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EFFECT OF ENVIRONMENTAL CONDITIONS ON GROWTH OF *PYTHIUM MYRIOTYLUM* CAUSING SOFT ROT OF TURMERIC

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ABSTRACT

Effect of different pH levels, temperature, light were tested against the growth of *P. myriotylum* under *in vitro* conditions. The results of experiment indicated that the growth of *P. myriotylum* was maximum in pH range of 6.00- 6.50 and temperature range of 25 - 30°C. The exposure of the fungus to alternate cycles of 12 hour light and 12 hour darkness resulted in the maximum mycelial growth of *P. myriotylum* compared to continuous light and dark.

INTRODUCTION

Turmeric is spice derived from the rhizomes of *Curcuma longa*, is an erect perennial herb viz. grown annually.Turmeric is one of the important medicinal plants, among 32 prioritized medicinal plants according to the National Medicinal Plants Board (NMPB) under the ministry of Health and Family Welfare. The turmeric is an important ingredient in the preparation of medicinal oils, ointments and poultice. The active component of the turmeric plant is curcumin which have considerable anti-inflammatory activity due to their ability to inhibit prostaglandin synthesis [1]. More recently, evidence that curcumin may have antioxidant and anticancer activities has renewed scientific interest in its potential to prevent and treat the disease [2]. The extract of turmeric rhizome can be used to cure bacterial, viral, parasitic and fungal infections by stimulating the immune system. The crop is being debilitating by an array of opportunistic pathogens. Among all, *Pythium myriotylum* which causes rhizome soft rot disease is a major limiting factor in production, leading to heavy losses [3]. An understanding of the role of environmental conditions and its effect on infection and survival of the pathogen is necessary to develop cultural disease management practices. Therefore, the objectives of this study includes isolation, purification and identification of pathogenic fungus causing rhizome soft rot disease of turmeric and determine optimal conditions for the mycelia growth of the fungus including pH, temperature and light.

MATERIALS AND METHODS

- **Isolation, Purification and Identification of Pathogen:**

The pathogen was isolated by tissue segment method [4] on PDA medium.Turmeric rhizome showing characteristic of soft rot symptom were cut into small pieces of 1.0 to 1.5 cm, surface sterilized with 0.1 per cent mercuric chloride for one minute and washed in sterile distilled water thrice and blot dried with sterilized filter paper. The sterilized rhizome bits were placed in Petri plates containing sterilized PDA medium. The plates were incubated at 28±2°C for four days and observed for the fungal growth. The fungus was purified by single spore isolation technique [5] and the purified isolates were maintained on PDA slants for further studies. Total of seven isolates were isolated from different growing areas.The pathogen was identified up to species level based on their cultural and morphological characters. The identification was further confirmed from Agharkar Research Institute, Pune, Maharashtra.

- **Pathogenicity Test:**

The pathogenicity of all seven purified isolates of *P. myriotylum*was confirmed by Koch's postulates. Sprout of turmeric were raised by planting 20g bits of seed rhizomes in steam sterilized standard potting media consisting of soil: sand: farm yard manure in 3:1:1 ratio. Forty five old plants were used for inoculation. A control treatment was maintained without inoculums. Giant cultures of inocula of *P. myriotylum*were inoculated to turmeric plant. Observation was made every alternate day regarding development of wilt symptoms. When the plant showed wilt symptoms such plants were carefully uprooted and the pathogens were reisolated by standard tissues isolation method. The pathogens reisolated were compared with original cultures.

- **Environmental Factors affecting to the Growth of Isolates:**

The effect of pH, incubation temperature and lighton the mycelial growthof *P. myriotylum in vitro* in agar plates was studied.

- **Effect of Hydrogen Ion Concentration (pH):**

The effect of pH on the growth of the pathogen was studied followed the method described by Kiryu [6] using PDA medium. Potato dextrose broth was prepared in 250 ml Erlenmeyer flask, each containing 100 ml broth. The pH of the broth was adjusted to 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 with a help of digital pH meter using 0.1 N Hydrochloric acid and 0.1 N Sodium hydroxide. The required quantity of agar was added to each flask and sterilized. The sterilized media of different pH levels were poured in the sterilized Petri plates in about 20 ml quantities and allowed to solidify. Five mm discs from the actively growing ten day old cultures of different isolates were placed on the centre of the Petri plates. The plates were incubated at 28 ± 2°C for 10 days then the mycelia growth diameter was measured. Three replications were maintained for each treatment.

- **Effect of Temperature:**

Petri plates containing 20 ml of PDA medium were inoculated with five mm mycelia disc from ten day old culture of different isolates. The inoculated plates were incubated at different temperature: 5, 10, 15, 20, 25, 30 and 35°C. The colony diameter was measured ten days after inoculation. Three replications were maintained for each treatment.

- **Effect of Light:**

The pathogen cultures of different isolates on PDA were exposed to continuous light, dark and 12 h light and 12 h dark in an environment chamber maintained at 30°C. Mycelial disc of nine

mm of each isolate was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in environment chamber and light intensity was adjusted to required level. The mycelial growth was measured in each case ten days after inoculation.

RESULTS AND DISCUSSION

- **Isolation, Purification and Identification of Pathogen:**

The process of isolation resulted in seven isolates ofpathogen collected from different regions. All the isolateswere confirmed by morphological and cultural charactersas isolates of *P. myriotylum.*

- **Pathogenicity Test:**

Inoculated plants exhibited symptoms within 15 days of inoculation, lower most leaf exhibited yellowing symptoms. Later, rhizomes were rotted, emitted foul smell.Re-isolation trails revealed that the isolated fungi from diseased seed rhizomes are found to be identical with those used for artificial inoculation.

- **Effect of Hydrogen Ion Concentration (pH) on the Mycelial Growth:**

Fungi generally utilize substrates in the form of solution only if the reaction of solution conducive to fungal growth and metabolism Kiryu [6]. This brings importance of hydrogen ion concentration for better fungal growth. Of all the eleven pH levels, pH 5.0 was found to be ideal and produced the maximum mean mycelial growth of 88.50 mm followed by pH 5.5 (86.60 mm) and pH 6.0 (75.00 mm). Themycelial growth was lowest at pH 3.0 which recorded 25.00 mm (Fig. 1). The pH below six and above seven was noticed to be inhibitory to the growth. The results of experiment indicated that *P. myriotylum*prefers pH range of 5.00- 5.50. Kraft and Erwin [7]opined that the optimum pH requirement for the growth of *P.aphanidermatum*was in between pH 5.0 to 5.5.Kumar and Grover [8] also noted that pH 5.5 to be most optimum for harvesting highest mycelial growth of *P.aphanidermatum.*Rajan and Singh [9] proved that growth of *Pythiumaphanidermatum*was maximum when the pH ranges from 5 to 6.

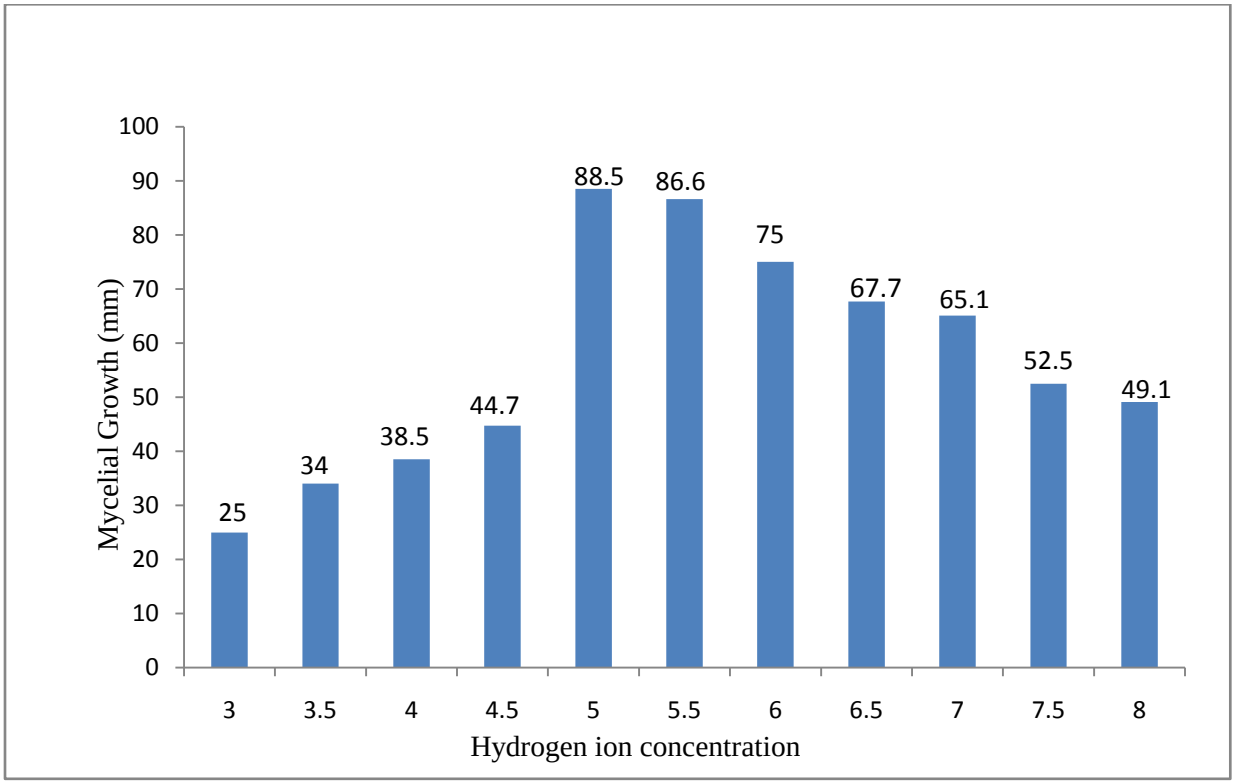

Fig. 1: Effect of hydrogen ion concentration (pH) on mycelial growth of *P. myriotylum*

- **Effect of Different Temperature on the Mycelial Growth:**

Temperature is most important physical environmental factor for regulating the growth and reproduction of fungi. All the seven isolates grew well at temperature of 30°C (89.45 mm) followed by 25°C (86.11) and 35°C (70.27). The least growth was observed at 5°C (9.7) (Table1). From the study, it is clear that temperature ranging from 25 - 30°C is better for the growth of*P. myriotylum*. The results are supported byLutrell and Mc Carter [10] who reported temperature requirement for the growth of *P. aphanidermatum* and *P. myriotylum* ranged between 27 -30º C. Lumsden and Ayers [11] noted that, the optimum temperature for the growth of *P. myriotylum* was 30º C.

Table 1: Effect of different temperatures on the mycelial growth of *P. myriotylum* isolates

Isolates	MycelialGrowth (mm)						
	Temperature(˚C)						
	5	10	15	20	25	30	35
PM1	10.2	18	42	62	85	90	68
PM2	8.3	19	38	54	88	90	64.5
PM3	11	23.5	46	61	89	90	67.2
PM4	9	18.2	43.5	60	90	90	72.5
PM5	11	19	44	62	88	89.5	69.4
PM6	10	20.2	48	61	83	89	72.5
PM7	9.5	18.5	48.5	63	83	88	68
Mean	9.7	19.2	46.3	65.2	86.11	89.45	70.27

- **Effect of Light on the Mycelial Growth:**

Light has profound effect on the mycelial growth of*P. myriotylum*. The exposure of the fungus to alternatecycles of 12 h light and 12 h darkness for 10 days resultedin the maximum mycelial growth of *P. myriotylum*(88.73 mm) which was significantly superior over other twotreatments tested (Table 2). The mycelial growth offungus exposed to continuous light resulted in 37.10 mmand continuous darkness resulted in 22.72 mm growth. The study agreed well with results of ArunKumar [12] thatalternate cycles of 12 h light and 12 h darkness resulted inmaximum growth of *A. solani*.

Table 2: Effect of light on mycelial growth of *P. myriotylum*isolates

Isolates	Mycelial Growth (mm)		
	Light	Dark	12 h alternate dark and light
PM1	35.4	24	89.8
PM2	36.5	23	88
PM3	35.00	25	84
PM4	38.6	23.4	86
PM5	37.2	23	88.5
PM6	38.6	28	88.5
PM7	37.2	23.8	88
Mean	37.10	22.72	87.73

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