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VARIABILITY IN GROWTH OF *COLLETOTRICHUM GLOEOSPORIOIDES* ISOLATES PATHOGENIC ON CACAO IN RESPONSE TO FUNGICIDES, ANTIBIOTICS AND DETERGENT*

R. CHANDRA MOHANAN, K. M. KAVERIAPPA[1] and K. K. N. NAMBIAR[2]
Central Plantation Crops Research Institute, Regional Station, Vittal 574 243, *Karnataka, India*

ABSTRACT

Growth rates of three virulent isolates of *Colletotrichum gloeosporioides* Penz. causing foliar diseases *viz.*, blight, shot hole and irregular spot on cacao were compared on potato dextrose agar medium containing different concentrations of fungicides (Carbendazim and Mancozeb), antibiotics (Nystatin and Actidione) and a detergent (Cetavlon). The three isolates varied markedly from each other in their growth response in the presence of these chemicals. Of the two fungicides tested, the isolates were more sensitive to Carbendazim than Mancozeb. The growth of all the three isolates was completely inhibited in the presence of Carbendazim at 5 and 10 μg/ml medium, whereas the growth of irregular spot isolate was completely inhibited even at 3 μg/ml medium. The isolates were found to be sensitive to Nystatin and Actidione though complete inhibition of growth was not observed at any of the concentrations tested. All the three isolates also showed marked variation in growth in response to Cetavlon. The irregular spot isolate was more sensitive to Cetavlon than the other two.

INTRODUCTION

Colletotrichum disease of cocoa (*Theobroma cacao* L.) is wide spread in South India. It has been found in greater intensities in some of the cacao growing areas. In India, *Colletotrichum gloeosporioides* Penz. has been reported to cause rotting of immature pods and produce three kinds of foliar symptoms *viz.*, blight, shot hole and irregular spot (Chandra Mohanan and Kaveriappa, 1983). The isolates of *C. gloeosporioides* collected from various cacao growing areas of South India were classified into leaf blight, shot hole and irregular spot isolates based on the symptoms produced on cacao leaves. There was great variation in pathogenicity among the isolates of the three groups (Chandra Mohanan and Kaveriappa, 1984). To use fungicides, antibiotics and other antimicrobial compounds more effectively in disease control, it is important to know the intraspecific variations in response to fungicides and other antimicrobial compounds.

MATERIALS AND METHODS

Among the 299 pathogenic isolates of *C. gloeosporioides* collected from different cacao growing areas of Southern India, the most virulent isolates causing blight, shot hole and irregular spot symptoms on cacao leaves were selected for the present studies. The three most virulent isolates TN I/139, Ke II/55 and Ka III/9 causing blight, shot hole and irregular spot on cacao leaves respectively were tested on potato dextrose agar medium (PDA) containing different concentrations of fungicides, antibiotics and a detergent.

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* *Contribution No.* 673, *Central Plantation Crops Research Institute, Kasaragod, Kerala*
1 *Department of Applied Botany, Mangalore University, Mangalagangotri* 574 199, *Karnataka*
2 *Central Plantation Crops Research Institute, Kasaragod* 670 124, *Kerala, India.*

Fungicides: (1) Carbendazim (Bavistin WP) – (technical 99.5%) at 3, 5 and 10 μg/ml medium; (2) Mancozeb (Dithane M–45) (technical 80%) at 500, 800 and 1000 μg/ml.

Antibiotics: (1) Nystatin (mycostatin, B–grade) at 20, 30 and 40 μg/ml of medium; (2) Actidione GR (Cycloheximide Naramycin) at 40, 50 and 60 μg/ml.

Detergent: (1) Cetavlon (Cetyltrimethyl ammonium bromide) – 800, 900 and 1000 μg/ml medium.

The range of concentrations ot the above chemicals were selected on the basis of their effect on other fungi and bacteria and by conducting a pilot study using a range of concentrations. Suspensions of the fungicides, antibiotics and the detergent were added to autoclaved and cooled PDA, immediately, before pouring into petri plates, so as to obtain required concentrations.

The PDA containing the chemicals was dispensed uniformly into 90 mm diam. petriplates at 15 ml per plate. To determine the mycelial growth, each plate was inoculated with a 6 mm diam. disc from the periphery of 10–day–old cultures of each isolate maintained on PDA. Each disc was placed upside down in the centre of the petri plate. Three replications were used for each concentration of the chemical.

The plates were incubated at room temperature (27 $\pm$ 2°C) for 10 days and radial growth of colonies was measured in two directions at right angles to each other and the mean values were taken as the colony diameter for each plate. The mean values were used to find out the per cent inhibition of growth and to find out the variability in the growth rate. The per cent inhibition of growth was calculated by using the following equation (Vincent, 1927) : $I = \frac{100\,(C-T)}{C}$

where, I = inhibition of fungal growth; C = growth in check; and T = growth in treatment.

RESULTS AND DISCUSSION

The three isolates of *C. gloeosporioides* varied markedly from each other in their growth response in the presence of fungicides, antibiotics and the detergent (Fig. 1).

Among the two fungicides tested, the isolates were more sensitive to Carbendazim than Mancozeb. The growth of the isolate Ka III/9 was completely inhibited in the presence of Carbendazim at 3, 5 and 10 μg/ml whereas TN I/139 and Ke II/55 exhibited

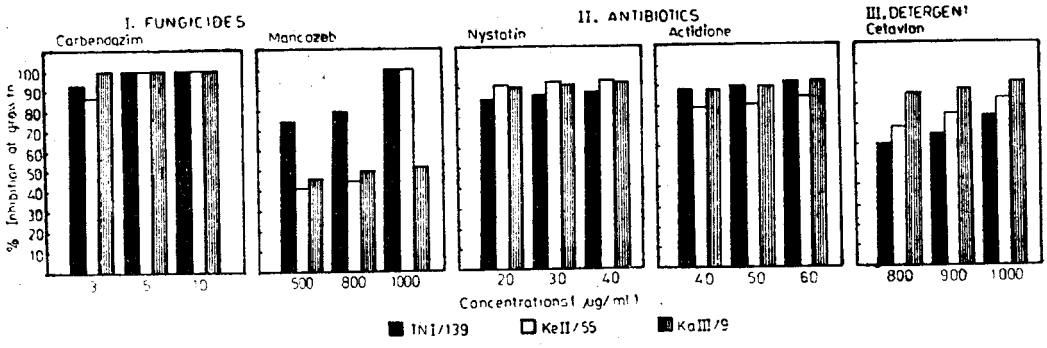

Fig. 1. Variability in growth of three isolates of *C. gloeosporioides* in presence of fungicides, antibiotics and a detergent

slight growth at 3 μg/ml. While Ka III/9 was moderately tolerant to Mancozeb at 1000 μg/ml, the growth of other two isolates was completely inhibited at 1000 μg/ml concentration. The isolate TN I/139 was more sensitive to Mancozeb, the growth being inhibited to an extent of 74 to 79 per cent at 500 and 800 μg/ml respectively. Isolates tolerant and sensitive to Carbendazim have been reported in *Helminthosporium maydis* Nishikado & Miyake pathogenic on maize (Bains and Mohan, 1982). The isolate Ka III/9 showed a differential reaction in response to Carbendazim and Mancozeb. It was highly sensitive to Carbendazim and less sensitive to Mancozeb. Such differential reactions have also been reported with some isolates of *Phytophthora cinnamomi* Rands in response to antibiotics (Leary et al., 1982).

All the isolates were sensitive to Nystatin and Actidione. Complete inhibition of growth of the isolates was not observed in the presence of these two antibiotics at the concentrations tested. The rate of growth of the isolates Ke II/55 and Ka III/9 did not vary much in the presence of Nystatin at 20, 30 and 40 μg/ml. They were more sensitive than TN I/139. In the presence of Actidione the growth was more in Ke II/55 than the other two isolates at the three concentrations tested. Most of the studies on variability in the growth of the isolates in response to antibiotics were conducted on *Phytophthora* sp.(Zentmyer, 1955; Vaarataja, 1960; Leary et al., 1982). Majority of the isolates of *P. cinnamomi* showed complete inhibition in growth in the presence of 10 μM Cycloheximide. On the other hand the growth of all *P. cinnamomi* isolates tested was faster on media containing Nystatin than any normal media (Leary et al., 1982). In the present study, though all the isolates of *C. gloeosporioides* were sensitive to the two antibiotics tested TN I/139 was comparatively more tolerant to Nystatin and Ke II/55 was more tolerant of Actidione. In the presence of Nystatin Ke II/55 and Ka III/9 showed similarity in growth while in the presence of Actidione TN I/139 and Ka III/9 showed a close similarity in growth.

All the isolates showed variation in growth in the presence of Cetavlon. The inhibition was more in Ka III/9 followed by Ke III/55 and TN I/139. Thus there was marked variation in the inhibition of growth of the three isolates on PDA incorporated with Cetavlon at 800, 900 and 1000 μg/ml concentration. Inhibition of growth in the presence of detergents has also been reported in pathogenic fungi like *Sclerotium rolfsii, S. oryzae* and *Macrophomina phaseolina* (Mukherjee, 1974, 1976).

Most of the studies on tolerance to fungicides have been reported from the fungal isolates collected from localities where the fungicides have been in use (Bolton, 1976; Davidse, 1981; Gutter et al., 1981; Papavizas and Bowers, 1981; Webster, Ogawa and Bore, 1970). In such cases the pre-existing tolerance in the natural population could not be determined because the chemicals had already been in use for varying periods before the isolates were collected. Large scale application of fungicides may favour dominance of fungicide-tolerant strains and in due course these tolerant strains may predominate in the natural population posing problems in effective implementation of fungicidal control of diseases. Such problems have been noticed when Benomyl was used in large scale to control plant diseases (Schroeder and Provvidenti, 1969; Bollen and Scholten, 1971; Dekker, 1976). The fungicidal control of *Colletotrichum* disease of cacao has not yet begun in India. There fore, the response of the isolates of *C. gloeosporioides* to the fungicides, antibiotics and detergent noticed in the present studies can be considered as their natural tolerance

rather than the induced tolerance in response to the application of these chemicals.

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