Effect of pH on the growth and protein content of dikaryon and fusant protoplast mycelium of *Pleurotus sajor-caju* at different incubation periods under submerged condition

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J. Mycopathol. Res. 60(4) : *635-639, 2022;*
ISSN 0971-3719

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J. Mycopathol. Res. **60(4)** : 635-639, 2022; (ISSN 0971-3719)
doi:10.57023/JMycR.60.4.2022.635

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Received : 17.10.2022 Accepted : 18.11.2022 Published : 26.12.2022

In this study the effect of pH on the growth and protein content of dikaryonand fusant protoplast mycelium of *Pleurotussajor-caju* at different incubation periods under submerged condition was determined sequentially. The respective mycelia were grown separately at 5, 10, 15 and 20 days of incubation period. All those incubation periods were studied at pH 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5. The dry weight and protein content of the respective mycelia were determined. The data revealed that the pH 5.5 at 15 days incubation period was the best for the growth of both dikaryon and fusant protoplast mycelia as well as the yield of protein. pH 5.5 also showed best result at different incubation periods of different mycelium.

Key words: Dikaryon, fusant protoplast, mycelia, *Pleurotus sajor-caju*, protein

INTRODUCTION

With the advent of rapid industrialization and urbanization, the human civilization has been experiencing drastic population growth that has led the situation to food scarcity, malnutrition and undernourishment. Especially the under developed and developing countries have been suffering from this food problem severely. Low protein content in the diet is a serious issue in India. Animal protein, due to its high market price, is beyond reach to a large sector of population living below poverty level. Even, some religious factors are there which prohibit a significant population to intake animal protein. In this situation, mushrooms are good protein supplement to the economically weaker section as well as the people with vegan diets Mushrooms are high source of protein diet and have proven to be boon to human health and nutrition (Barman *et.al.*2018). Besides, mushrooms have enormous medicinal value due to the presence of several biologically active compounds including polysaccharide, tri-terpenoids, lentinan, adenosine. These compounds exert medicinal influences on their uses. The consumption of mushrooms in India and all over the world has been a practice since the centuries. The tribal people and people of hill regions collects different types of mushrooms from the wild habitat with the help of their traditional knowledge, but, sometimes this leads to the death due to consumption of non-edible poisonous species.

Scientific cultivation of mushroom individually and industrially, by utilizing organic wastes is an easy process for any person with a little demonstration and this process is quite safe and secure for consuming edible ones. Mushroom farming has now become one of the most proven income generating enterprise in different parts of the country to double the farmers income within a year (Thakur and Singh, 2020). Moreover, it can counteract the malnutrition, low protein diet and provide economic sustainability. Out of many edible cultivated species in India, *Pleurotus sajor-caju* is cultivated extensively and commercially right now. Attempts have also been made to utilize pruned

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tea leaves for cultivation of *P. sajor-caju* in North Bengal (Roy and Chakraborty, 2018).

Different parameters affect the cultivation of mushrooms. pH is one of the major parameters that affect the growth of the mycelium and protein content in the mushrooms. Different studies show that *Pleurotus* sp. is acid loving and the maximum Yield can be received within the range of pH 5.0-6.0. (Berne *et al*. 2005; Nwoko *et al*.2022; Sardar *et al*. 2015). In the present study, the effect of different pH on the growth of two types of mycelia (dikaryon and fusant protoplast) of *Pleurotus sajor-caju* has been determined.

MATERIALS AND METHODS

Fresh and healthy basidiocarps of *Pleurotus sajor-caju* were collected from the natural habitat of Calcutta and its adjoining area of West Bengal.

Pileus tissue culture was prepared by transferring a small portion of tissue on PDA slants. The composition of PDA medium was- potato (peeled) - 400 gm, dextrose – 20 gm, agar -25 gm and distilled water – 1000 ml.The medium thus prepared was transferred to the culture tube in 15 ml amount, properly plugged and sterilized in autoclave at 121^{0}C for 20 minutes.The surface of the basidiocarps were cleaned with sterilized distilled water and 0.1% mercuric chloride solution and rinsed with sterile distilled water. Then a small portion of tissue from the junction of stipe and pileus was transferred aseptically into a PDA slant and incubated for 7 days at 25^{0}C. By this way, by repeated sub-culturing, the pure tissue culture of the fungus was prepared.

The slant cultures were sub-cultured aseptically on PDA medium and incubated at 25^{0}C for 7 days and then stored in the refrigerator at 15^{0}C. Subculturing was made regularly at every 30 days intervals in order to keep these mycelia fresh and in growing condition. In order to conduct the experiment the mushroom culture was allowed to grow in Glucose- Asparagine medium. The composition of medium is Glucose – 10 gm., L-Asparagine -2 gm, KH_2PO_4 – 2 gm, $MgSO_4,7H_2O$ – 1 gm, $FeSO_4,7H_2O$ – 2 mg, $ZnSO_4,7H_2O$ – 2 mg, $MnSO_4,7H_2O$- 1 mg, Biotin – 5 ìg, Thiamine HCL – 100 ìg and distilled water -1000 ml. The pH of the medium was adjusted to pH 6.0.

A small portion of growing mycelium from the PDA slant of the fungus was aseptically transferred to 150 ml. Erlenmeyer flasks containing 30 ml of sterile Glucose-Asparagine liquid medium. The inoculated flasks were incubated for 7 days with continuous shaking (120 rpm) at 25ºC. After harvesting the filtration, the mycelia were washed with sterile distilled water to make them free of the medium and were fragmented into small pieces in a sterile waring blender. Then the fragmented mycelium was suspended in phosphate buffer medium (pH 6.0) for 24 h to overcome the shock encountered during blending. An aliquot of 1 ml of this cell suspension was used as inoculum for experimental studies. The mycelium obtained directly after isolation from the mushroom tissue was the source of dikaryon while fusant mycelium was obtained after protoplast fusion from the mother culture.

Glucose-Asparagine (GA) liquid medium was prepared and pH was adjusted to 6.0. The medium was distributed among several 150 ml. Erlenmeyer flasks containing 30 ml of medium. The flasks were sterilized in an autoclave at 121^{0}C for 20 minutes and inoculated with 1.0 ml of mycelia cell suspension of mushroom.

Several such flasks were inoculated in order to have three replications for each treatment.

After the required incubation period of each treatment three flasks of mushrooms were harvested by filtration through a tarred sintered funnel (IG -3, Jena). The mycelium was washed several times with distilled water to make it completely free adherent medium. The harvested mycelium was dried in a vacuum oven at 60^0 C for 24 h and cooled in desiccators and weighed. The dry weight of mycelium was taken as index of growth.

The total nitrogen content of the mycelium was determined by the method of Folin and Wu (1919). Crude protein was calculated by using a factor of 4.38 on the basis of 22.83% nitrogen content of mushroom protein (Crisans and Sanda, 1978).

To find out the effect of pH on the mycelia growth and yield of protein of the fungus, 30ml of GA basal liquid medium was poured into each Erlenmeyer flask (150 ml). These flasks were then sterilized at 121^{0}C for 20 min. The sterilized flasks were then inoculated with 1.0 ml of mycelia suspension inoculums of the fungus and incubated for 20 days at 26^0 C. The pH of the medium was adjusted to

different pH by buffering with phosphate and acetate buffer. The different pH grades adjusted were 5.0, 5.5, 6.0, 6.5, 7.0 and 7.4. The sterilized flasks of each grade were then inoculated separately with 1.0 ml of mycelia suspension of the fungus and incubated for 20 days at 25^0 C. Harvesting was done as 5,10,15 and 20 days. Every fifth day a set of flasks was harvested and mycelia growth and yield of protein were determined.

Sufficient numbers of flasks were inoculated in order to have three replications for each treatment.

RESULTS AND DISCUSSION

The data in Table 1 and Figs. 1 & 2 reveal that pH 5.5 at incubation period of 15 days was the best for the growth and yield of protein by the dikaryon mycelia of *Pleurotus sajor-caju* (90.20 mg/30ml and 26.42% respectively).

At 5 days incubation period, the maximum growth of the dikaryon mycelia was observed at pH 5.5 (38.20 mg/30ml) and the minimum at pH 7.5 (16.26 mg/ 30ml). At 5 days incubation period the yield of protein by the mycelia also showed similar trend as that of mycelial growth with a maximum at pH 6.0 (20.20%) and minimum at pH 7.5 (12.78%). The growth and protein contents of mycelia increased with the incubation days, peaking at 15 days and then showing a downward trend, with pH effects being same as that of 5 days. Maximum growth observed at 15 days incubation period at pH 5.5 was 90.20 mg/ 30ml and maximum protein content was also obtained at pH 5.5 (26.42%).

A study by Nwoko *et al.* (2022) on variability effect of pH on yield optimization and mycochemical compositions of *Pleurotus ostreatus* had shown the similar result where pH value was found effective in acidic value. The same findings had also been reported by Okwulehie *et al* (2018). Sardar *et al.*(2015) also reported that maximum mycelia growth had been exhibited at pH 6.0, while an earlier study by Yadav (2001) had concluded that *Pleurotus sajor-caju* could tolerate a wide range of pH (5.0-8.0) for their good mycelia growth.

Table 1: Data (average)[a] showing the effect of pH on the growth of dikaryon mycelium and yield of protein by the dikaryon mycelium of *Pleurotus sajor-caju* at different incubation periods under submerged conditions

pH	Incubation Periods							
	5 Days		10 Days		15 Days		20 Days	
	Dry wt. of mycelium (mg/30ml)	Protein Content (%)	Dry wt. of mycelium (mg/30ml)	Protein Content (%)	Dry wt. of mycelium (mg/30ml)	Protein Content (%)	Dry wt. of mycelium (mg/30ml)	Protein Content (%)
5	19.00 ± 2.10	18.16 ± 1.18	48.20 ± 2.60	20.42 ± 1.24	71.00 ± 2.86	23.60 ± 1.36	58.00 ± 2.62	22.20 ± 1.42
5.5	38.00 ± 2.26	21.08 ± 1.24	62.00 ± 2.52	23.42 ± 1.28	90.00 ± 4.20	26.60 ± 1.66	71.50 ± 2.60	25.4 ± 1.62
6	29.80 ± 2.32	19.32 ± 1.20	49.20 ± 2.48	21.10 ± 1.40	79.60 ± 3.96	23.12 ± 1.62	63.00 ± 2.74	21.8 ± 1.54
6.5	23.00 ± 2.40	16.36 ± 1.22	44.00 ± 2.54	18.88 ± 1.36	73.00 ± 3.48	20.42 ± 1.52	52.00 ± 2.80	19.48 ± 1.46
7	20.00 ± 2.10	15.80 ± 1.24	36.12 ± 2.48	17.12 ± 1.32	52.44 ± 2.96	18.32 ± 1.48	34.10 ± 2.22	17.42 ±1.38
7.5	16.60 ± 2.14	13.82 ± 1.18	30.40 ± 2.44	14.86 ± 1.26	48.00 ± 2.56	16.48 ± 1.36	28.00 ± 2.14	15.48 ± 1.32

[a] Data are average of three replicates.

The data in Table 2 and Figs. 3 & 4 reveal that the maximum growth and yield of protein by the fusant protoplasts mycelia of *Pleurotus sajor-caju* were obtained at pH 5.5 and 15 days of incubation period (90.00 mg/30ml and 26.60% respectively).At 5 days of incubation period, the maximum growth of

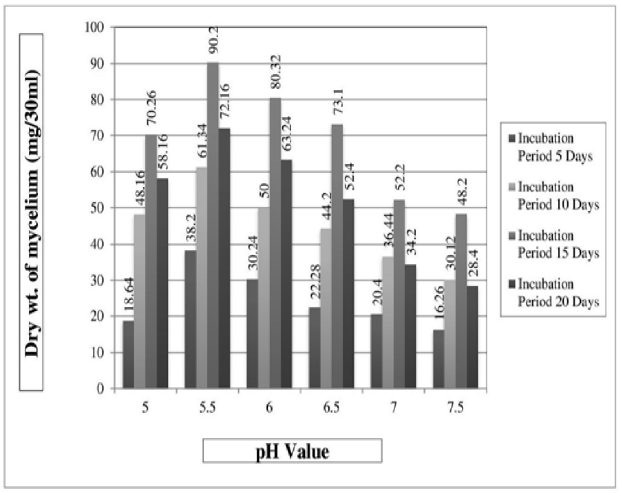

Fig. 1: Effect of pH and incubation period on the growth of dikaryon mycelium of *Pleurotus sajor-caju*

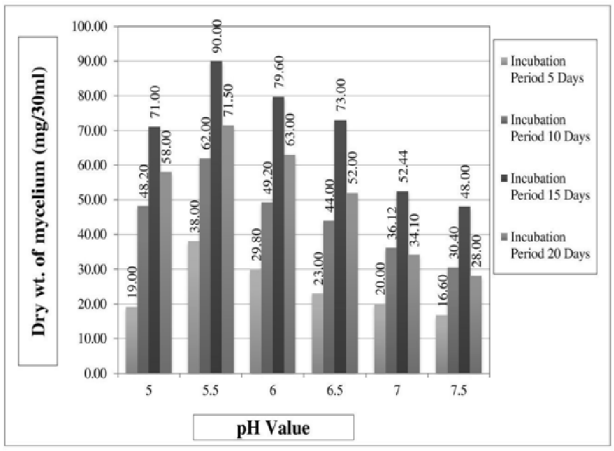

Fig. 3: Effect of pH and incubation period on the growth of fusant mycelium of *Pleurotus sajor-caju*

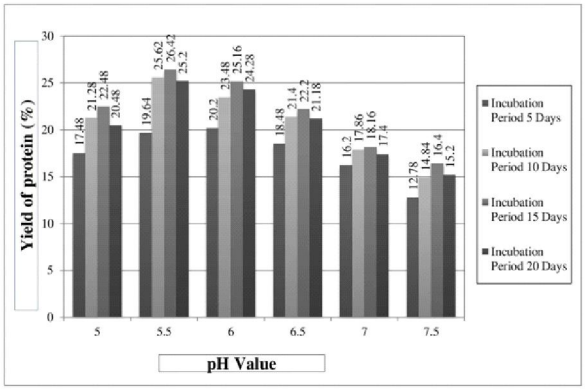

Fig. 2: Effect of pH and incubation period on the protein content of dikaryon mycelium of *Pleurotus sajor-caju*

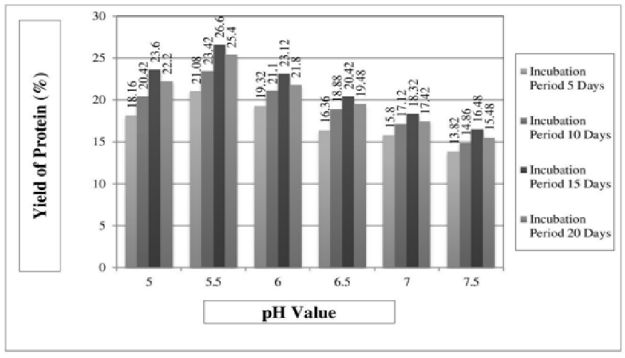

Fig. 4: Effect of pH and incubation period on the protein content of fusant mycelium of *Pleurotus sajor-caju*

Table 2: Data (average)[a] showing the effect of pH on the growth of fusant protoplast mycelium and yield of protein by the fusant protoplast mycelium of *Pleurotus sajor-caju* at different incubation periods under submerged condition

pH	Incubation Periods							
	5 Days		10 Days		15 Days		20 Days	
	Dry wt. of mycelium (mg/30ml)	Protein Content (%)	Dry wt. of mycelium (mg/30ml)	Protein Content (%)	Dry wt. of mycelium (mg/30ml)	Protein Content (%)	Dry wt. of mycelium (mg/30ml)	Protein Content (%)
5.0	19.00 ± 2.10	18.16 ±1.18	48.20 ± 2.60	20.42 ±1.24	71.00 ± 2.86	23.60 ± 1.36	58.00 ± 2.62	22.20 ± 1.42
5.5	38.00 ± 2.26	21.08 ±1.24	62.00 ± 2.52	23.42 ±1.28	90.00 ± 4.20	26.60 ±1.66	71.50 ± 2.60	25.40 ± 1.62
6.0	29.80 ± 2.32	19.32 ±1.20	49.20 ± 2.48	21.10 ±1.40	79.60 ± 3.96	23.12 ±1.62	63.00 ± 2.74	21.80 ± 1.54
6.5	23.00 ± 2.40	16.36 ± 1.22	44.00 ± 2.54	18.88 ±1.36	73.00 ± 3.48	20.42 ±1.52	52.00 ± 2.80	19.48 ± 1.46
7.0	20.00 ± 2.10	15.80 ±1.24	36.12 ± 2.48	17.12 ±1.32	52.44 ± 2.96	18.32 ±1.48	34.10 ± 2.22	17.42 ± 1.38
7.5	16.60 ± 2.14	13.82 ±1.18	30.40 ± 2.44	14.86 ±1.26	48.00 ± 2.56	16.48 ±1.36	28.00 ± 2.14	15.48 ± 1.32

[a] Data are average of three replicates.

mycelia was obtained at pH 5.5 (38.00 mg/30ml) and least at pH 7.5 (16.60 mg/ 30ml). The yield of protein also showed similar trend. At 15 days of incubation period also , the maximum mycelia growth was obtained at pH 5.5 and minimum at pH 7.5 (48.00 mg/30 ml). Protein content also showed similar pattern. Growth and protein content declined after 15 days. Interestingly, both dikaryon and fusant mycelia showed similar results.

Similar results has also been computed by Nwoko *et al* (2022). Aziz *et al*. (2018) also studied the growth of 3 species of *Pleurotus* in different media and different temperatures. In addition, preparation, fusion and regeneration of protoplast of another edible paddy straw mushroom – *Volvariellela volvacea* has been reported (Chatterjee and Samajpati, 2021).

CONCLUSION

Data received during the study revealed that pH 5.5 was the best for the growth of both the dikaryon mycelia and fusant protoplast mycelium at 15 days incubation period. At the same time, the yield of protein by both dikaryon mycelium and fusant protoplast mycelium was best at the pH 5.5 at 15 days incubation period. The overall better result had been found in the range of 10 days to 20 days incubation period at pH 5.5. The lowest growth and yield of protein had been observed at pH 7.5. That data could be utilized in commercial cultivation process of *Pleurotus sajor-caju* for better growth and yield of protein.

ACKNOWLEDGEMENT

Author is grateful to Professor N. Samajpati, Department of Botany, Ballygunge Science College, University of Calcutta for his valuable guidance and providing the lab facilities.

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