Int. Journal of Universal Science and Technology
ISSN: 2454-7263, Volume: 05 No: 04 Published: Jan., 2019
Paper ID: IJUP3135, Web: www.universalprint.org
Title Key: Study on the production of some enzymes by …

Study on the production of some enzymes by *Colletotrichum capsici* causing fruit rot of Chillies

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Abstract

A Virulent *Colletotrichum capsici* produced more cellulolytic enzymes than avirulent ones. The activity of these enzymes increased with the increase in age of culture. These pathogens also produced some non-specific toxic metabolites in culture filterate. These metabolites reduces seed germination, shoot length, root length and vigour index of the seedlings of chilli, rice, mungbean, maize, cotton, groundnut. Okra, egg plant, cucumber and tomato respectively. These toxic metabolites reduced seed germination which caused mortality of chilli seedlings. They also produced phytotoxic symptoms in the treated ripe and green chilli fruits and leaves.

Keywords: Cellulolytic enzymes, vigour index, Virulent, Avirulent etc.

Introduction:

Fruit rot of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) It causes severe losses both in yield and quality of the product. The disease is most common in almost all major chilli growing areas.It is reported to cause 25–48% loss in different parts of India (Muthulakshmi 1990; Datar 1995; Ekbote 2001).

Cell wall degrading enzymes released by pathogens are known to be responsible for pathogenesis. The ability of a pathogen to produce cellulolytic enzymes which determines the degree of degradation of cell wall during pathogenesis and inhibition of these enzymes ultimately affects the disease development. A numbers of cell wall degrading enzymes are shown to be produced by plant pathogens (Chenglin *et al.* 1996), which are known to facilitate cell wall penetration and tissue maceration in host plants. Apart from these, several toxins produced by microorganisms were also reported which are responsible for the induction of diseases in plants. These microorganisms produce toxic metabolites in culture media and plant tissues which were involved in the disease syndrome (Wood *et al*. 1972). Several species of *Colletotrichum* were known to produce different types of toxic metabolites (Bhaskaran and Kandaswamy 1978; Sriram *et al*. 2000). Present study deals with the production of cell wall degrading enzymes and isolate the toxins produced by *C. capsici* and study its effect on plant and seed germination.

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MATERIALS AND METHODS

Production of cell wall degrading enzymes:

The most virulent and avirulent isolates of *C. capsici* were used for this study. To study the *in vitro* production of cellulolytic enzymes, the pathogens were grown on Czapek-Dox liquid medium (pH 7–7.5) where the carbon source was substituted with 1% 1% carboxy methyl cellulose (for cellulolytic enzymes). The media were inoculated with 8 mm diameter culture disc of the pathogens. The culture filtrates were obtained after incubation at room temperature (27 ± 1°C) for 5, 10, 15 and 20 days and centrifuged at 3000 rpm for 20 min. The culture filtrates as such were used for the assay of cellulases.

Assay of cellulolytic enzymes:

Cellulase (C_1) activity:

Cellulase (C_1) activity was assayed by the method of Norkrans (1950). The assay mixture contained 1 ml of cellulose solution (the concentration which was adjusted to give approximately 0.85 absorbance at 610 nm), 4 ml of 0.1 M phosphate buffer (pH 7.0) and 5 ml of enzyme source. The absorbance of the assay mixture was determined at 610 nm in a Spectronic – 20 colorimeter immediately upon the addition of the enzyme source and again after the incubation period of 24 h at 27°C. The enzyme activity was expressed in units (1 unit = change in absorbance of 0.01).

Cellulase (C_x) activity:

Cellulase (C_x) activity was assayed by the viscosimetric method of Hancock *et al*. (1964). Two ml of enzyme extract was added to 4 ml of 1.2% carboxy methyl cellulose (CMC) solution buffered at pH 5.0 with sodium citrate buffer. The loss of viscosity of the CMC solution was determined by means of an Ostwald-Fenske viscosimeter size 150 at 5 min intervals up to 15 min. Enzyme source boiled for 10 min at 100°C served as check. The results were expressed as the per cent loss in viscosity in 15 min.

$$V = \frac{T_0 - T_1}{T_0 - T_w} \times 100$$

where, V – per cent loss of viscosity, T_0 – flow time in seconds at zero time, T1 – flow time of the reaction mixture at time T_1 and T_w – flow time of distilled water.

Result and Discussion:

Colletotrichum capsici pathogens produced cellulolytic enzymes *in vitro*. The enzyme production increased with the increase of incubation period. The virulent strains of *C. capsici* produced more cellulolytic enzymes (C1 and Cx) than the avirulent ones. The C_1 activity was found to be greater (9.00 units) in 20-day-old culture filtrate of virulent strains of *C. capsici* than the avirulent ones (0.75 units). Similarly, the Cx enzyme activity was found to be greater (83.97% loss of viscosity) in 20-day-old culture filtrate of virulent strains of *C. capsici* than the avirulent ones (21.11%). Mycelial dry weight also increased with the increase of incubation period. The dry weight of mycelium was higher in virulent strains of *C. capsici* (500 mg) as compared to the avirulent ones (80 mg) (Table 1).

Incubation days	*Colletotrichum capsici*					
	Virulent pathogen			Avirulent pathogen		
	C_1	C_x	Mycelial dry weight (mg)	C_1	C_x	Mycelial dry weight (mg)
5	1.80	15.00 (22.97%)	110	0.10	5.00 (12.92%)	40.0
10	4.10	22.57 (27.73%)	270	0.15	8.10 (16.45%)	62
15	7.50	70.87 (59.26%)	450	0.50	16.50 (23.98%)	72.0
20	9.00	83.97 (66.40%)	500	0.70	21.11 (27.35%)	80.0
SD	1.50	3.50	3.90	0.21	1.32	2.40

In the present investigation, the pathogen *C. capsici* produced cellulolytic enzymes *in vitro*. For successful pathogenesis, the pathogen has to overcome the host barriers like cell wall, pectin layer and protein matrix. The elaboration of an array of cell wall splitting enzymes helps the pathogen for easy penetration of the host cell wall and subsequent colonization (Goodman *et al*. 1967).Cellulose is a major structural constituent of the cell wall of host plants. Many phytopathogenic fungi produce cellulases in culture adaptively which hydrolyse cellulose and its derivatives (Marimuthu *et al*. 1974; Muthulakshmi 1990). The results obtained in the present study indicate that *C. capsici* produced C_1 and Cx *in vitro* and the activity of these enzymes increased with increase of the culture age. The virulent isolates of *C. capsici* produced more cellulolytic (C_1 and Cx) enzymes than the avirulent ones. Mehta *et al*. (1974, 1975). Another interesting observation in the present study is that the pathogens (*C. capsici*) produced cellulolytic enzymes which degraded CMC and cellulose. Walch and Khulwein (1968) also reported that *Ganoderma resinaceum* and *G. pfeifferi* produced extracellular enzymes which degraded CMC and cellulose.

The production and activity of cellulolytic enzymes detected *in vitro* suggest their active role in disease development by the pathogen in chilli fruits. Singh and Jain (1979) reported that the bottle gourd anthracnose pathogen (*C. lagenarium*) produced cellulolytic enzymes. Since both *C. capsici* are intercellular in the host, the productions of these enzymes appears to facilitate the dissolution of host cell wall and middle lamella and help entry and establishment of the pathogen in the host and are possibly responsible for playing a vital role in pathogenesis through cell wall degradation and disintegration of tissues. In the present study, the virulent pathogens produced more cellulolytic enzymes than the avirulent ones indicating the importance of the cell wall degrading enzymes in pathogenesis.

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