JOURNAL OF PLANT PROTECTION RESEARCH
Vol. 48, No. 4 (2008)

PRODUCTION OF CELL WALL DEGRADING ENZYMES AND TOXINS BY *COLLETOTRICHUM CAPSICI* AND *ALTERNARIA ALTERNATA* CAUSING FRUIT ROT OF CHILLIES

Theerthagiri Anand, Ramanujam Bhaskaran, Thiruvengadam Raguchander Gandhi Karthikeyan, Manikam Rajesh and Govindasamy Senthilraja*

Department of Plant Pathology, Centre for Plant Protection Studies,
Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

Received: September 18, 2007
Accepted: March 7, 2008

Abstract: The virulent isolates of *Colletotrichum capsici* and *Alternaria alternata* produced more cellulolytic enzymes *viz*., C_1 and C_x *in vitro* than the avirulent ones and the activity of these enzymes increased with the increase in age of culture. The virulent isolates of *C. capsici* and *A. alternata* produced more pectinolytic enzymes (macerating enzymes, pectin methyl esterase and endo polygalacturonase) than the avirulent ones. All the pectinolytic enzymes were highly active in 10-day-old culture and the activities decreased with the increase of culture age. Whereas the activity of enzymes produced by avirulent isolate of pathogens did not decrease and these enzyme activities increased with the increase in the age of culture. These pathogens also produced nonspecific toxic metabolites in culture filtrate which reduced seed germination, root length, shoot length and vigour index of the seedlings of chilli, rice, mungbean, maize, cotton, groundnut, okra, egg plant, cucumber and tomato. The toxins of the pathogens reduced seed germination and caused mortality of chilli seedlings in pot culture. The toxins also produced phytotoxic symptoms in the treated ripe and green chilli fruits and leaves.

Key words: Cellulolytic and pectinolytic enzymes, vigour index, virulent, avirulent

Abbreviations: PME – pectin methyl esterase, Endo-PG – endo polygalacturonase

INTRODUCTION

Fruit rot of chilli (*Capsicum annuum* L.) caused by *Colletotrichum capsici* (Syd.) Butler and Bisby and *Alternaria alternata* (Fr.) Keissler causes severe losses both in yield

*Corresponding address: barathiana@yahoo.com

and quality of the product. The disease is prevalent in almost all major chilli growing areas and 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 and pectinolytic enzymes 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 have been 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. In addition, several toxins produced by microorganisms were reported to be 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* and *Alternaria* were known to produce different types of toxic metabolites (Bhaskaran and Kandaswamy 1978; Sriram *et al*. 2000). Attempts were made to assess the production of cell wall degrading enzymes and isolate the toxins produced by *C. capsici* and *A. alternata* and study its effect on plant and seed germination.

MATERIALS AND METHODS

Production of cell wall degrading enzymes

The most virulent and avirulent isolates of *C. capsici* and *A. alternata* were used for this study. To study the *in vitro* production of pectinolytic and cellulolytic enzymes, the pathogens were grown on Czapek-Dox liquid medium (pH 7–7.5) where the carbon source was substituted with 1% pectin (for pectic enzymes) or 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. For the assay of pectinolytic enzymes, the culture filtrates were dialysed for 18 h against distilled water at 40°C. The dialysate served as enzyme source. As dialysis was found to reduce the activity of cellulolytic enzymes (Bateman 1964), 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, T_1 – flow time of the reaction mixture at time T_1 and T_w – flow time of distilled water

Assay of pectinolytic enzymes

Macerating enzymes

Macerating enzyme activity was assessed by the method described by Mahadevan (1965). Potato discs of 8 mm diameter and 30 µ thickness were obtained by using a hand microtome. Ten potato discs were placed in a sterile Petri dish and 10 ml of the culture filterate was added. The uninoculated medium served as control. The coherence of the potato discs was tested at different time intervals using a glass rod and the enzyme activity was expressed as the time taken in hours for maceration of potato discs.

Pectin methyl esterase (PME)

Pectin methyl esterase activity was estimated following the procedure described by Gupta (1970). Pipetted out 20 ml of pectin solution and its pH was adjusted to 7.0 using 1 N sodium hydroxide. To this, 10 ml of enzyme solution was added and its pH was adjusted immediately to 7.0 in the pH meter by adding 1 N NaOH. The enzyme substrate mixture was incubated for 24 h and pH was readjusted to 7.0 with 0.02 N NaOH, which was equal to the enzyme activity and the enzymatic activity was expressed in terms of units (one unit is 0.1 ml of 0.02 N NaOH used).

Endo polygalacturonase (endo-PG)

Endo-PG activity was estimated by the standard viscosimetric method (Hancock *et al*. 1964) using 3 ml of enzyme source, 1 ml of 1.2% sodium polypectate and 1 ml of 0.5 M ammonium acetate buffer. The loss in viscosity of the pectate solution was determined by means of the Ostwald-Fenske vicosimeter 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.

Extraction of toxin

The toxins produced by *C. capsici* and *A. alternata* were extracted using the procedure given by Arthur *et al*. 1974. The effect of toxin on seed germination and seedling growth was tested under laboratory conditions (Ludwig 1957). Seeds of rice, maize, mungbean, groundnut, cotton, tomato, egg plant, okra and cucumber were surface sterilized with 0.1% mercuric chloride solution for 30 sec washed in repeated changes of sterile distilled water and treated with toxin (100 ppm). Twenty five seeds were

placed at equidistance on the sterilized filter paper kept inside the sterile Petri dishes and soaked with 10 ml of sterile distilled water. Three replications were maintained. The seeds soaked in sterile distilled water served as control. After 5 days the per cent germination was calculated. The length of shoot and root of the seedlings was also recorded after 5 days. The vigour index was calculated.

Effect of toxin on seed germination and seedling vigour of chilli in pot culture

The surface sterilized toxin (100 ppm) treated chilli seeds were sown in 15 cm pots containing 1.75 kg of sterilized soil. Twenty five seeds were sown in each pot with five replications. The pots were watered regularly. Healthy seeds sown similarly served as control. The germination percentage, symptoms, if any, on seedlings were observed.

Effect of toxin on chilli leaves and ripe and green chilli fruits

The chilli leaves as well as ripe and green chilli fruits were treated with toxin at 100 ppm. Sterile water served as control. The symptoms were recorded after 24 h of incubation.

RESULTS

Production of cell wall degrading enzymes

In vitro* production of cellulolytic enzymes by *C. capsici* and *A. alternata

Both pathogens produced cellulolytic enzymes *in vitro*. The enzyme production increased with the increase of incubation period. The virulent strains of *C. capsici* and *A. alternata*, produced more cellulolytic enzymes (C_1 and C_x) than the avirulent ones.

The C_1 activity was found to be greater (9.00 and 12.00 units) in 20-day-old culture filtrate of virulent strains of *C. capsici* and *A. alternata* respectively than the avirulent ones (0.75 and 0.90 units). Similarly, the C_x enzyme activity was found to be greater (83.97% and 76.00% loss of viscosity) in 20-day-old culture filtrate of virulent strains of *C. capsici* and *A. alternata,* respectively than the avirulent ones (21.11% and 0.90%). Mycelial dry weight also increased with the increase of incubation period. The dry weight of mycelium was higher in virulent strains of *C. capsici* and *A. alternata* (510 and 480 mg) as compared to the avirulent ones (85 and 95 mg) (Table 1).

In vitro* production of pectinolytic enzymes by *C. capsici* and *A. alternata

In the virulent isolates of *C. capsici* and *A. alternata*, production of the pectinolytic enzymes *viz*., macerating enzymes, PME and Endo-PG increased up to 10 days and thereafter the activities of the enzymes decreased. Maximum enzyme activity was observed in the culture filtrate 10 days after incubation. *C. capsici* produced more of PME and endo-PG than *A. alternata* and there was no distinct difference in the activity of macerating enzymes between the two pathogens. In the avirulent isolates, the enzyme production increased with the increase of incubation period (throughout the experimental period) but was much less than in the virulent isolates (Table 2). The mycelial weight increased with the increase of incubation period in both virulent and avirulent isolates of *C. capsici* and *A. alternata*.

Table 1. Activity of cellulolytic enzymes produced by *C. capsici* and *A. alternata*

Incubation time [days]	*C. capsici*					
	Virulent isolate			Avirulent isolate		
	C_1*	C_X**	mycelial dry weight [mg]	C_1*	C_X**	mycelial dry weight [mg]
5	1.50	15.00 (22.79)	115	0.10	5.00 (12.92)	45.0
10	4.00	22.57 (28.37)	270	0.10	8.10 (16.54)	62.0
15	8.50	73.87 (59.26)	455	0.50	16.52 (23.98)	73.0
20	9.00	83.97 (66.40)	510	0.75	21.11 (27.35)	85.0
SD	1.60	3.50	3.87	0.21	1.32	2.40
	A. alternata					
5	6.50	42.50 (40.69)	240	0.20	0.50 (4.05)	70.0
10	7.30	51.80 (46.03)	375	0.20	0.70 (4.80)	75.0
15	10.50	71.00 (57.42)	475	0.70	0.90 (5.44)	85.0
20	12.00	76.00 (60.67)	480	0.90	0.90 (5.44)	95.0
SD	1.60	3.65	3.50	0.17	0.28	2.51

*enzyme activity in units, ** per cent loss of viscosity, mean of three replications, Data in parentheses are arc sine transformed values

Table 2. Activity of pectinolytic enzymes produced by *C. capsici* and *A. alternata*

Incubation time [days]	*C. capsici*							
	Virulent isolate				Avirulent isolate			
	macerating enzymes*	PME**	Endo-PG***	mycelial dry weight [mg]	macerating enzymes*	PME**	Endo-PG***	mycelial dry weight [mg]
5	12.00	4.50	14.50 (22.38)	240	no maceration	2.50	5.00 (12.92)	32.0
10	12.00	18.50	69.97 (56.77)	360	no maceration	5.00	11.10 (19.46)	58.0
15	18.00	14.00	42.00 (40.40)	555	36.00	7.00	25.70 (30.46)	80.0
20	24.00	13.50	38.50 (38.35)	605	36.00	8.50	28.66 (32.37)	85.0
SD	1.15	1.90	2.80	3.15	–	0.96	2.47	2.56
	A. alternata							
5	15.00	4.50	23.50 (29.00)	250	no maceration	1.00	4.30 (11.97)	60.0
10	15.00	10.75	59.70 (50.59)	385	no maceration	2.75	10.10 (18.53)	75.0
15	18.00	8.75	40.00 (39.23)	590	no maceration	5.75	20.36 (26.82)	89.0
20	24.00	8.00	24.00 (29.33)	695	no maceration	5.75	22.24 (28.14)	110.0
SD	1.33	1.85	2.70	3.44	–	0.70	2.35	3.06

* time taken for maceration (h), ** enzyme activity in units, *** per cent loss of viscosity, mean of three replications
Data in parentheses are arc sine transformed values

Toxin assay

Effect of toxin of *C. capsici* on seed germination, root and shoot elongation and vigour index

Among the seeds of different crop plants tested for the effect of toxin, the highest inhibition of seed germination was observed in chilli when compared to other crops. Toxin treated chilli recorded the lowest seed germination of 26.66% as compared to 93.33% in the control (Fig. 1). The reduction of shoot length was the highest in chilli (77.35%) followed by mungbean (69.45%) and groundnut (64.28%), (Fig. 2). Treatment of the seeds with the toxin caused the highest reduction of root length in chilli (76.41%) followed by groundnut (67.03%) and okra (57.68%) while in rice and cotton lesser per cent reduction of the root length *viz*., 24.04 and 15.58%, respectively was observed (Fig. 3). The vigour index values based on the per cent germination and root length of seedlings from the healthy seeds and seeds treated with the toxin of the pathogen revealed that chilli recorded the maximum (93.26%) reduction in the vigour index being of 31.2 and 462.9, respectively for the healthy and the treated seeds (Fig. 4).

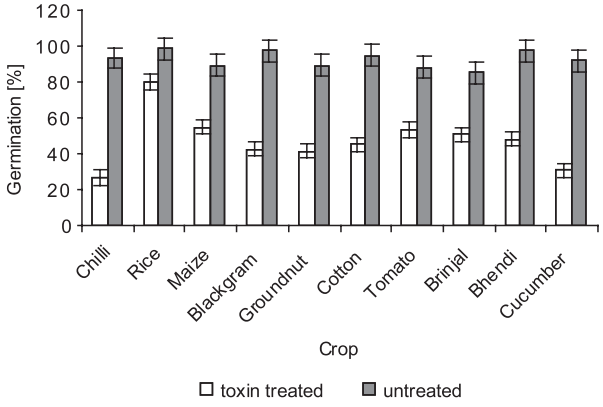

Fig. 1. Effect of toxin of *C. capsici* on seed germination

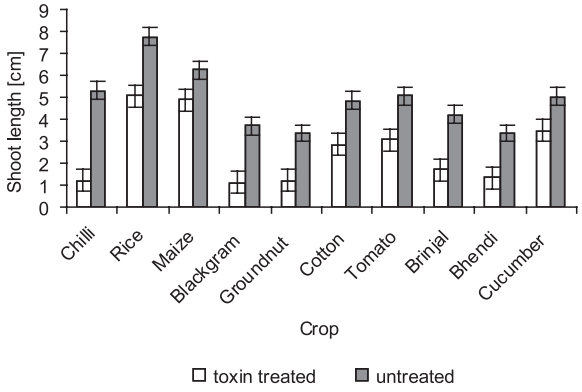

Fig. 2. Effect of toxin of *C. capsici* on shoot length

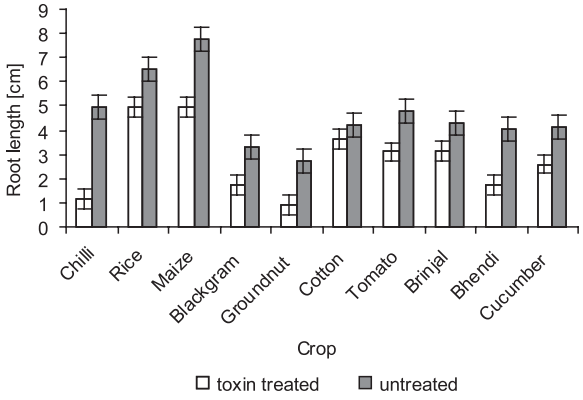

Fig. 3. Effect of toxin of *C. capsici* on root length

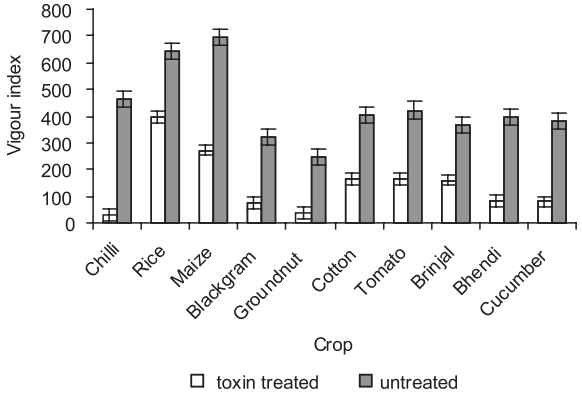

Fig. 4. Effect of toxin of *C. capsici* on vigour index

Effect of *A. alternata* toxin on seed germination, root and shoot elongation and vigour index

Among seeds tested for the effect of toxin of *A. alternata,* chilli, bhendi and cotton recorded very low seed germination of 26.66, 30.66 and 30.66% compared to 93.33, 97.33 and 94.66% in control, respectively (Fig. 5). When the seeds were treated with toxin, shoot elongation was inhibited. The reduction of shoot length was the highest in chilli (81.13%) followed by other crops (Fig. 6). Treatment of the seeds with the *A. alternata* toxin caused the highest reduction of root length (91.33%) in chilli followed by other crops (Fig. 7). The vigour index values based on the germination per cent and root length of the seedlings raised from the healthy seeds and seeds treated with the toxin of the pathogen showed the maximum reduction (97.52%) in vigour index in chilli. The toxin treated chilli seeds exhibited the vigour index of 11.5 as against 462.9 for the untreated seeds (Fig. 8).

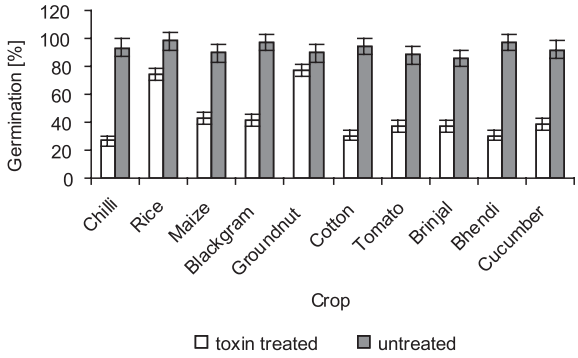

Fig. 5. Effect of toxin of *A. alternata* on seed germination

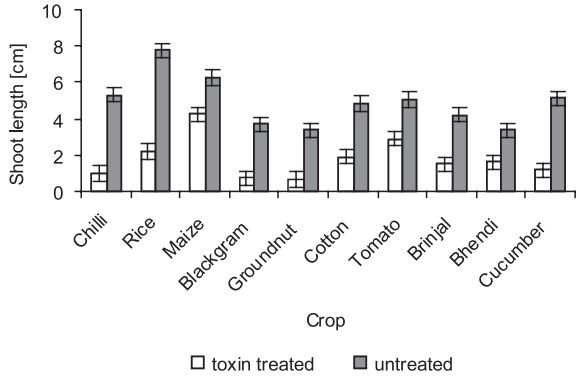

Fig. 6. Effect of toxin of *A. alternata* on shoot length

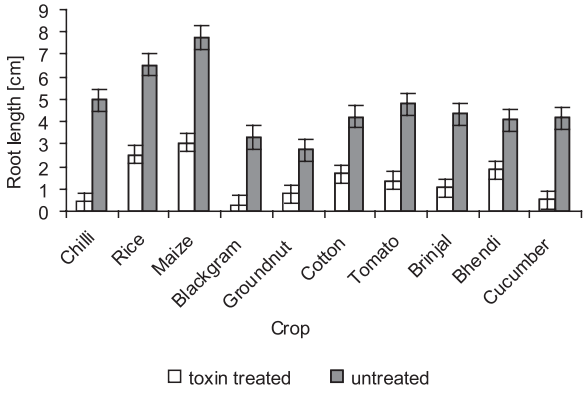

Fig. 7. Effect of toxin of *A. alternata* on root length

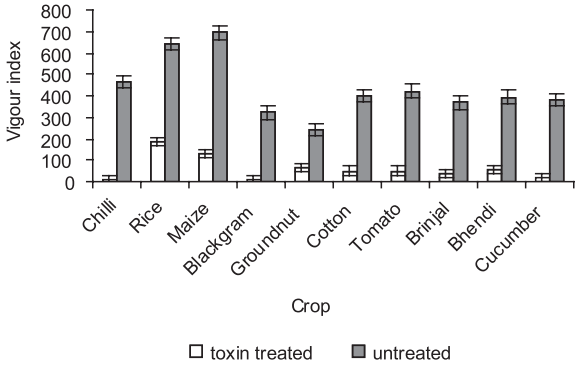

Fig. 8. Effect of toxin of *A. alternata* on vigour index

Effect of toxin on chilli seed germination in pot culture

The results recorded on the effect of toxins revealed that there was a marked inhibition in chilli seed germination. When the toxins of both pathogens (*C. capsici* and *A. alternata*) were mixed and in chilli seeds were treated with the inhibition of seed germination was greater than when the seeds were treated with the toxins of the pathogens separately. When the toxins of *C. capsici* and *A. alternata* were applied separately to chilli seeds, the per cent seed germination was 12.00 (86.95% reduction over control) and 16.00 (82.60% reduction over control) respectively, whereas chilli seeds treated with the mixture of toxins recorded only eight per cent seed germination compared to 92.00% in control (Table 3).

Table 3. Effect of toxins of *C. capsici* and *A. alternata* on seed germination of chilli in pot culture

Treatments	Per cent seed germination*	Per cent reduction over control	Remarks
Toxin of *Colletotrichum capsici*	12.00 (20.27)	86.95	lanky growth
Toxin of *Alternaria alternata*	16.00 (23.58)	82.60	lanky growth
Toxin of both the pathogens	8.00 (16.43)	91.30	lanky growth
Control	92.00 (73.57)		
SD	3.27		

* Mean of five replications
Data in parentheses are arc sine transformed values

When chilli leaves were treated with the toxin of *C. capsici* necrotic symptoms were observed in the leaf lamina. *A. alternata* toxin treated leaves showed vein chlorosis. In ripe and green chilli fruits, the toxin of the pathogens (*C. capsici* and *A. alternata*) caused necrotic symptoms.

DISCUSSION

Cell wall degrading enzymes

In the present investigation, the pathogens (*C. capsici* and *A. alternata*) produced both cellulolytic enzymes and pectinolytic enzymes *in vitro*. For successful pathogenesis, the pathogen has to overcome the host barriers like cell wall, pectin layer and protein matrix (Williams and Heitefuss 1976). 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* and *A. alternata* produced C_1 and C_x *in vitro* and the activity of these enzymes increased with increase of the culture age. The virulent isolates of *C. capsici* and *A. alternata* produced more cellulolytic (C_1 and C_x) enzymes than the avirulent ones. Mehta *et al*. (1974, 1975) also reported high cellulase activity in the culture filtrate of virulent *A. tenuis* and *A. solani*. Another interesting observation in the present study is that the pathogens (*C. capsici* and *A. alternata*) 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. Muthulakshmi (1990) also found that *A. tenuis* produced C_x enzyme which degraded cellulose.

In the present investigation, the virulent isolates of *C. capsici* and *A. alternata* produced more macerating enzymes, PME and endo-PG *in vitro* than the avirulent ones. All the pectinolytic enzymes were highly active in the culture filtrate up to 10 days of age and thereafter the activity decreased. The maceration of potato discs increased with increasing age of the culture *in vitro* up to 10 days. Symptoms of fruit rot appeared in chilli fruits within 11 days after inoculation. Maximum pectinolytic enzymes in the culture filtrate occurred up to 10 days indicating the role of these enzymes in pathogenecity. This corraborated with the observation of Muthulakshmi (1990) in the case of *A. tenuis* causing fruit rot of chilli. The enzyme PG hydrolytically cleaves pectin in such a manner that the α1, 4-glycosidic bonds of the chain are split (Bateman and Miller 1966). PME removes the esterified methyl group from the pectin chain hydrolytically (Goodman *et al*. 1967).

All these cell wall splitting enzymes are mostly adaptive, secreted by the pathogen in the presence of appropriate substrates. Pectinolytic enzymes were produced only in the presence of pectin in the medium and cellulolytic enzymes were produced only in cellulose containing medium. Jha and Gupta (1988) reported that the combination of glucose and pectin (or) polypectate induced secretion of endo-PG and endo-PMG in *A. triticina* infected wheat.

The production and activity of pectinolytic and 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 both pectinolytic and cellulolytic enzymes. Since both *C. capsici* and *A. alternata* 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 and pectinolytic enzymes than the avirulent ones indicating the importance of the cell wall degrading enzymes in pathogenesis.

Toxins

Gaumann (1950) stated that microorganisms are pathogenic only if they are toxigenic. A vast array of toxins, host specific and non-specific, are produced by plant pathogens and their role in symptom production has also been established (Das Gupta 1986; Ou Yang *et al*. 1993). Production of toxin *in vitro* by *C. capsici* has been reported by Subbaraja and Pillayarswamy (1973), Thirupathiah and Subramanian (1974) and Das Gupta (1986). In the present study, *C. capsici* produced a toxic metabolite in the culture filtrate and it was inhibitory to the seed germination and shoots and root elongation of seedlings of chilli. The toxin also affected the seed germination and shoots and root elongation of rice, mungbean, maize, cotton groundnut, okra, cucumber, tomato and brinjal. Inhibitory effect of a toxic metabolite of *C. capsici* in chilli seeds and seedlings has been reported earlier by Subbaraja and Pillayarswamy (1973) and Kumar and Mahmood (1986). Ou Yang *et al*. (1993) reported that the toxic metabolite of *C. capsici* inhibited the radicle growth of chilli, green gram, pea and cowpea cultivars. Jeyalakshmi (1996) stated that the culture filtrate of *C. capsici* inhibited the seed germination and shoot and root elongation of chilli, blackgram, rice, pearl millet and tomato. Bhale *et al*. (1998) also reported that the culture filtrate of *C. dematium* inhibited the seed germination and root and shoot elongation of chilli.

The toxin produced by *C. capsici* in the present study is an extracellular host non-specific toxin, since the metabolite has inhibitory effect on non-hosts also. Das Gupta (1986) found that the toxin secreted by *C. capsici* causing anthracnose of betelvine in Frie's medium affected the root elongation of the sprouted rice seeds. The production of host non-specific toxins by the fruit rot pathogen has bean cited by Subbaraja and Pillayarswamy (1973), Kumar and Mahmood (1986) and Jeyalakshmi (1996). The vigour index calculated on the basis of germination percentage and root length revealed that chilli seeds recorded least vigour index. The vigour indices of other toxin treated crops were also less. The toxin of *C. capsici* affected the germination of chilli seeds and caused lanky growth of the seedlings in pot culture. The toxin also produced phytotoxic symptoms on chilli fruits and leaves. Mathur (1995) also reported that the culture filtrate of *C. capsici* caused inhibition of seed germination, wilting of seedlings and fruit damage.

Several species of *Alternaria* are known to produce host specific toxins (Pringle and Scheffer 1964). Production of non-specific toxins by *Alternaria* spp. has also been reported (Subbaraja and Pillayarswamy 1973; Vijayalakshmi and Rao 1988). The role of toxin in triggering disease has been the subject of intense investigation by many workers as evidenced by the publication of large number of research papers and reviews (Vidhyasekaran *et al*. 1970; Muthulakshmi 1990; Singh *et al*. 1996).

The present study revealed that the toxin of *A. alternata* was highly inhibitory to the germination of chilli seeds and shoots and root elongation. It also affected the germination and shoots and root elongation of tomato, brinjal, cucumber, okra, rice, mungbean, maize, cotton, and groundnut but to a smaller extent when compared to chilli. Muthulakshmi (1990) found that the culture filtrate of the pathogen inhibited

seed germination and root and shoot elongation in chilli, sorghum and paddy. Bhale *et al*. (1998) also made similar observations in chilli. Recently, Amaresh and Nargund (2005) reported that the toxins produced by *A. helianthi* inhibited the seed germination, root and shoot length of sunflower and sorghum. In the present study, the toxin of *A. alternata* inhibited the seed germination in pot culture and was responsible for severe seed rot and seedling decay. Similar observations were made by Bhale *et al*. (1998) in chilli.

The mixture of toxins of *C. capsici* and *A. alternata* was much more inhibitory to seed germination and caused more seed rot and seedling decay than the individual toxins. It is concluded that the inhibitory effect of the culture filtrate of the pathogen on seed germination and seedling growth of chilli and other crops tested in the present investigation might be due to the production of non-specific phytotoxic substances by the fungi and released into the culture medium.

REFERENCES

Amaresh V. S., Nargund V.B. 2005. Isolation of toxin from *Alternarai helianthi* causing blight in sunflower. J. Mycol. Pl. Pathol. 35 (2): 367–369.

Arthur L.K., Dale B.K., Strobel G.A. 1974. Isolation and partial characterization of four host specific toxins of *Helminthosporium maydis* (Race T). Pl. Physiol. 53: 250–257.

Bateman D.F. 1964. Cellulase and the *Rhizoctonia* disease of bean. Phytopathology 54: 1372–1377.

Bateman D.F., Miller R. 1966. Pectic enzymes in tissue degradation. Annu. Rev. Phytopath. 4: 119–146.

Bhale U., Bhale M.S., Khare M.N. 1998. Influence of culture filtrate of seed borne *Colletotrichum dematium* and *Alternaria alternata* on chilli seed germination. J. Mycopatholo. Res. 36: 81–84.

Bhaskaran R. Kandaswamy T.K. 1978. Production of a toxic metabolite by *Alternaria helianthi in vitro* and *in vivo*. Madras Agric. J. 65: 801–804.

Chenglin Y., William S.C., Carl E.S. 1996. Purification and characterization of a polygalacturanase produced by *Penicillium expansum* in apple fruit. Phytopathology 86: 1160–1166.

Das Gupta B. 1986. Role of toxin secretion by *Colletotrichum capsici* on the expression of leaf spot symptoms in Betelvine. J. Plant. Crops 14: 36–41.

Datar V.V. 1995. Pathogenicity and effect of temperature on six fungi causing fruit rot of chilli. J. Mycol. Pl. Pathol. 25: 312–313.

Ekbote S.D. 2001. Survey for chilli disease in Haveri district of Karnataka. J. Agric. Sci. 15: 726–728.

Gaumann E. 1950. Principles of Plant Infection. Hafner, New York, 123 pp.

Goodman R.N., Kiraly Z., Zaitlin M. 1967. The Biochemistry and Physiology of Infectious Plant Disease. D. Van Nostrand Co., Inc. Princeton, New Jersey, 354 pp.

Gupta T. 1970. *In vitro* and *in vivo* production of pectolytic and cellulolytic enzymes by four isolates of *Colletotrichum.* p. 197–207. In: "Physiology of Parasitism" (G.P. Agarwal, K.S. Bilgrami, eds.). Today and Tomorrow's, New Delhi.

Hancock J.G., Miller R.L., Lorbeer J.W. 1964. Pectolytic and cellulolytic enzymes produced by *Botrytis allii*, *Botrytis cinerea* and *Botrytis squamosa in vitro* and *in vivo*. Phytopathology 54: 928–931.

Jeyalakshmi C. 1996. Studies on fruit rot and die-back disease of chilli (*Capsicum annuum* L.) incited by *Colletotrichum capsici* (Syd.) Butler and Bisby. M.Sc. (Ag.) Thesis, Tamil Nadu Agric. Univ., Madurai, India, 216 pp.

Jha D.K., Gupta D.P. 1988. Production of pectolytic enzymes by *Alternaria triticina.* Indian Phytopath. 41, p. 652.

Kumar S., Mahmood M. 1986. Bioassay of culture filtrates of isolates of *Colletotrichum dematium* on seeds, seedlings and fruits of chilli. Indian Phytopath. 39: 282–284.

Ludwig R.A. 1957. Toxin production by *Helminthosporium sativum* and its significance in disease development. Can. J. Bot. 35: 291–303.

Mahadevan A. 1965. Production of protopectinase by *Fusarium vasinfectum.* Curr. Sci. 19: 566–568.

Marimuthu T., Bhaskaran R., Shanmugam N., Purushothaman D. 1974. *In vitro* production of cell wall splitting enzymes by *Alternaria sesami*. Labdev. J. Sci. Tech. 12B: 26–28.

Mathur K. 1995. Bioassay of culture filtrate of isolates of *Colletotrichum capsici* on seeds, seedlings and fruits of chilli. J. Mycol. Pl. Pathol. 25: 195–197.

Mehta P., Vyas K.M., Saksena S.B. 1974. Effect of native carbon sources and pH on the cellulases of *Alternaria solani* and *Alternaria tenuis*. Sci. Cult. 41: 400–402.

Mehta P., Vyas K.M., Saksena S.B. 1975. Production of pectolytic enzymes by *Alternaria solani* and *Alternaria tenuis* on different culture media. J. Indian Bot. Soc. 54: 200–206.

Muthulakshmi P. 1990. Studies on fruit rot of chilli (*Capsicum annuum* L.) caused by *Alternaria tenuis* Nees. M.Sc. (Ag.). Thesis, Tamil Nadu Agric. Univ., Madurai, India, 139 pp.

Norkrans B. 1950. Influence of cellulolytic enzymes from hyphomycetes on cellulose preparations of different crystallinity. Physiol. Plantarum 3: 75–85.

Otani U.S., Nishimura Kohmoto K. 1974. Nature of specific susceptibility of *Alternaria kikuchiana* in Nijisseiki cultivar among Japanese pears. III. Chemical and thermal protection effect of host specific toxin. Ann. Phytopathol. Soc. Japan 40: 66–69.

Ou Yang F., Xie B.Y., Ou Yang B.Y., Lu F.C. 1993. *Colletotrichum capsici* toxin. Acta Mycol. Sinica 12: 289–296.

Owens L.D. 1969. Toxins in plant disease. Structure and action. Science 165: 18–25.

Padmanaban P., Narayanasamy P. 1977. Factors influencing toxin production by *Alternaria macrospora* Zimm. Madras Agric. J. 64: 177–180.

Pringle R.B., Scheffer R.P. 1964. Host specific toxins. Annu. Rev. Phytopath. 2**:** 133–156.

Singh G., Jain S.C., Singh G. 1996. Effect of culture filtrate of chilli seed mycoflora on seed germination and root and shoot elongation. Indian Cocoa, Arecanut and Spices 20: 10–11.

Singh R.B., Jain J.P. 1979. *In vitro* production of pectinolytic and cellulolytic enzymes by *Colletotrichum lagenarium* the causal organism of bottle gourd anthracnose. J. Mycol. Pl. Pathol. 9: 277–278.

Sriram S., Babu S., Jaisankar R., Raguchander T., Vidhyasekaran P., Muthukrishnan S., Samiyappan R. 2000. Degradation of phytotoxin produced by *Rhizoctonia solani*, the sheath blight pathogen in rice. Can. J. Microbiol. 46 (6): 520–524.

Subbaraja K.T., Pillayarswamy K. 1973. Studies on toxin production by four fungi causing fruit rot of chilli. Annamalai Univ. Agric. Res. Annual. 5: 107–116.

Thirupathiah V., Subramanian D. 1974. A 'killing factor' produced by *Colletotrichum capsici* (Syd.) Butler and Bisby in culture. J. Indian Bot. Soc. 53: 261–264.

Vidhyasekaran P., Subramanian C.L., Govindaswamy C.V. 1970. Production of toxins by seed-borne fungi and its role in paddy seed spoilage. Indian Phytopath. 23: 518–525.

Vijayalakshmi M., Rao A.S. 1988. Toxin production by *Alternaria alternata* pathogenic to brinjal (*Solanum melongena* L.). Curr. Sci. 57: 150–151.

Walch H., Khulwein H. 1968. On cellulolytic activity in the genus *Ganoderma*. Arch. Microbiol. 61: 373–380.

Wheeler H., Luke H.H. 1963. Microbial toxins in plant diseases. Annu. Rev. Microbiol. 17: 223–242.

Williams P.H., Heitefuss R. 1976. Physiological Plant Pathology. Springer Verlag, Berlin, 306 pp.

Wood R.K.S., Balio A., Graniti A. 1972. Phytotoxins in Plant Disease. Academic Press, New York, 530 pp.

POLISH SUMMARY

WYTWARZANIE ENZYMÓW DEGRADUJĄCYCH ŚCIANY KOMÓRKOWE ORAZ TOKSYN PRZEZ *COLLETOTRICHUM CAPSICI* I *ALTERNARIA ALTERNATA*, CZYNNIKI SPRAWCZE ZGNILIZNY OWOCÓW CHILI

Wirulentne izolaty *Colletotrichum capsici* i *Alternaria alternata* wytwarzały więcej enzymów celulolitycznych C_1 i C_x niż awirulentne izolaty *in vitro*, a aktywność tych enzymów wzrastała z wiekiem kultury. Wirulentne izolaty wytwarzały więcej enzymów pektolitycznych (enzymy powodujące maceracje, metylestraza pektynowa i poligalakturonaza), niż izolaty awirulentne. Wszystkie enzymy wytwarzane przez izolaty wirulentne były wysoce aktywne w 10-dniowej kulturze i ich aktywność spadała wraz z wiekiem kultury. Jednocześnie aktywność enzymów wytwarzanych przez izolaty wirulentne nie zmniejszała się, a wzrastała wraz z wiekiem kultury. Powyższe patogeny wytwarzały także niespecyficzne toksyczne metabolity obecne w filtratach kultur, które redukowały kiełkowanie nasion, długość korzenia, długość pędu i wskaźnik wigoru chili, ryżu, fasoli tycznej, kukurydzy, bawełny, orzecha ziemnego, oberżyny, ogórka, pomidora i *Hibiscus aesculentus.* Toksyny badanych patogenów powodowały również zamieranie siewek w wazonach oraz powstawanie objawów fitotoksyczności na zielonych i dojrzałych owocach i liściach chili.