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Some Physiological Studies on *Phytophthora incotianae* var. *Parasitica*, The Causal Organisms of Collar Rot of Brinjal

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Abstract

Studies on pathogenicity and some of the physiological aspects of the fungus *Phytophthora incotianae* var. *parasitica* were carried out. It produced typical symptoms of disease after 20 days of inoculation. A temp of 25°C, pH of 6 and continuous light was found to be the optimum for its growth after 72 hours of incubation.

Introduction

In Pakistan, Brinjal (*Solanum melongena* L.) an important vegetable crop is cultivated over an area of 8.97 thousand acres with an annual production of 42.73 thousand tones. This crop is attacked by a number of bacterial, fungal, nematode and viral diseases like bacterial wilt (*Pseudomonas solanacearum*), fruit and collar rot (*Phytophthora incotianae* var. *parasitica*) and root knot disease (*Meloidogyne javanica* and *M. incognita*) have been described by Hafiz (1986) from Pakistan. However, a serious and new disease of brinjal root naming collar rot appeared during 1991 causes severe damage to crop. Majeed *et al.* (1989) have shown that the disease was caused by *Phytophthora incotianae* var. *parasitica*.

Materials and Methods

Isolation of the fungus: Brinjal plants showing typical symptoms of collar rot were collected from the experimental area of Ayub Agri Res Inst (AARI), Faisalabad. For isolation of the pathogen, infected portion of the stem blackened at collar region were cut into small pieces, surface disinfected by dipping in 5 per cent sodium hypochlorite soln. for 1-2 minutes and dried with a sterile paper towel. They were plated on PARP (Pymaricin, Ampicilin, Rifampicin, Penta chloro nitro benzene) a selective medium for the isolation of pathogen species (Kannwisher and Michell, 1978).

Pathogenicity: An experiment was conducted to test pathogenicity of the fungus by inoculating the stem of brinjal plant with fungal. Earthen pots (15 x 15 cm) were filled with sterilized soil and one-two weeks old healthy brinjal nursery plant was transplanted to each pot. After three days of transplantation, brinjal plants in five pots were inoculated with the fungus culture, by giving small longitudinal incision on the stem, at collar region. Inoculum from 3-week-old culture was placed on the incision and wrapped with cellophane tape, while the remaining five pots served as control. Inoculated plants were examined for the symptoms of disease after 20 days of incubation.

Effect of temp on the mycelial growth: To determine the opt. Temp for the growth of *P. nic* var. *par*, each petri plates (90 mm) containing corn meal agar medium was inoculated with a single 5 mm disc of the fungus by placing it in center of each plate. The inoculated petri plates were incubated at 20, 25, 30 and 35 (+2)°C.

Effect of light on mycelial growth: To determine the effect of light duration on mycelial growth of *P. nic* var. *par*, culture plates containing PDA medium were inoculated with mycelial plugs (5 mm diameter) of the fungus and incubated at 25 ± 1°C in:

- i. 24 hrs continuous light
- ii. 12 hrs light with 12 hrs darkness
- iii. 24 hrs continuous darkness

Effect of pH on mycelial growth: To determine the effect of various pH levels on the mycelial growth, PARP medium was used as the substrate for the growth of the fungus. A quantity of 250 ml of PARP medium taken a flask was autoclaved and adjusted to pH levels of 4, 5, 6, 7, 8 and by the addition of appropriate amounts of N/2 HCl normal NaOH soln. The medium with experiments were run in quadruplicate. Petriplates were inoculated with 5 mm discs of the fungus and were incubated at 25 ± 1°C. Radial growth of the fungus was recorded after 24, 48 and 72 hrs of incubation and analyzed statistically. Temperature readings were taken. There was no production of sporangia in Petriplates. Therefore, for sporangia production experiment was conducted using soil extract as substrate for the growth of the fungus where 5 mm disc of the fungus culture on PDA were added to the extract.

Results and Discussion

Pathogenicity test: All the inoculated brinjal plants in pots developed characteristic "Collar rot" symptoms after 20 days of inoculation. No symptom developed on uninoculated plants. The isolations made from the affected stems yielded *P. incotianae* var. *parasitica*. Present res

Table 1: Effect of different temperature, light duration and pH levels on mean mycelial growth (in mm) of *P. incotianae* var. *parasitica*.

Temp °C	Treatments				
	Growth	Light	Growth	pH	Growth
20	30.33 c	Continuous light	88.4 a	4	31.40 e
25	85.50 a	Continuous darkness	42.9 b	5	56.66 b
30	69.9 b	12 hrs light and 12 hr. darkness	55 c	6	57.13 a
35	30.8 d			7	56.20 b
				8	54.40 c
				9	52.20 d

correlate with those of Majeed *et al.* (1993), who isolate the same fungus from brinjal plants and confirmed its pathogenicity.

Effect of temperature of mycelial growth of *P. incotianae* var. *parasitica*: The fungus was grown at 20, 25, 30 and 35 ± 2 C. The most suitable temp for mycelial growth was found to be 25 ± 2 C as the colony growth of the fungus at 14 temp was 86.9 mm in dia after 72 hrs of inoculation (Table 1). Below this temp the growth of the fungus was significantly less and it was only 32.4 mm at 20 C. At 30 C the growth of the fungus decreased and it was 69.9 mm, however at 35 C the growth of the fungus decreased significantly and it was only 30.8 mm in diameter. These results are almost in agreement with those reported earlier by Rao *et al.* (1969), Selvaraj *et al.* (1973), Perezpenaranda *et al.* (1989), Thareja *et al.* (1989), Haung *et al.* (1991) who observed that 22-25 C was the optimum temp for the growth of *P. incotianae* var. *parasitica*.

Effect of light on the mycelial growth: The effect of light on the mycelial growth of *P. incotianae* var. *parasitica* but varied with the light duration (Table 1). Continuous light was found to be the most suitable for maximum growth of the fungus. When the fungus was exposed to continuous light for 72 hrs, colony diameter was 88.6 mm as compared to 26.2 mm and 55 mm with exposure to continuous darkness and 12 hrs light + 12 hrs darkness respectively. The fungus growth was statistically different at continuous light and continuous darkness. Perez *et al.* (1989) also found better mycelial growth of *P. incotianae* var. *parasitica* in continuous light. However, according to Chauhan and Singh (1991) light has inhibitory effect on germination of zoospores with max germination (84.7 %) in complete darkness.

Effect of different pH levels on mycelial growth: The growth of *P. incotianae* var. *parasitica* varied significantly at various pH levels, but is increased greatly with an increased in incubation period (Table 1). However, when the effect of pH levels on fungus growth was compared within 72 hrs of incubation, a pH of 6 of was observed to be the best for fungus growth as at this pH a max growth of 57.13 mm was obtained. Below and above this pH, growth of the fungus decreased significantly, indicating that slightly acidic pH favor the mycelial growth and sporangia production of this fungus.

Similarly when the effect of pH levels sporangia production was compared at 48 hrs of incubation, the pH of 6 was found to be the best for sporangia production as well. Perez

et al. (1989) studied sporulation of 10 Agane isolates of *P. incotianae* var. *parasitica* and found that sporulation was max at pH 6. Similar results were also obtained by Allen and Nandra (1975). Below and above this pH, production of the fungus decreased. After 48 hrs of incubation period at pH 9, 8, 7, 6, 5 and 4, the mean sporangia production was 4, 4.80, 7, 11.80, 9.6 and 6 sporangia per plate respectively.

Unique morphological, genetically and physiological features, combined with the wide variety of disease caused on a large number of plants make *Phytophthora* one of the most fascinating subjects for investigation.

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