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Original Research Article

Optimization of Mycelia Growth Parameters for *Pleurotus florida* and *Pleurotus sajor-caju*

Santosh Kumar[1*], Amarendra Kumar[1], Gireesh Chand[1], Md. Nadeem Akhtar[2] and Tribhuwan Kumar[3]

[1]Department of Plant Pathology, Bihar Agricultural University, Sabour, Bhagalpur-813210, Bihar, India
[2]Krishi Vigyan Kendra, Agwanpur, Saharsa, Bihar Agricultural University, Sabour, India
[3]Department of Molecular Biology and Genetic Engineering (Bio-Technology), Bihar Agricultural University, Sabour, Bihar, India
**Corresponding author*

A B S T R A C T

Effects of different culture media, temperature and carbon & nitrogen source were optimised for maximum radial mycelial growth in both species i.e. *Pleurotus florida* and *P. sajor-caju.* Wheat extrct agar was the most ideal culture media for mycelial growth followed by Potato dextrose agar and malt extract agar. *P. florida* showed maximum growth (63.68 mm) at temperature 22.5°C and next at 25°C (60.15 mm). 27.50°C (63.04 mm) was found most optimum temperature for the growth of *P. sajor-caju* followed by 30°C (57.46 mm). Minimum radial mycelial growth of both mushroom species has been observed on 15°C. Fructose was the best carbon source for enhancing radial mycelial growth of *P. florida*, while starch for *P. sajor-caju*. This study is very useful for the pure culture and mother spawn production to be utilised in the commercial cultivation and higher production of oyster mushroom.

Keywords

Pleurotus spp., culture media, temperature, carbon and nitrogen source, mycelial growth

Introduction

Mushrooms are highly nutritious and are important features of human diet worldwide. Their nutritional value relies on relatively high protein, minerals, vitamins and essential amino acid content. Moreover, edible mushroom have low calories. *Pleurotus* is an efficient ligno-cellulose decomposing species of white rot fungi. "Oyster mushroom" is a common name which refers to several species of edible mushroom pertaining to the genus *Pleurotus*. This mushroom cultivation is an economic agribusiness due to being edible and its excellent flavour and delicious taste (Shah *et al.,* 2004). The cultivation of oyster mushrooms has come of age around the globe, because of their nature to grow at a wide range of temperatures and cheap ligno–cellulosic substrates (Khan and Garcha, 1984). Mushroom myceliums grow on natural or semi- synthetic composts and absorb nutrients for their survival. The mycelium branches produce enzymes which digest complex carbohydrate, lipids and protein, which are further easily absorbed by their hyphae. The mycelium penetrates the compost during spawn run stage and store energy until fruiting bodies are formed. The

maintenance and production of a reliable pure culture mycelium with magnificent qualities is a key operation and is the first critical stage towards the success of spawn production and mushroom cultivation. Physico-chemical parameters for the most efficient mycelial colonization of different edible mushrooms need to be elucidated to develop a data base for mushroom germplasm repositories.

Present study is aimed at optimizing, parameters such as nutrient media, temperature range and carbon and nitrogen sources for mycelial growth of *Pleurotus* species.

Materials and Methods

Pure mycelial culture of two *Pleurotus* spp. *viz. P. florida*, *P. sajor- caju*, were taken to optimise the effect of different culture media, temperature and carbon and nitrogen sources in nutrient media for radial mycelial growth. The culture was maintained on PDA for further studies.

Effect of culture media and temperature on mycelial growth

Six culture media were used in this study i.e. wheat extract agar, potato dextrose agar, malt extract agar, Hawkers, Richards and Czpack dox. For radial mycelial growth on culture media, Petri dishes containing 20 ml of sterilized medium were inoculated with 5 mm disc of pure culture of studied mushroom species with initial pH adjusted to seven and incubated at 25^{o}C.

For the study of effect of temperature on radial mycelial growth, 5 mm discs of each test mushroom was inoculated in the sterilized Pertri dishes containing PDA and incubated at different temperatures *viz,* 15, 17.5, 20, 22.5, 25, 27.5 and 30^{o}C.

Effect of carbon and nitrogen on mycelial growth

Potato dextrose agar medium was used as the basal medium for the study of carbon and nitrogen sources. Eight carbon sources namely glucose, dextrose, fructose, maltose, manitol, lactose, sucrose and starch and eight nitrogen sources namely sodium nitrate, ammonium nitrate, potassium nitrate, ammonium phosphate, ammonium ferrous sulphate, ammonium chloride, glycine and proline were taken to optimized the growth of *Pleurotus* spp. Quantity of individual substance in the basal medium was calculated, and the quantity equivalent to that was singly substituted in the basal medium by replacing the corresponding original one. All data were analyzed by one-way analysis of variance (ANOVA) and conducted in completely randomized design (CRD) with three replications. The observations were recorded as number of days to complete 90 mm radial growth of *P. florida*, *P. sajor- caju*.

Results and Discussion

Five different culture media, seven temperature and eight carbon and nitrogen sources were optimized for the radial mycelial growth of *P. florida*, *P. sajor- caju.* Among them the results on the effect of different culture media on radial mycelial growth of *P. florida*, *P. sajor-caju* are presented in table 1. Maximum average mycelial growth of *P. florida* was observed on wheat extract agar *(*65.36 mm) after six days followed by potato dextrose agar (56.01 mm) and malt extract agar (56.01 mm). Similarly maximum average mycelia growth (61.97mm) of P. *sajor-caju* was observed on wheat extract agar followed by potato dextrose agar (50.13 mm) and malt extract agar (37.75 mm). While minimum mycelial growth of both species of

mushroom were observed on Richards. This finding is near to the finding of Furlan *et al.*, (1997), who found that wheat extract agar as a better culture medium for *A. bitorquis* than PDA. Singh *et al.,* (2000) has also observed wheat extract agar medium as the best substrate for the radial mycelial growth of different *Pleurotus* spp. However, in one of the study carried out by Suharban and Nair (1991), the mycelial growth rate of *Pleurotus* species on different culture media is influenced by agar based media. The mycelial growth of the *P. florida* and *P. sajor- caju* were also optimised on seven different temperature as presented in table 2. *P. florida* showed maximum growth (63.68 mm) at temperature 22.5^{o}C followed by 25^{o}C, which was 60.15 mm while *P. sajor-caju showed* maximum growth (63.04 mm) at temperature 27.50^{o}C followed by 30^{o}C which was 57.46 mm. Minimum radial mycelial growth of both mushroom species was observed on 15^{o}C. Bhatt *et al.*, 2008 who reported that hybrids of *Pleurotus* and parental species exhibited maximum growth at 25^{o}C, slowest at 15^{o}C. This may be due to genomic effect of other species in the hybrid.

Table.1 Effect of different culture media on mycelial growth of *Pleurotus florida* and *Pleurotus sajor-caju* mushroom fungus at 25^{0} C

Medium	Radial Mycelium growth (mm)*			
	Pleurotus florida		***Pleurotus Sajor-caju***	
	3 days	**6 days**	**3 days**	**6 days**
Wheat Extract Agar	25.51	65.36	23.68	61.97
Potato Dextrose Agar	22.72	56.01	21.03	50.13
Malt Extract Agar	16.41	39.17	13.07	37.75
Hawkers	02.99	04.55	02.70	03.68
Richards	01.64	02.35	01.28	02.20
Czpack dox	6.73	13.38	10.38	16.14
CD (P=0.05)	1.60	2.00	0.91	02.07
CV (%)	7.061	3.702	4.22	4.02

*Mean of three replications

Table.2 Effect of Temperature on radial mycelial growth of *Pleurotus florida* and *Pleurotus sajor-caju* after 7 days

Temperature	Radial Mycelium growth (mm)*	
	Pleurotus florida	***Pleurotus sajor-caju***
15.0	30.71	20.71
17.5	47.52	35.32
20.0	49.25	46.33
22.5	63.68	45.56
25.0	60.15	33.89
27.5	57.77	63.04
30.0	53.19	57.46
CD (P=0.05)	3.58	3.45
CV (%)	3.91	4.52

*Mean of three replications

Table.3 Effect of carbon sources on radial mycelial growth of *Pleurotus florida* and *Pleurotus sajor-caju* after 7 days

Carbon sources	Radial Mycelium growth (mm)*	
	Pleurotus florida	***Pleurotus sajor-caju***
Glucose	43.95	54.45
Dextrose	42.94	51.80
Fructose	74.18	69.95
Maltose	62.23	61.22
Manitol	63.37	67.66
Lactose	19.85	19.45
Sucrose	63.93	70.20
Starch	69.07	73.61
CD (P=0.05)	2.24	2.48
CV (%)	2.33	2.43

*Mean of three replications

Table.4 Effect of nitrogen sources on radial mycelial growth of *Pleurotus florida* and *Pleurotus sajor-caju* after 7 days

Carbon sources	Radial Mycelium growth (mm)*	
	Pleurotus florida	***Pleurotus sajor-caju***
Sodium Nitrate	39.71	47.09
Ammonium nitrate (II)	64.37	60.86
Potassium nitrate	53.34	40.49
Ammonium phosphate	57.45	52.77
Ammonium ferrous sulphate	11.82	11.10
Ammonium chloride (I)	67.40	70.08
Glycine	45.51	41.59
Proline	25.52	18.06
CD (P=0.05)	3.17	3.78
CV (%)	3.97	3.43

*Mean of three replications

However Moorthy (1993) has found that 25 to 28˚C were found to be the optimum for *P. sajor-caju* in *in vitro* studies. Optimum temperature range for the growth of different *Pleurotus* species *viz*., *P. sajor-caju, P. ostreatus, P. florida, P. flabellatus, P. sapidus, P. membranaceous, P. cystidiosus, P. abalones* and *P. eryngii* has also been optimised and is reported at 25-30^{o}C (Sohi and Upadhyay, 1989 and Bugaraski *et al.*, 2002). Similarly a number of researchers have also reported that 25^{o}C temperature is the best for radial mycelial growth of *Pleurotus* spp. (Yadav, 2001; Pandey *et al.*, 2003 and Joshi, 2004). But in case of *P. florida* maximum growth 63.68 mm at temperature 22.5^{o}C refers that this one is ideal species for the growth at lower temperature in cold season.

Results on the effect of different carbon and nitrogen sources on radial mycelial growth of *P. florida, P. sajor-caju* after seven days are presented in table 3 and 4 respectivelly.

Fructose (74.18 mm) was found to be the best carbon source for *Pleurotus florida* followed by starch and sucrose, which was 25. 52 mm and 45.51mm respectively, while in case of *P. sajor- caju,* starch support medium showed better growth i.e. 73.61 mm followed by sucrose and fructose, which was 70.20 mm and 69.95 respectively. Glucose has also been found to be the best carbon source for *Pleurotus ostreatus* followed by maltose and starch (Nwokoye *et al.,* 2006). We are of the opinion that isomer and polymer of glucose are suitable carbon source for the mycelia growth of fungal species. Ammonium chloride was the most suitable nitrogen source for *P. florida* and *P. sajor- caju* which was 67.40 mm and 70.08 mm respectively. The second best nitrogen source was ammonium nitrate for *P. florida* and *P. sajor- caju* which was 64.37 mm and 60.86 mm respectively. Ammonium is the most suitable nitrogen source for most of edible mushroom fungi (Rangel-Castro *et al.,* 2002). The effect of carbon and nitrogen source on the mycelia growth depends on species, culture media and conditions (Lin, Yang, 2006). Therefore, it can be deduced that reduced form of nitrogen is more suitable for the mycelia growth.

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