# Influence of culture filtrate of seed-borne Colletorichum demetium and Alternaria alternata on chilli seed germination

USHA BHALE, M.S. BHALE AND M.N. KHARE

Department of Plant Pathology, J.N. Krishi Vishwa Vidyalaya, Jabalpur 482004

Colletotrichum dematium and Alternaria alternata were found to be associated with chilli seeds in Madhyay Pradesh and were responsible for severe seed rot and seedling decay. The production of toxic metabolities of these two seedborne pathogens was determined by adopting the bioassay method inhibitory to seed germination and root/shoot elongation. Culture filtrate of C. dematium obtained from 13 day incubation in potato-sucrose broth was most inhibitory (50%) to seed germination and seedling development while culture filtrate of A.. alternata from 15 day incubation in Richards medium was most inhibitory (69.42% inhibition).

Key words: Colletotrichum dematium, Alternaria alternata, culture filtrate, influence, chilli seed

## INTRODUCTION

The concept that seed-borne mycoflora cause diseases by producing toxic substances is well established (Neergaard, 1977; Owens, 1969; Ciegler et al., 1971; Sadashivan and Kalyansundaram, 1965). If is already known that Colletotrichum dematium and Alternaria alternata reported to cause severe seed-rot and seedling decay of crops under central Indian conditions. The seed-borne nature of C. dematium and A. alternata in chillies had been observed (Kulshreshtha et al., 1976; Siddiqui et al., 1977; Grover and Bansal, 1970; Mridha and Siddiqui, 1989; Showkat et al., 1978; Hashmi, 1989). The pathogens were also found responsible for causing leaf spot and fruit rots limiting the profitable cultivation of chillies in recent years. The present investigation was undertaken to study the influence of toxic effect of culture filtrate of these two fungi on seed germination and seedling development.

# MATERIALS AND METHODS

A bioassay method based on the inhibition of root and shoot elongation (RL and SL) was used (Das and Shrivastava, 1969). An aliquot of 50 ml of potato sucrose broth (PSB) was taken in 150 ml Erlenmeyer flask, inoculated with a disc (5 mm diameter) cut out from developing culture of *C. dematium* and incubated at room temperature (28°C) for different period of time. Richard's medium was used in place of PSB for *A. altrnata* 

The crude culture filtrate (CF) was obtained by filtering through muslin cloth, cotton pad and finally seitz filter after various incubation periods. Twenty five surface sterilized

chilli seeds free from any apparent infection by *C. dematium* and *A. alternata* were soaked for 4 h in sterile plates containing the CF. The non-association was already confirmd by testing through incubation on moist blotters. The CF treated seeds were plated on moist blotters following standard blotter method (ISTA, 1985). The seed soaked in sterile distilled water served as control. Parallel studies were also made by using different dilutions of CF in sterile distilled water.

## RESULTS AND DISCUSSION

Influence of culture filtrate of C. dematium

Higher germination (90%) was observed in control seeds soaked in sterile water as compared to those soaked in all ages of CF. Most effective inhibition (50%) was recorded when the chilli seeds were soaked in 13 days old CF. However, the extent of inhibition was lower in CF from 17 days old CF onwards and germination increased (Table 1) progressively indicating gradual loss in toxicity of the older CFs.

Table 1: Influence of culture filtrate of Colletorichum dematium on germination of chilli seeds

Age of CF	Seed germin	nation (%)	Reducation of seed
(days)	CF	SW	grmination. (%)
5	72	'90	20.0
9	59	90	34.5
13	45	90	50.0
17	57	90	36.6
21	65	90	27.7

CF: Crude culture filtrate

SW: Sterile water

The most effective culture filtrate obtained after 13 days incubation when diluted (25% and 50%) exhibited lower toxicity in terms of RL and SL elongation. The RL (0.3 cm) and SL (0.5 cm) was recorded in seeds soaked in cent per cent CF while in control RL (5.6 cm) and SL (3.10 cm) was recorded (Table 3).

Table 2: Influence of culture filtrate of Alternaria alternata on germination of chilli seeds

Age of CF	Seed germination (%)		Reduction of seed	
(days)	CF	SW	germination (%)	
luit .				
5	58.0	85.0	31.77	
10	42.0	85.0	50.59	
15	26.0	85.0	69.42	
20	31.0	85.0	63.53	
25	39.0	85.0	54.12	
30	43.0	85.0	49.42	

CF: Crude culture filtrate

SW : Sterile water

Table 3: Effect of culture filtrate of seed-borne C. dematium and A. alternata on root-shoot elongation

Dilution of	fCF	Culture filtrate				
(%)		C. dematium		A. alternata		
		RL	SL	RL	SL	
Control	0	5.60	3.10	4.90	3.25	
	25	3.90	1.50	4.90	3.15	
	50	2.20	1.00	1.15	2.00	
	100	0.30	0.50	0.25	0.47	

RL: Root length (cm)

SL: Shoot length (cm)

Influence of culture filtrate of A. alternata

Higher (85%) seed germination was recorded when seeds were soaked in sterile water as compared to seeds soaked in CF of different ages. Maximum inhibition (69.42%) in seed germination was observed when seeds were soaked in CF of 15 days old culture. However, seed germination gradually increased when seeds were treated with CF of more than 15 days old culture (Table 2).

Here also the most inhibitory CF obtained from 15 days old culture when diluted (25% and 50%) exhibited lower toxicity in terms of RL and SL (Table 3).

In the present studies it was observed that both the seed-borne fungi produced toxic metabolities which inhibited the seed germination and also had the inhibitory influence on root and shoot development. Colletorichum fuscum was reported by Lewis and Goodman (1962) to produce toxin colletotin whereas Powell and Whalley (1959) and McMillan and Pryce (1968) mentioned the production of macrocyclic carcenogenic lactones as colletodiol by C. capsici. Progressive inhibition of seed germination, root elongation and production of non-host specific toxins was also observed by Sahni et al. (1974). Alternaria alternata was also reported to produce toxins (tentoxin) which caused chlorosis while Meronuck et al. (1972) recorded production of tenuazonic acid. The production of toxin and its effect was studied by Siddaramaiah and Hegde (1984), Templeton et al., (1967) while Mehan and Murphy (1947) and Sahni et al., (1974) observed the progressive loss in seed grmination and root elongation.

#### REFERENCES

Ciegler, A., Kadis, S. and Aji, S.J. (eds.) (1971). A comprehensive treatise. Microbial Toxins VI. Fungal Toxins. Academic Press, New York. p. 563.

Das. A.M. and Shrivastava, D.N. (1969). Production and action on wheat seedlings of toxic metabolites of *Helminthosprium sativum*. Ann. Phytopath. Soc. Japan, 35: 275-281.

Grover, R.K. and Bansal, R.D. (1970). Seed-borne nature of *Colletotrichum capsici* on chilli seeds and its control by seed dressing fungicides. *Indian Phytopathol.* **23(4)**: 664-668.

Hashmi, M.H. (1989). Seed-borne mycoflora of *Capsicum annuum* L. *Pak. J. Bot.* **21(2)**: 302-308. International Seed Testing Association (1985). *International Rules for Seed Testing. Rules.* 1985 (ed.) S.R. Draper. International Seed Testing Association Zurich, Switzerland p. 1-570.

0

Kulshrestha, D.D., Mathur, S.B. and Neergaard, P. (1976). Indentification of seed-borne species of *Colletotrichum. Friesia* 11: 116-125.

Lewis, S. and Goodman, R.N. (1962). Morphological effects of colletotin on tomato and *Digitalis* foliage. *Phytopathology*, **52**: 1272-1276.

McMillan, J. and Pryce, B.J. (1968). Tetrahedron letters In Comprehensive treatise Microbiol. Toxins VI Fungal Toxins (ed. Ciegler et al., 1971). Academic Press, New York p. 563.

Meehan, F. and Murphy, M.C. (1947). Differential phyto toxicity of metabolic byproducts of *Helminthosporium victorae*. Science 106, 270.

Meronuck, R.A., Steel, J.A., Mircha, C.J. and Chriestensen, C.M. (1972). Tanuazonic acid, a toxin produced by Alternaria alternata. Appl. Microbiol. 23(3): 613-617.

Mridha, M.A.U. and Siddiqui, A.B.M. (1989). Fruit rot disease of chilli in relation to seed infection. Seed Res. 17(1): 80.

Neergaard, P. (1977). Seed Pathology. McMillan Press, London, UK.

Owens, L.D. (1969). Toxin plant disease structure and mode of action. Science 165: 18-25.

Powell, J.W. and Whalley, W.B. (1959). Fungal toxins. *In Comprehensive treatise microbial toxin* VI (eds. Ciegler *et al.*, 1971). Academic Press, New York p. 563.

Sadashivan, T.S. and Kalyansundaram, R. (1965). Phytotoxin and toxaemia. *Beit. Bioch. Physiol.*Naturst. 65: 379-386.

Sahni, S.S., Chahal, D.S. and Singh, N. (1974). In vitro production of toxic metabolities by different species of Colletotrichum. Ind. J. Mycol and Plant Pathol 4: 222-223.

Showkat, A.L.B., Michail, S.H., Tarabeih, A.M. and Alza rari, A.J. (1978). Alternaria fruit rot of pepper. Acta Phytopath. Acad. Sci. Hunga. 13(3/4): 349-355.

Siddaramaiah, A.L. and Hegde, R.K. (1984). Ocurrence of *Alternaria alternata* (Kr.) on safflower and sesame from India. *J. Oilsed Res.* 1:83.

Siddiqui, M.R., Singh, D. and Gaur, A. (1977). Prevelance of chilli anthracnose fungus on seeds and its effective control. *Seed Res.* **5(1)**: 67-72.

Templeton, G.E., Grabbe, C.L., Fulton, N.D. and Bollenbacher, K. (1967). Factors affecting amount and pattern of chlorosis caused by metabolities of *Alternaria tenuis*. *Phytopathology* 57: 516-518

(Accepted for publication 9 September 1998)