

EFFECT OF PRE- AND POST-INOCULATION FACTORS ON INFECTION OF COCOA BY *COLLETOTRICHUM GLOEOSPORIOIDES**

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Abstract : Five-day old cultures of *Colletotrichum gloeosporioides* were more virulent. The infection increased linearly with the increase in time of exposure to high RH and exposure to high humidity for 72 h was ideal. Oat meal agar was the best medium for sporulation. Conidia produced in this medium showed higher germination and degree of infection. Conidia germination was maximum in tap water as also the degree of infection. Disease development was the highest on 18-day old cocoa seedlings.

Keywords : Pre- and Post-inoculation factors, *Colletotrichum gloeosporioides*, Cocoa

Colletotrichum disease of cocoa (Reddy and Chandra Mohanan, 1976; Sarma and Nambiar, 1976) has in the recent years, increased in all the cocoa-growing areas of South India (Chandra Mohanan, 1983). Earlier studies have revealed that the culture media, age of the culture, humidity, temperature and age of the host plant play a major role in the disease development (Chand *et al.*, 1968; Collins, 1974; Welty and Collins, 1979; Eisensmith *et al.*, 1982). During the studies on pathogenicity of *Colletotrichum gloeosporioides* Penz. on cocoa, a high degree of variation in the disease development was observed even when seedlings raised from the beans of the same pod were used for inoculation. This may produce inconsistent results while screening cocoa germplasm for resistance to *C. gloeosporioides* infection. Hence, the present study was undertaken to find out the influence of various factors on foliar infection of cocoa seedlings inoculated with *C. gloeosporioides*.

MATERIALS AND METHODS

In the first set of experiments, the influence of age of the culture and duration of incubation of inoculated seedlings under high humidity for disease development was determined.

Age of the culture : *C. gloeosporioides* was isolated from cocoa leaves showing shot hole symptoms. Cocoa seedlings were raised by sowing pregerminated beans (Chandra Mohanan and Kaveriappa, 1984) and twenty-day old seedlings (ten) were inoculated with a suspension of 1×10^7 conidia/ml prepared in sterile distilled water (SDW) separately from 5-, 10- and 15-day old cultures on potato dextrose agar (PDA) medium. Both the surfaces of leaves were inoculated by spraying the conidial suspension. Equal number of seedlings sprayed with SDW alone served as control. After inoculation, each seedling

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was covered with a polythene bag containing two pieces of cotton pads moistened with 50 ml SDW to maintain high humidity. The inoculated seedlings were transferred to a moist chamber built of areca palm leaves on top and four sides (Chandra Mohanan and Kaveriappa, 1984). The polythene covers were removed from the seedlings 48 h after inoculation. The temperature and RH inside the moist chamber were recorded continuously with a thermohygrograph.

The seedlings were scored eight days after inoculation, when maximum lesions developed (Chandra Mohanan and Kaveriappa, 1984). The infection index of each seedling was calculated (McKinney, 1923). The mean infection index of 10 seedlings was recorded. Since the seedlings inoculated with 5-day old cultures showed maximum infection, such cultures were employed in further experiments.

High humidity : All the treatments were replicated thrice with 10 seedlings per replication. Since maximum disease index was obtained by 72 h incubation under high humidity, the same period of incubation was used for further experiments.

Culture media, conidial suspension and age of seedlings : Oatmeal agar (OMA) and PDA were prepared by following the standard methods (Kiraly *et al.*, 1974). For tender cocoa leaf medium (TCL), pale green tender leaves were collected from a 'Forestero' cocoa plant and cut into fine bits; lg of leaf was taken in each test tube with 2.5 ml distilled water and sterilized at 15 p.s.i. for 15 min. For the assessment of sporulation and germination of conidia, four ml SDW was added separately to the cultures in each of the three media. The test tubes were shaken well for five minutes on a mechanical shaker to dislodge the conidia and spore concentration in each of these was determined by a haemocytometer. Three samples were drawn from each test tube and three tubes were maintained for each medium. The average of the counts of three tubes was taken for the assessment of sporulation.

The representative samples of spores from the above cultures were taken and then germination was tested in tap water (TW), SDW and 1 per cent dextrose (1 per cent D). The per cent germination was determined from a total of 150 conidia per replication.

Pathogenicity : The experiment was laid out in a factorial randomized block design with three culture media \times three types of spore suspension \times three ages of seedlings \times three replications (Table 1). Ten seedlings were included in each replication. Disease severity was rated eight days after inoculation and the disease index was calculated. The data were analyzed statistically.

RESULTS AND DISCUSSION

Mean, minimum and maximum temperatures which prevailed inside the moist chamber during inoculation trials were 24.6, 19.0 and 29.0°C respectively while the corresponding RH was 89.5, 55 and 100 per cent respectively.

Age of culture

The disease index was maximum (13.4 per cent) when 5-day old cultures were used; whereas the disease index was 9.5 and 7.16 per cent when 10- and 15-day old cultures were used respectively. Collins (1974) concluded that spores from different cultures

TABLE 1 : Effect of culture media, conidial suspension and age of cocoa seedlings on *C. gloeosporioides* infection

Medium	Sporulation† (× 10,000)	Media for conidial suspension	Germination* of conidia (per cent)	Infection index (per cent)*			Mean of three groups
				15-day old	18-day old	20-day old	
PDA	10.98	Tap water	68.51	33.33	41.67	16.67	30.56
		Sterile distilled water	48.54	27.78	33.33	13.89	25.00
		1 per cent dextrose	62.96	22.22	30.55	13.89	22.22
		Mean	60.0	27.78	35.18	14.82	25.93
OMA	31.14	Tap water	97.59	55.55	76.39	22.22	51.39
		Sterile distilled water	97.38	27.78	38.89	16.67	27.78
		1 per cent dextrose	95.50	27.78	41.67	19.45	29.63
		Mean	96.82	37.03	52.32	19.44	36.27
TCL	15.72	Tap water	10.47	30.55	38.89	13.89	27.78
		Sterile distilled water	1.69	19.45	25.00	11.11	18.52
		1 per cent dextrose	75.93	22.22	23.61	8.33	18.05
		Mean	29.36	24.07	29.17	11.11	21.45
Mean	—	—	29.63	38.89	15.12	—	

Media	Sporulation	Germination	Infection index
CD (P = 0.05) for media	2.38	2.96	3.45
CD (P = 0.05) for conidial suspension	—	2.96	3.45
CD (P = 0.05) for age group of seedlings	—	5.13	3.45
CD (P = 0.05) for media × conidial suspension	—	—	5.98
CD (P = 0.05) for media × age group of seedlings	—	—	5.98
CD (P = 0.05) for conidial suspension × age group of seedlings	—	—	5.98
CD (P = 0.05) for media × conidial suspension × age group of seedlings	—	—	5.98

*Mean of 3 replications
†mean of 9 replications

(*Colletotrichum trifolii* Bain. & Essary) which are more than 7-day old should not be used for disease screening. Five-day old cultures of *Gloeosporium fructigenum* Berk. gave maximum infection on apple fruits and virulence of the fungus decreased with aging (Chand *et al.*, 1968).

High humidity

When inoculated seedlings were incubated for different periods under high humidity, those incubated for 72 h developed a maximum infection of 17.33 per cent, whereas the disease index was 1.95 and 11.75 per cent when incubated for 24 and 48 h respectively. The seedlings could not be exposed to high humidity for more than 72 h as the tender leaves became turgid and showed symptoms like wilting when incubated for 96 h. The degree of infection also did not increase after 72 h. When inoculated seedlings were not covered with moist polythene bags, no infection was observed. Thus maximum infection was obtained with 5-day old cultures and 72 h incubation under high humidity. The seedlings covered with polythene bags to maintain high humidity always formed a thin film of water on the surface of leaves, which might have facilitated the conidial germination and subsequent infection. This is possibly the reason why no infection was observed on seedlings which were kept without polythene coverings outside the moist chamber. Singh (1973) has reported that in *Colletotrichum falcatum* Went., high humidity and contact with water were necessary for germination of conidia.

Effect of culture media, conidial suspension and age of seedlings

Analysis of the data (Table 1) indicated that infection index varied significantly with the media used, medium of conidial suspension and age of the seedlings. Among the three media used, sporulation was significantly higher on OMA followed by TCL.

Welty and Collins (1979) reported lima bean agar as the best medium for culturing *C. trifolii* for pathogenicity tests on alfalfa. Interaction between culture media and media for conidial germination was significant. Even the percentage germination of conidia varied with the culture media when tested on TW, SDW or 1 per cent D (Table 1). The germination of conidia obtained from OMA was the highest. Conidia from OMA showed maximum germination in TW and was closely followed by SDW. The germination of conidia from PDA was significantly high in TW followed by 1 per cent D, whereas conidia from TCL showed significantly high germination in 1 per cent D. The sporulation was significantly high on OMA and germination of conidia produced on this medium was also high. In general, conidia in TW gave higher germination. Barnes (1972) used tap water for preparing conidial suspension for inoculation trials with *Colletotrichum orbiculare* since distilled water was found to reduce conidial germination to a greater extent.

Pathogenicity

The infection was better with the inoculum obtained from OMA followed by PDA and was significantly higher when TW was used for preparing conidial suspension in all the treatments (Fig. 1). Disease index on 18-day old seedlings was significantly high followed by 15-day old seedlings in all the treatments. The 15-day old seedlings had comparatively very small leaves and thus lesser leaf area for infection. The disease index was significantly high on 18-day old seedlings when OMA was used for growing the fungus and TW for preparing the spore suspension.

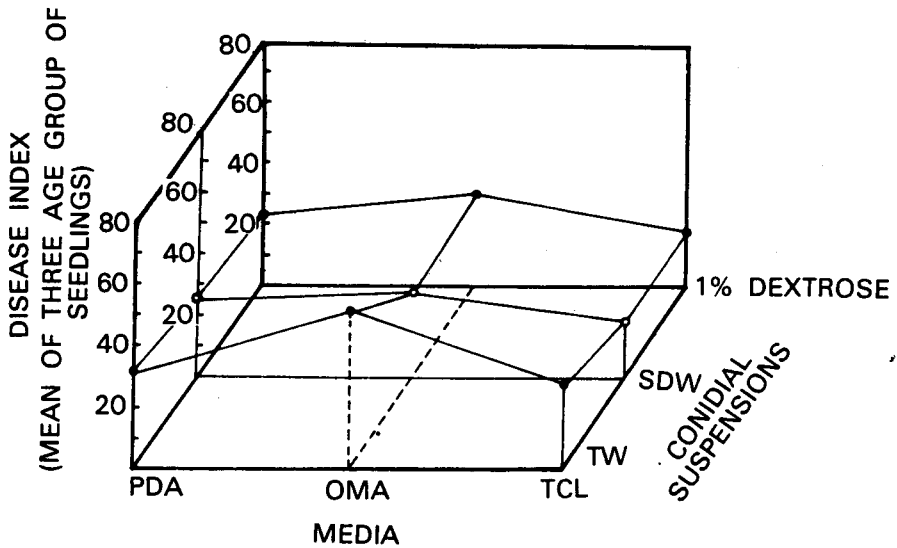


Fig. 1 : Effect of culture media and media for conidial suspension on infection.

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REFERENCES

- Barnes, G. L. (1972). Differential pathogenicity of Oklahoma isolates of *Colletotrichum orbiculare*. *Plant Dis. Repr.* 56 : 35-38.
- Chand, J. N., Kondal, M. R. and Agarwal, R. K. (1968). Epidemiology and control of bitter rot of apple caused by *Gloeosporium fructigenum* Berk. *Indian Phytopath.* 21 : 257-263.
- Chandra Mohanan, R. (1983). Diseases of cocoa and their control measures. *Indian Cocoa Arecanut Spices J.* 7 : 35-37.
- Chandra Mohanan, R. and Kaveriappa, K. M. (1984). Variation in pathogenicity among the isolates of *Colletotrichum gloeosporioides* on cocoa in India. *Proc. 9th International Cocoa Research Conference, Lome, Togo*, 323-326.
- Collins, H. B. (1974). *The nature and inheritance of anthracnose (Colletotrichum trifolii (Bain & Essary) Resistance in alfalfa, Medicago sativa L.* Ph.D. Thesis, N. C. State Univ. Raleigh, 105 pp.
- Eisensmith, S. P., Sjulín, T. M., Jones, A. L. and Cress, C. E. (1982). Effects of leaf age and inoculum concentration on infection of sour cherry by *Coccomyces hiemalis*. *Phytopathology* 72 : 574-577.
- Kiraly, Z., Klement, Z., Solymosy, F. and Voros, J. (1974). *Methods in Plant Pathology*. Elsevier Scientific Publishing Co. London, 509 pp.
- McKinney (1923). Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. *J. agric. Res.* 26 : 195-217.

- Reddy, M. K. and Chandra Mohanan, R. (1976). Occurrence of pod rot of cacao caused by *Colletotrichum gloeosporioides* in India. *J. Plant. Crops* 4 : 14-15.
- Sarma, Y. R. and Nambiar, K. K. N. (1976). Leaf spot of cacao. *Curr. Sci.* 45 : 466.
- Singh, P. (1973). Conidial germination in *Colletotrichum falcatum*—Study of physical factors and standardization of germination techniques. *Acta Phytopath. Acad. Sci. Hungaricae* 8 : 19-41.
- Welty, R. E. and Collins, H. B. (1979). Loss of viability of spores of *Colletotrichum trifolii* produced on lima bean agar. *Plant Dis. Repr.* 63 : 486-489.