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EFFECT OF BIOCONTROL AGENTS AGAINST *COLLETOTRICHUM CAPSICI* CAUSING ANTHRACNOSE OF CHILLI (*CAPSICUM ANNUUM* L.)

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ABSTRACT

Chilli is an important vegetable and spice crop worldwide and one of the most important vegetables in India. The chief constituent of chilli, Capsaicin has antioxidant, antibacterial and anti-cancerous properties. Anthracnose caused by *Colletotrichum capsici* .is a major problem in India and one of the more significant economic constraints to chilli production worldwide, especially in tropical and subtropical regions. In the present study effective isolates of *Pseudomonas* sp. were screened out against *Colletotrichum capsici*. Two isolates of *Pseudomonas* sp were tested against *Colletotrichum capsici* on PDA by dual culture technique. Both the isolates showed more than 70% inhibition of the radial growth of the test pathogen *Colletotrichum capsici*. Isolate P1 showed 78% of reduction whereas isolate P6 showed 89% of the radial growth of the test pathogen *Colletotrichum capsici*. A standard culture of *Pseudomonas fluorescens* obtained from Agriculture University, Tamilnadu serve as the standard culture.

Keywords: *Capsicum annuum, Colletotrichum capsici, Pseudomonas,* Dual Culture

INTRODUCTION

Chilli crop suffers from many diseases like damping off, foot rot, anthracnose, dieback, fruitrot, wilt, leaf spots, powdery mildew etc. Anthracnose caused by *Colletotrichum* spp.is a major problem in India and one of the more significant economic constraints to chilli production worldwide, especially in tropical and subtropical regions. The disease causes both pre- and post-harvest fruit decay. Chilli anthracnose usually develops under high

humid conditions when rain occurs after the fruits have started to ripen with reported losses of up to 84%. Economic losses caused by the disease are mainly attributed to lower fruit quality.

Presently, greater emphasis should be placedon biological control of soil and seed borne pathogens, in order to reduce the environmental hazards, to avoid the development of resistant strains and to reduce the cost of cultivation. Biological control of soil borne pathogens offers environmentally safe, durable and cost effective alternative to chemicals **[1]**.

Pseudomonas sp. and its products have been studied and used for biocontrol in many countries. Most of effective strains produce both cell wall lytic enzymes and secondary metabolites against the disease causing fungi. Several strains of *Pseudomonas* have been found to be effective as biocontrol agent of various soil and seed borne plant pathogenic fungi.

The present study was proposed with an objective to access the isolate and identify the causal organism of anthracnose of chilli and select effective isolate of *Pseudomonas* sp. against *Colletotrichum capsici.*

MATERIALS AND METHODS

The experiment was conducted to screen out biological control agents against chilli anthracnose caused by *Colletotrichum capsici* at School of Biosciences, Mahatma Gandhi University, Kottayam, Kerala, India.

Isolation and Identification of the Causal Organisms

Infected fruit specimens were collected from Kerala Agriculture University, Vellayani. The fruitspecimens were washed with tap water, the discolored parts cut into small pieces (5 mm), sterilized with 0.1% NaOCl for t wo minutes and rinsed in sterilized water for three times and dried between folds of sterilized filter paper. The sterilized fruit pieces were transferred on sterilized PDA plates and incubated at room temperature for 5 days. The fungal isolates were purified,identified by standard protocol and stored in the PDA slants for further use.

Pathogenicity Test

In vitro Condition

C. capsici was cultured on PDA for 3 days. Then 0.7 cm agar plug contained with mycelia of *C. capsici* was placed on pierced area on chili fruit (*Capsicum anuum* L.) obtained from chilli field. All inoculated fruits were incubated in moist plastic chamber, kept at room temperature (27$\pm$oC). Fruits inoculated with sterile distilled water serve as control. Disease severity of anthracnose infection was recorded at 5 days after incubation by measuring size of diseased

lesion on chili fruit **[2]**. Pathogen was reisolated from diseased fruits and compaired with the original culture.

***In vivo* Condition**

Conidial suspension of twelve day old PDA grown cultures is sprayed on one-month-old chilli plants. The inoculated plants are covered with plastic bags for two days to maintain humidity. The plants are assayed for disease seven days after inoculation and continued to be so for up to 20 days **[3]**. Pathogen was reisolated from diseased fruits and leaves and compaired with the original culture.

Dual Culture Assay

Both the isolates were tested for their inhibitory activity against the fungal pathogen Colletotrichum capsici by dual culture technique **[4]**. Thefungal pathogen was grown on a PDA plate till it covered the whole surface of the agar. With the help of a sterile cork borer, a disc of fungal growth from this plate was taken and placed at the center of a fresh PDA plate. Twenty four hour old culture of each bacterial strain was then streaked parallelly on either side of the fungal disc 3 cm away from the disc. The plates were kept for incubation at 30^{o}C for 96 hours. Visual observations on the inhibition of growth of fungal pathogen were recorded after 96 hours of incubation in comparison with the PDA plate simultaneously inoculated with only the fungal pathogen. Percentage of reduction in growth was calculated following the formula:

$$\frac{\text{Growth of fungi in control plate- Growth of fungi in test plate} \times 100}{\text{Growth of fungi in control plate}}$$

RESULTS AND DISCUSSION

Colletotrichum capsici was isolated from infected fruit specimens collected from Kerala Agriculture University, Vellayani **(Figure 1)**. The isolate was confirmed by SEM analysis and colony morphology **(Figure 2 and 3)**. The pathogenicity of the fungus was proved under both *in vitro* and *in vivo* condition **(Figure 4 and 5)**.

Dual Culture Assay

Two cultures of *Pseudomonas* were tested against the test pathogen *Colletotrichum capsici.* Both the isolates showed more than 70% inhibition of the radial growth of the test pathogen *Colletotrichum capsici*. Isolate P1 showed 78% of reduction whereas isolate P6 showed 89% of the radial growth of the test pathogen *Colletotrichum capsici*. A standard culture of *Pseudomonas fluorescens* obtained from Agriculture University, Tamilnadu serve as the standard culture. The standard showed 81% of reduction of radial growth of the fungi.

Significant reduction of mycelial growth of *Colletotrichum* sp.in presence of *Pseudomonas* spp. were also reported by

many other workers **[5, 6]** and the results of the present investigation are in agreement with the above mentioned report.

Many investigators have suggested the rhizospheric bacteria *Pseudomonas* spp. as very interesting sources for the identification of antimicrobial compounds and their practical use as biopesticides **[7, 8]**.

Figure 1: Diseased Sample

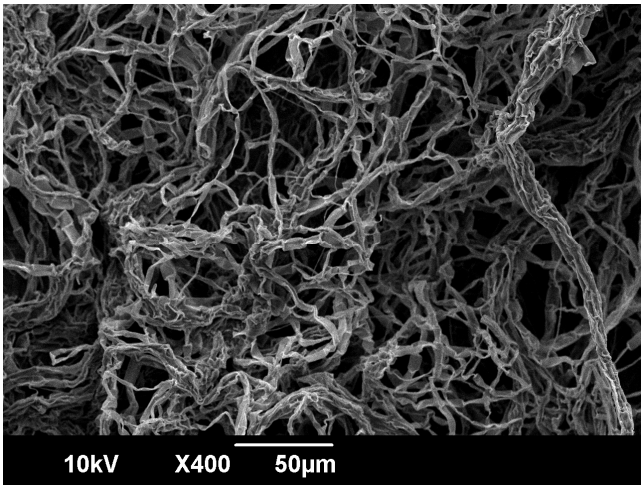

Figure 2: SEM View of the Isolate

Figure 3: Growth of *Colletotrichum capsici* in PDA

Figure 4: Pathogenicity test under *in vitro* condition

Figure 5: Pathogenicity Test Under *in vivo* Condition

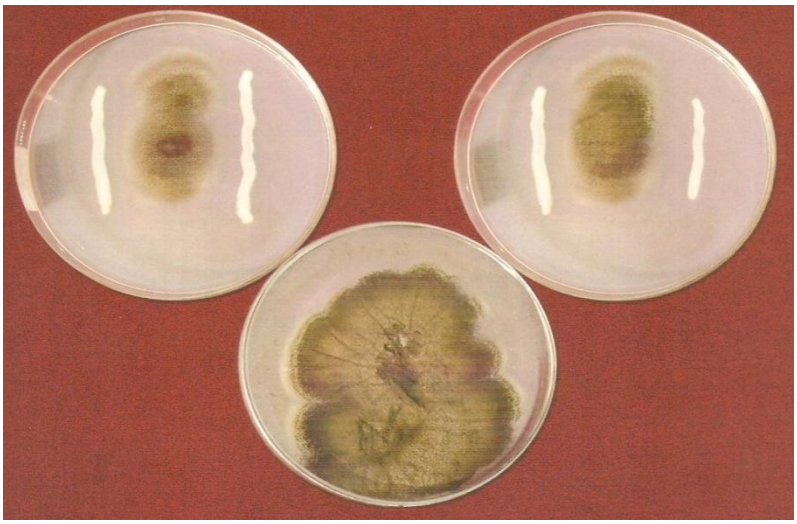

Figure 6: Antifungal Activity of Ps 1 Against *Colletotrichum capsici*

Figure 7: Antifungal Activity of Ps 6 Against *Colletotrichum capsici*

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