



Physiological Studies of Aquatic Fungi, Purana Ghat, Betwa River, Hamirpur (U.P.)

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ABSTRACT

*The present study was conducted in Betwa River along a stretch of 40 km from its origin to downstream of the river near old Betwa Ghat area to assess the presence of fungi in the river water. The study reveals the physiological growth of the aquatic fungi with respect to the pH and temperature. pH of all the stations ranged between 7.0 to 9.0, which fell under neutrally alkaline and constantly alkaline group. 6.5 was the optimum pH for growth and sporulation of *Pythium* sp. and *Saprolegnia* sp. but growth can occur at all pH treatment from 3.5 to 9.5 thus providing a wide pH range tolerance from highly acidic to highly alkaline. Temperature favour various growth activities of fungi. Moderate to poor vegetative growth for *Pythium* sp. ranges between 10°C to 30°C and for *Saprolegnia* sp. ranges from 22°C-27°C.*

Keywords: Betwa river, Growth, Sporulation, pH, Temperature.

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INTRODUCTION

The main line of investigation was to study the effect of pH and Temperature variation over the Growth and Sporulation of aquatic fungi *Pythium* sp. and *Saprolegnia* sp. from the water samples and phytoplanktons of purana ghat, Betwa river, Hamirpur, Uttar Pradesh.

The Hamirpur district falls in the humid sub-tropical zone. The climate of the district has four broad seasons. The winter generally spread over from December to February. The period from March to June is summer. Hot and rainy season generally extends from July to September. October and November exhibit autumn. Winter months are too cold. The maximum rainfall is observed in the district during July to August and minimum in the months of April and October. The hottest month is May and coldest month is January in the district. maximum and minimum temperatures recorded in the district ranges from 20° to 40°C. Normal rainfall in Hamirpur district is 851 mm. About 90 % of the rainfall occurs during the southwest monsoon, lasting from about June to September. The relative humidity ranges from 40 % to 90% in the area. The district situated in drainage basin of river Betwa and Ken which are two important right bank tributaries of river Yamuna. Hydrogeologically, the district falls in southern peninsular zone.

Aquatic hyphomycetes (also known as freshwater hyphomycetes, amphibious fungi or Ingoldian fungi) are a polyphyletic group of true fungi [1-2]. Their taxonomy and identification have traditionally been based on the morphology and development of asexually produced spores. There is no unambiguous definition of what makes a fungus an aquatic hyphomycete, but two crucial characteristics are the ability (1) to sporulate under water and (2) to thrive on deciduous leaves decaying in streams and rivers. Majority of aquatic hyphomycetes produce relatively large, multiradiate (often tetraradiate) or sigmoid spores [3-4], whose tips may be covered with sticky mucilage [5]. These properties facilitate the attachment of the spore to leaves and other smooth surfaces [6-8]. Two type of fungi has been identified, uniflagellate and biflagellate, under Uniflagellate fungi, the members of Rhizidiaceae, Phlyctidiaceae, Cladochytriaceae, Chytridiaceae, Blastocladiaceae and Megachytriaceae included whereas, under Biflagellate fungi members of Saprolegniaceae, Pythiaceae and Moniliaceae (Hyphomycetes) were isolated.

It has been found that pH and temperature are two important criteria for understanding the physiology of aquatic hyphomycetes. It has been found that growth of fungi affected by 'Hydrogen ion concentration'

(pH) variation in a medium in which aquatic hyphomycetes grows, either directly by its action on the cell surfaces or indirectly by its effect on the availability of nutrients.

MATERIAL AND METHODS

The two chytrids employed in this study, *Pythium sp.* (Sporangia filamentous, undifferentiated from the vegetative Hyphae without inflations. Sometimes Sporangia produced terminally with warty wall and *Saprolegnia sp.* (Hyphae stout, coenocytic, branched), from purana ghat, Betwa river, during summer 2018 [fig. (a) and (b)]. Stock cultures of the chytrids to be used as inoculum were maintained on slants of a YPSS medium, solidified with agar. As both fungi are monocentric, their development on solid substrata is determinate. To facilitate their spread over the entire surface of the slant it was found expedient to employ a low concentration of agar. This permitted a small quantity of liquid to collect at the base of the slant. Rolling the liquid over the agar several times after the 3rd day of inoculation inundated the sporangia and dispersed the zoospores.

Composition of YPSS (Yeast Powder Soluble Starch Agar) Medium:

K₂HPO₄ - 1.0gm

MgSO₄.7H₂O - 0.5gm

Starch (soluble) - 15gm

Yeast Extract (Difco) - 4gm

Agar Agar - 15gm

Water - 1litre

After an incubation period of 7 days the fluid evaporated and the dense creamy to crumbly fungus growth could be skimmed from the surface of the slant without disturbing the underlying agar. The thalli were washed thoroughly and permitted to settle to the tapered base of a 40-ml centrifuge tube containing 25 ml of YPSS medium from which the organic components had been omitted. After 1 hr, numerous sporangia discharged their contents and the resulting zoospore suspension was used to inoculate the experimental flasks. Preliminary experiments designed to test the extent to which the inoculation procedures had been standardized demonstrated that, when 2-ml samples of inoculum were used, the observed differences between the means derived from duplicate experiments could have arisen from random sampling errors in more than 30 per cent of the trials. Furthermore, no coefficient of variation was found to exceed 10 per cent. Since autoclaving glucose together with the other components of the medium was found to exert neither a deleterious nor a stimulatory effect upon these organisms, this procedure was followed routinely. Nutrients were sterilized by autoclaving at 121°C (15 lb) for 15 min. The liquid media employed were dispensed into Erlenmeyer flasks in amounts that brought the volume to 40 ml per flask after inoculation.

The organisms were incubated at different temperature and pH. The cultures were harvested on the 15th day following inoculation, their growth indicated by the maximum yields during that period and declined markedly thereafter. Camera lucida drawing prepared and examined comparatively. Hydrogen ion concentrations were measured by Beckman model G, pH meter.

RESULT AND DISCUSSION

Temperature requirements: Stationary liquid cultures were utilized for the study of temperature effect on the growth and sporulation of the isolates. *Pythium sp.* gave maximal yields at temperatures ranging from 22°C to 30°C. The fungus grew poorly at 15°C, 16°C and 20°C and did not grow at 32°C to 40°C. The harvests of *Saprolegnia sp.* were maximum at 22°C. Poor yields were obtained at 10°C, 11°C, 16°C, 20°C, 23°C, 25°C, 28°C and 30°C, whereas virtually no growth occurred at 15°C, 28°C and 31°C.

Hydrogen ion and phosphate tolerance: An attempt to stabilize the pH of the nutrient solutions by increasing the concentration of the K₂HPO₄-KH₂PO₄ buffer system revealed that *Pythium sp.* shows maximum growth at pH 6.5 and minimum at pH 3 while *Saprolegnia sp.* shows maximum growth at pH 5 and minimum at pH 9.5 of the 15th day of incubation [Table 1].

Table 1: Effect of pH on the growth and sporulation of *Pythium sp.*

pH	Days of incubation					
	5 th Day		10 th Day		15 th Day	
	Growth (in cm)	sporulation	Growth (in cm)	sporulation	Growth (in cm)	Sporulation
3.0	0.4	-	1.0	-	1.2	-
3.5	1.3	-	1.9	-	2.3	-
4.0	1.5	-	1.96	Poor	2.56	Poor
4.5	1.5	Poor	2.2	Mod	2.45	-
5.0	1.6	Poor	1.96	Good	2.25	-
5.5	1.55	Mod	2.17	Good	2.90	-
6.0	1.80	Mod	2.3	Good	3.14	-
6.5	2.4	Good	3.20	Mod	3.30	-
7.0	1.1	Good	1.52	Mod	2.25	-
7.5	1.12	Mod	1.86	Poor	2.32	-
8.0	1.42	Poor	2.1	Poor	2.4	-
8.5	1.35	Poor	1.1	-	2.27	-
9.0	1.60	-	1.9	-	2.04	-
9.5	1.44	Poor	1.80	-	2.15	-
10.0	1.1	-	1.40	-	1.8	-
Control	1.4	Poor	1.6	Good	1.6	-

Analysis of the Results = (Growth = colony diameter), pH(Growth)

6.5 (3.30) > 6.0 (3.14) > 5.5 (2.90) > 4.0 (2.56) > 4.5 (2.45) > 8.0 (2.40) > 7.5 (2.32) > 3.5 (2.30) > 8.5 (2.27) > 5.0 (2.25) > 7.0 (2.25) > 9.5 (2.15) > 9.0 (2.04) > 10.0 (1.8) > 3.0 (1.2)

Table.2: Effect of pH on the growth and sporulation of *Saprolegnia sp.*

pH	Days of incubation					
	5 th Day		10 th Day		15 th Day	
	Growth (in cm)	sporulation	Growth (in cm)	sporulation	Growth (in cm)	Sporulation
3.0	1.0	-	1.30	-	1.34	-
3.5	1.30	-	1.33	-	1.40	-
4.0	1.40	Mod	1.55	Poor	1.58	-
4.5	1.62	Good	1.88	Poor	1.77	-
5.0	1.81	Mod	1.85	Good	2.25	-
5.5	1.73	Mod	1.75	Good	1.76	-
6.0	1.92	Mod	1.98	Good	2.14	-
6.5	2.4	Mod	2.50	Mod	2.60	-
7.0	1.7	Poor	1.82	Good	2.20	-
7.5	1.44	Poor	1.66	Poor	1.68	-
8.0	1.5	Mod	1.53	Good	1.62	-
8.5	1.45	Good	1.55	Mod	1.57	-
9.0	1.2	-	1.4	-	1.5	-
9.5	0.54	Poor	0.99	-	0.99	-
10.0	0.7	-	-	-	-	-
Control	1.7	Mod	1.9	Good	2.6	-

Analysis of Results = (Growth = colony diameter) pH (Growth)

6.5 (2.60) > 5.0 (2.25) > 7.0 (2.20) > 6.0 (2.14) > 4.5 (1.77) > 5.5 (1.76) > 7.5 (1.68) > 8.0 (1.62) > 4.0 (1.58) > 8.5 (1.57) > 9.0 (1.50) > 3.5 (1.40) > 3.0 (1.34) > 9.5 (0.99)

CONCLUSION

Increased water temperature increases the rate of metabolic activity within a system. Which leads to faster microbial nutrient cycling and growth. As the temperature increases, pH also increases which directly affect the diversity and growth of the aquatic fungi in the ecosystem. Slightly acidic medium has been found suitable for the growth of fungi at 22°C.

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