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Optimization of Growth of Two Wild Species of Pycnoporus Collected from Foothill of Uttarakhand

Vasundhra Sharma* **and A.K. Jaitly**
Microbiology Laboratory, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University Bareilly, Uttar Pradesh, India.
*Corresponding author email id: researchdhara@gmail.com

Abstract **– Optimum culture condition for mycelia production of wild *Pycnoporus sanguineus* and *Pycnoporus cinnabarinus* has been investigated with respect to the culture media and incubation temperature. Four media used were Potato Dextrose Agar, Czapek-Dox Agar, Malt Extract Agar and Yeast powder Starch soluble Agar. The average radial growth rate of mycelium ranged between 0.49cm/day and 0.22cm/day with the highest radial growth obtained for *Pycnoporus sanguineus* on Potato Dextrose Agar. Mycelia grow minimal on Czapek- Dox Agar as 3.4cm for *Pycnoporus sanguineus* and 4.8cm for *Pycnoporus cinnabarinus*. Maximum mycelia radial growth rate 0.49cm/day was recorded for *Pycnoporus cinnabarinus* at 25±2^{0}C. In conclusion the data obtained in this study provides useful information for further metabolic investigation of the two tested species of mushroom.**

Keywords **– Culture Media, Growth Rate, Mushroom, Mycelia, Temperature.**

I. INTRODUCTION

Macro-fungi with fleshy, sub fleshy or sometimes leathery fructifications, bearing their spore producing surface either on lamellae (gills) or lining the tubes, opening out by means of pores falls under the category of mushroom [1]. Mushroom are seasonal fungi, which occupy diverse niches in nature in the forest ecosystem. Mushroom has been recorded as a source of food and medicines for human beings all through the world from the times of history. Mushroom have squandered by man with tenacity probably for their weave and pleasing flavor [2]. They have been informally categorized among the "white vegetables", which have recently been recognized as a "forgotten source of nutrients" [3]. Mushroom fruiting bodies became important as nutraceutical and pharmaceutical agent due to the adequacy of producing high protein content with essential amino acids, vitamins, minerals, and exopolysaccharides with low cholesterol [4]. Many medicinal properties have been described from mushroom, as immunomodulatory, hepatoprotective, anticeceptive, antidiabetic, antiviral, antitumor etc. [5]. Moreover mushroom possess preventive role against human lifestyle related diseases such as hyperlipidemea and diabetes [6]. For this multifarious savvy mushroom have become more and more influential foodstuffs. In India the total recorded mushroom are approximately 850 species [7]. On the other hand less than twenty five mushroom species are widely exploited as edible and medicinal Mushroom; even few have attained commercial status[8]. Mushroom have chosen for this study because they comprise a vast and yet largely unbroached source of potential new pharmaceuticals having beleaguered history with gaps and punctuations.

Pycnoporus is a cosmopolitan genus of fungi belonging to phylum Basidiomycota, class Agaricomycetes and Polyporaceae order [9]. It is constituted by nine species reported till date [10] among which four are the most conformable, *Pycnoporus sanguineus* (L. Fr.) Murr., *P. cinnabarinus* (Jacq. Fr.) Karst, *P. puniceus* (Fr.) Ryv., and *P. coccineus* (Fr.) Bondartsev and Singer (1980). The genus *Pycnoporus* is characterized by an orange- red color on the surface, pileus and pores. This color arises from the synthesis of various pigments such as cinnabarin, cinnabarinic acid and 194 tramesanguin and acts as free radical scavengers, antifungal and antioxidants [11]. The pigments of filamentous fungi can be an alternative source as additive or color enhancer instead of those from chemical or synthetic dyes in industries like food, pharmaceutical and cosmetic.

Though, *Pycnoporus* genus is demonstrated as potential source of natural bioactive compounds, large scale production is the major constraints in order to fulfill the huge requirement of bioactive materials. Mycelium growth is the best tool to identify necessary components for the production of fruiting bodies as mycelium growth required short time in comparison with fruiting body development. The mycelial growth depends on several factors such as growth media, pH, temperature, nutrient element and some environmental factors [12]. Mycelium is an important part for mushroom production as well as for production of extracellular and / or intracellular bioactive compounds useful for formulation of nutraceutical and pharmaceuticals [13]. Mycelial biomass powder can be used to formulate various types of supplement tablets and capsules. Therefore, present study aimed to investigate the behavior of two collected and identified species of genus *Pycnoporus* i.e. *Pycnoporus sanguineus (M22)* and *Pycnoporus cinnabarinus (M20)* on different media and temperature for mycelial growth.

II. MATERIALS AND METHODS

A. Collection and Identification of Fruiting Bodies

Through several visits mushroom fruiting bodies were collected from different sites of the foothill forest zone of Uttarakhand state of India during April-August 2016. During the field survey, the macro fungi samples were collected with great care to avoid the damage to the base and other parts of the samples. Macroscopic details such as shape, size, color, color change on bruising or ageing, odors, spore deposition and fresh specimens and ecological characteristics of the sample were recorded and sample were photographed in their natural habitats.

Specimens were kept in the separate paper bags to avoid mixing and were taken to the laboratory. All specimens were brought to Microbiology Laboratory for identification and further studies. Out of all collected specimen two species were identified as of *Pycnoporus* genus on the basis of morphological and microscopic characteristics as summarized by [14]-[15]. The mycelial isolation was carried out on potato dextrose yeast agar by tissue culture at a temperature of 32^{0}C for seven days and the isolate was maintained in the same medium (slant) and stored at 4^{0}C. The slants were reactivated every periodically.

B. Optimization Experiments

Two experiments were conducted on the mycelial growth of identified mushroom species *Pycnoporus cinnabarinus* and *Pycnoporus sanguineus*. The first experiment was performed to evaluate the effect of different agar media and the second was performed to investigate effects of temperature on mycelial growth. Both the experiments were carried out under aseptic conditions at the Microbiology Laboratory. The experiments were laid out in a design with three replications in each treatment.

C. Experiment Number 1- Culture Media

Four different culture media were prepared and screened i.e. PDA (P), YPSSA (Y), MEA (M), and Czapek-Dox Agar (C) to identify the best culture media for the growth of wild *Pycnoporus cinnabarinus* and *Pycnoporus sanguineus*. Basal component of each medium was prepared mixing 20g of dextrose and 20g of agar and neutral pH was maintained. The mixture was boiled on until the agar dissolved completely and sterilized in an autoclave for 20 minutes under 15lbps. After cooling at room temperature 20ml of the media was poured into Petri dishes (150 mm) in a laminar hood under aseptic conditions. After solidification, the plates were inoculated in centre with the 5mm plug inoculums of *Pycnoporus cinnabarinus* and *Pycnoporus sanguineus* from pure slants. Plates were kept at 25±2^{0}C in an incubator for mycelium growth which was observed periodically.

D. Radial Growth Rate

Mycelial growth was determined by using a ruler across the plate and calculated the average of the vertical and horizontal colony radius. The radial growth was measured daily at every 24 hours, with a cent metric ruler. The measure being held three measures for each plate.

A. Average Growth Rate was measured using the following formula:

$$\text{Radial growth rate} = \frac{\text{maximum radius of mycelial colony } (cm)}{\text{total incubation time } (days)}$$

Mycelial Density

The mycelial density was also determined [16].

Experiment Number 2: Temperature

To screen the temperature values necessary for favorable growth of *Pycnoporus cinnabarinus* and *Pycnoporus sanguineus* the fungus was incubated for 15 days at four different temperatures. A 5 mm diameter plug of inoculums was removed with a cork borer from 5 day old pure culture, placed in the center of PDA plate at neutral pH, and incubated in the dark for stated days at 20, 25, 30 and 35ºC. Mycelial growth was measured by a cent metric ruler. After 15 days of incubation average radial growth rate and mycelia density of both *Pycnoporus* species were measured.

III. Results

On the basis of macroscopic and microscopic analysis specimen collected (Table I.) were identified as of *Pycnoporus* genus (Fig. 1.) were used for further study.

Table I. Source of the wild Pycnoporus species

Specimen Code	Source	Identification
M22*	Lalkuan	***Pycnoporus sanguineus***
M20*	Golabairaj	***Pycnoporus cinnabarinus***

Fig. 1. Collected Pycnoporus species

The Effect of Culture Media on the Mycelial Growth

The result (considering *Table II.)* on the 15th day showed that *P. sanguineus* had the highest mycelial colony radius 7.4cm on PDA media followed by MEA media with 6.9cm colony radius similarly *P. cinnabarinus* showed highest mycelia colony radius 7cm on PDA with a slight lesser mycelial growth 6.8cm being the colony radius on MEA. On YPSSA media *P. sanguineus* colony radius was 6.3cm and for *P. cinnabarinus* it was 5.5cm. The least growth was recorded on Czapek-Dox Agar media for both *P. sanguineus* and *P. cinnabarinus,* 5.9cm and 4.6cm being the colony radius (Fig. 2.1 & 2.2). May be the nutrients present in the potato agar rapidly gave the luxuriant growth with typical morphology and pigmentation.

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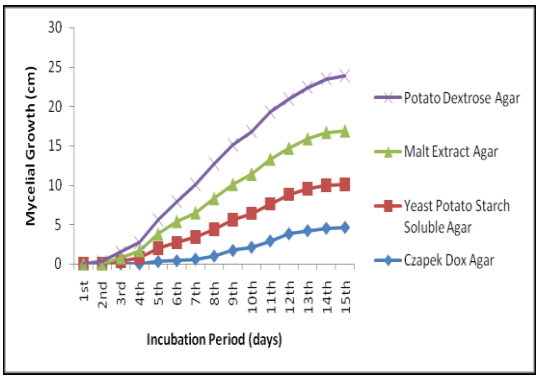

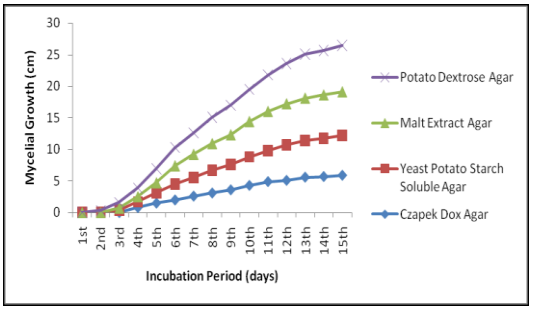

Fig. 2.1. & 2.2. Mycelial growth of *Pycnoporus cinnabarinus* and *Pycnoporus sanguineus* at different culture media

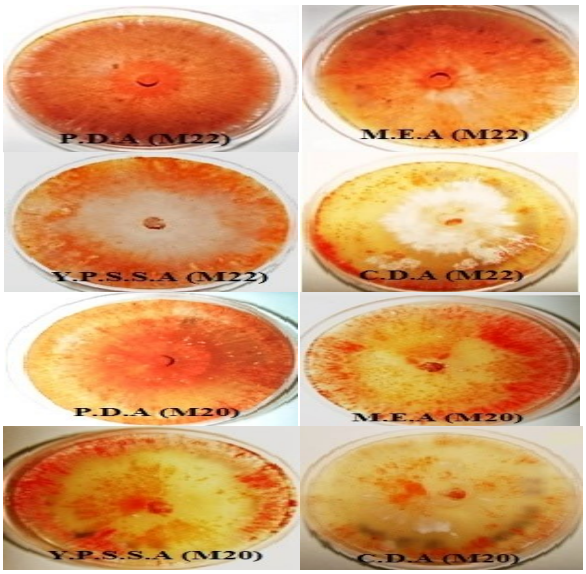

Fig. 3. Mycelial growth in different culture media

Effect of Temperature on the mycelial growth

Among different temperature levels *P. cinnabarinus* showed significant variation of mycelia growth slight higher as compared to *P. sanguineus*. The *sanguineus* spp. showed their highest mycelium growth at 25°C±2 (*Fig.3*). The fastest mycelial radial growth (7.4cm) was exhibited by *P. cinnabarinus* at 25^{0}C±2 followed by 7.2cm at 30^{0}C±2 (*Fig.4*). While the slowest mycelial growth was observed in *P. sanguineus* which was about 3.4cm on day-15 at 20±2°C in dark. However, at 35°C mycelium growth was negligible in both of the *Pycnoporus* species.

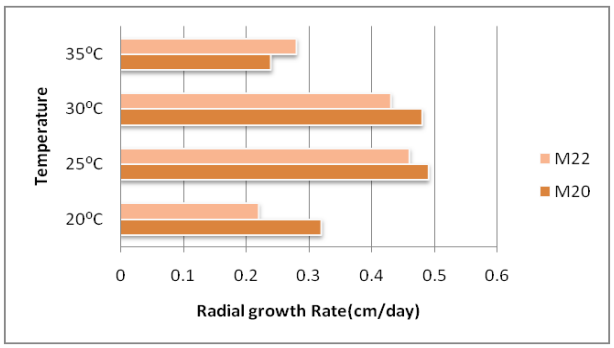

Fig. 4. Comparative Radial growth rate of *P. sanguineus* (M22) and *P. cinnabarinus* (M20) at different temperature levels

IV. Discussion

Fungi decompose the organic waste in order to extract useful nutrient for their growth and development with help of enzymes [17]. The ligno-cellulolytic activities of mushroom converts the complex organic substrate into fermentable substrate which ultimately result into production of bioactive materials [18]. Mushrooms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. Growth and activity of fungi including mushroom are affected by the substratum and also environmental condition in which they grow. During present investigation different media and temperature has been used to observe mycelial growth of *Pycnoporus sanguineus* and *Pycnoporus cinnabarinus* collected from Foothill forests of Uttarakhand.

Significantly the highest mycelial radial growth (7.4cm) of *Pycnoporus sanguineus* was observed in Potato Dextrose Agar which was statistically similar (6.9cm) to Malt extract agar media and the lowest mycelial radial growth (4.6cm) of *Pycnoporus cinnabarinus* was found on Czapek-Dox Agar medium. These are similar to [19] with pink oyster mushroom. Among different growing agar media used in this investigation i.e. PDA, MEA, and YPSSA, Czapek-Dox agar; PDA proved to be the best media (0.49cm/day) for the mycelial growth of both *Pycnoporus* species. This medium has been demonstrated to be highly supportive for the mycelial growth of mushroom by several authors [20]. This difference of mycelial growth on different agar media may be due to availability of different carbon sources and other required nutrients. PDA might exhibit higher nutrient sources for mushroom mycelia in Petri plate.

The comparative effect of mycelial growth rate at different temperatures was given in results (*fig.4*). The favorable temperature was 25-30ºC for growth of both wild *Pycnoporus* species. This result is supported by [21] they stated that the favorable temperature of mycelial growth was 20^{0}C-31^{0}C for *Pleurotus flabellatus and concluded* 25^0*C* to be the optimum or the hyphal growth. The range of 20^{0}C-31^{0}C of temperature as optimum temperature was reported by many researchers for several mushroom [22]-[23].

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V. Conclusion

Since mushroom are good source of bioactive compounds of anticancer, antifungal and anti-diabetic in nature, the mycelia may be used for the large scale production of the compounds as mushroom are seasonal. To make the bioactive production technology cost effective, present study may be useful in order to obtain more biomass ultimately to have bioactive compounds in hand. Further standardization regarding quantification of substrate as nutritional source for biomass production and its cost economics is required to reach more constructive conclusion.

Appendix

Table II. Radial Mycelial Growth of *Pycnoporus cinnabarinus* and *Pycnoporus sanguineus* in Different Medium

Media	Mushroom	Days																Mycelial Density
		1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th		
C	M20	0	0	0	0	0.3	0.4	0.6	1	1.7	2.1	2.9	3.8	4.2	4.5	4.6	+	
	M22	0	0	0	0.8	1.5	2	2.6	3.1	3.6	4.3	4.9	5.1	5.6	5.7	5.9	2+	
Y	M20	0	0	0.3	0.8	1.7	2.3	2.8	3.4	3.9	4.3	4.7	5	5.3	5.5	5.5	2+	
	M22	0	0	0.4	0.9	1.6	2.5	2.9	3.6	4	4.5	4.9	5.6	5.8	6	6.3	3+	
M	M20	0	0	0.5	0.9	1.8	2.7	3.1	3.9	4.5	5	5.7	5.9	6.4	6.7	6.8	4+	
	M22	0	0	0.3	0.8	1.6	2.9	3.7	4.2	4.7	5.6	6.2	6.5	6.7	6.9	6.9	4+	
P	M20	0	0.3	0.7	1	1.8	2.5	3.6	4.4	5	5.4	6	6.2	6.5	6.8	7	5+	
	M22	0	0.3	0.9	1.4	2.2	2.9	3.4	4.2	4.7	5.1	5.8	6.4	7	7.1	7.4	5+	

*C=Czapek-Dox Agar, Y=Yeast Potato Dextrose Agar, M=Malt Extract Agar, P=Potato Dextrose Agar
*+ very scanty, 2+ scanty, 3+ moderate, 4+ abundant, 5+ very abundant

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Authors' Profiles

Vasundhra Sharma, 07/10/1989
Ph.D (microbiology) M.J.P.R University, Bareilly, UP, India, 2016- contd.
She Research Scholar Microbiology Laboratory, Dept. of Plant Science, M.J.P.R. University, Bareilly, U.P., India
Mycology and Food Technology, Mrs. Sharma

Dr. Ajai Kumar Jaitly, 22/10/1958
Ph.D (microbiology) Lucknow Univ., Lucknow, UP, India,1983
He **Head** Dept. of Plant Sc., M.J.P.R. Univ., Bareilly, U.P., India, 2014-2017
Microbiology, Dr. Jaitly
Biotech. Overseas training Award 1992
UGC National Associate ship 1996