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Efficacy of methods in the detection of *Colletotrichum dematium* associated with chilli seeds

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The detection of *Colletotrichum dematium* (Pers, ex Fr.) Grove associated with chilli (*Capsicum annuum L.*) seeds was tried in 12 ways using standard blotter and agar plate methods and their modifications. A modified deep freeze blotter was the superior to other methods as it permitted to detect 31.0 percent *C. dematium* associated with seeds. The fungus was found responsible for seed rot, seedling decay, die back and fruit rot .

Key words: Detection methods, *Colletotrichum dematium,* chilli seeds

INTRODUCTION

Chilli *(Capsicum annuum* L.) is a major spice used as a condiment as well as vegetable in every household in India . The nutritive value and medicinal use of chilli are well accepted being the unique source of capsaicin, good source of vitamin C,A,P. and rutin (Hosmani, 1993; Tiwari, 1990; Singh , 1989). The crop suffers due to number of diseases which render its production into stake (Singh, 1987), many of them are seed-borne in nature (Nobel and Richardsoon, 1968).Seed rot, seedling decay, die back, fruit rot and anthracnose incited by *Colletotricum dematium* is a limiting factor in Madhya Pradesh (Agrawal *et al.* 1959; Verma, 1973). Considering the importance of *C. dematium* studies were undertaken to find a suitable and economical methods for its detection. The seed-borne nature of the fungus is established (Kulshreshtha *et al.*, 1976 ; Monoharachari and Padmavati, 1976 ; Siddiqui *et al.*, 1977).

MATERIALS AND METHODS

In a previous study 16 fungi were found associated with 36 chilli seed samples by us. Based on the maximum natural infection of *C. dematium* four sample were selected for detection. Detection was made under stereoscopic binocular microscope after incubation for 7 days under alternate cycles of 12 h light and 12h dark periods. Blotter and agar plate methods and their modification were tried.

Blotter methods

(1) *Standard Blotter method (*ISTA, 1993*)* : Twenty five seeds were plated on moist blotters in each plate and incubated for 7 days . Testing was done on untreated and seeds treated with 0.1 percent mercuric chloride followed by washing with sterile water.

(2) *2,4-D method* (Neargaard,1973) *:* The method was used in two ways : (a) seeds were soaked in 200 ppm, 2,4-Ddichlorophenoxy acetic acid for 10 minutes and placed on moist blotters in petridish and (b) the blotters were also soaked in 2,4-D solution of the same strength and seeds were plated .

(3) *Deep Freezing Blotters* (Limonard , 1966)*:* Seeds were plated as in the standerd blotter method, plates were kept in the incubation chamber for 24 h then transferred to freezer (-20°C) for the next 24 h, retransferred to the incubation chamber for five days. This method was modified by using 200 ppm streptopenicillin to soak the blotters . The rest of the method was the same.

(4) *Modified blotter method :* The pH of the water used for wetting the blotters was changed and pH levels of 6,7 and 8 were adjusted by 0.1N NaOH and O. 1N

HCL . The pH of the normal water was 7.2 used in standard blotter method .

Agar plate method

Potato-sucrose agar (PSA) medium was used instead of PDA . Twenty ml medium was used in each sterile plate and 10 pretreated seeds were plated at equal distance . Pretreatment of seeds with 0.1 percent mercuric chloride solution for 1 minute and three washing with sterile distilled water was done prior to plating . The method was also modified by incorporation of chilli seed extraction in the medium.

RESULTS AND DISCUSSION

Comparative efficacy of different methods for the detection of *C. dematium* associated with chilli seeds was tried in 12 ways (Table I). The association of *C. dematium* ranged from 11.0 to 31.0 per cent as detected by different methods. Minimum 11.0 per cent seeds exhibited the association of the fungus when tested by agar plate method. Modified deep freezing blotter method was the best as the maxium 31.0 per cent seeds exhibited the association of *C. dematium* where the blotters were dipped in streptopenicillin (200 ppm) and after 24 h of initial incubation, petriplates with seeds were transferred to low temperature for 24 h,then reincubated for 5 days in incubation chamber. The efficacy of this method is due to the reason that it did not allow the seed to germinate, because of low temperature the embryo was killed, however, the bacterial contamination due to condensation and other reasons was noticed. This was overcomed by use of streptopenicillin solution, which took care the growth of Gram-positive and Gram-negative bacteria in the plate and seed as well. This method provided maximum chances to *C. dematium* associated with chilli seeds,hence considered the best. Detection methods for *Colletotrichum* spp.have been tried in other crops (Singh, 1977) and recorded this method to be the best for *C. dematium* from urid (*Phaseolus* sp.) seeds while Hagborn *et al.* (1950) used 2,4-D method for *C. lindemuthianum*, whereas Agrawal *et al.* (1972) recommended blotter method for *C. truncatum.*

Table 1 Comparative efficacy of various methods in the detection of *Colletotichum dematium* associated with chilli seeds.

Methods	Per cent association of *C. dematium*		
	Average	Minimum	Maximum
Standard Blotter			
(i) Untreated seeds	23.2	20.0	27.0
(ii) Pretreated seeds	24.0	22.0	27.0
Agar Plate			
(i) On PSA	12.7	11.0	18.0
2 . 4-D (200 ppm)			
(i) Seed soak	24.7	17.0	29.0
(ii) Blotter dip	21.5	19.0	23.0
Deep Freeze Blotter	27.7	23.0	30.0
Modified Standard Blotter			
(Level of water)			
pH 6.00	20.7	18.0	23.0
7.00	20.7	17.0	24.0
8.00	19.2	18.0	20.0
Agar plate			
(i) PSA + Chilli seed extract	20.7	18.0	22.0
Modified Deep Freeze			
(i) Blotter dip in streptopenicillin (200 ppm)	28.2	27.0	31.0
(ii) sterile water	25.7	22.0	29.0

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