W (2001) Effect of three different culture media on mycelial growth of oyster and Chinese mushrooms, *Online Journal of Biological Sciences.* 1(12), 1130 -1133.

Qei. P. (1992) *Manual on Mushroom Cultivation Tool Foundation Amsterdam*, pp: 42-50.

Quimio, T.H. (1981) Philippine *Auricularia* taxonomy, nutrition and cultivation. *Mushroom Sci.* XI, 685-696.

Robinson, R.F. and R.S. Davidson (1959) The large scale growth of higher fungi. *Advance Applications of Microbiology.* 1:261-265.

Spore, C.T.A. (2006) Information for agricultural development in ACP Countries. Issue 124 (August).

Stanley, H.O. (2010) Effect of Substrates of Spawn production on mycelial growth of Oyster mushroom species, *Agriculture and Biology Journal of North America* 1(5): 817-820.