UNIVERSITI PUTRA MALAYSIA

COLLETOTRICHUM DISEASE OF COCOA (THEOBROMA CACAO L.) AND ITS CONTROL

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COLLETOTRICHUM DISEASE OF COCOA (*Theobroma cacao* L.) AND ITS CONTROL

By

YEE MING FATT

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for the Degree of Master of Agriculture Science
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Supervisor : Assoc. Prof. Dr. Sariah Meon

Faculty : Agriculture

Key Words : *Colletotrichum gloeosporioides*, *Theobroma cacao*

Isolation of disease pathogen from infected cocoa leaves and pods associated with leaf spot and pod rot disease yielded *Colletotrichum gloeosporioides*.

No distinct differences in cultural and morphological characteristics were noted from the various isolates. Further investigation by polyacrylamide vertical gel electrophoresis indicated that protein, esterase and peroxidase patterns were unable to differentiate strain variation within *C. gloeosporioides*.

The fungus was found to grow and sporulate well at 30°C. Cocoa Leaf Extract Agar (CLEA) was the best medium for mycelial growth while Potato Dextrose Agar (PDA) appeared to favour

sporulation. Better mycelial growth was also achieved on substrates of neutral condition while extreme alkaline condition induces sporulation.

Both cocoa leaves and injured pods were liable to infection by C. gloeosporioides. Three-week old cocoa seedlings and cherelles were noted as the most susceptible stage.

The development of Colletotrichum leaf spot disease invariably involved three phases of activity viz., pre-penetration phase which involved spore germination and formation of appressoria, penetration phase involving intercellular and intracellular hyphal penetration and post-penetration phase resulting in disintegration of cells and the formation of acervuli.

Several fungicides were screen for their activity against the leaf spot pathogen. The pathogen was extremely sensitive to Benlate (R), Difolatan 4F (R) and Bayleton (R)

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat keperluan untuk ijazah Master Sains Pertanian.

PENYAKIT *COLLETOTRICHUM* PADA KOKO (*Theobroma cacao* L.) DAN PENGAWALANNYA

Oleh

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Mac 1991

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Perkataan Utama : *Colletotrichum gloeosporioides*, *Theobroma cacao*

Pemencilan patogen penyakit daripada daun dan lenggai koko yang dijangkiti sehubungan dengan penyakit bintik daun dan reput lenggai menghasilkan *Colletotrichum gloeosporioides*.

Tiada perbezaan yang ketara ditemui dalam ciri-ciri kultur dan morfologi di antara beberapa pencilan. Kajian lanjutan dengan elektroforesis gel tegak poliakrilamid menunjukkan bahawa corak protein, esterase dan peroxidase tidak dapat membezakan strain di dalam *C. gloeosporioides*.

Kulat didapati hidup dan mengeluarkan spora dengan baik pada suhu 30°C. Agar ekstrak daun koko merupakan media yang terbaik untuk pertumbuhan miselium manakala agar dekstros kentang didapati sesuai untuk pensporulan. Pertumbuhan

miselium yang lebih baik juga didapati pada bahan yang berkeadaan neutral manakala keadaan beralkali meransang pensporulan.

Kedua-dua daun dan lenggai koko yang luka boleh dijangkiti oleh C. gloeosporioides. Anak benih koko yang berumur 3 minggu dan 'cherelles' merupakan peringkat yang paling mudah dijangkiti.

Perkembangan penyakit bintik daun Colletotrichum melibatkan tiga fasa aktiviti iaitu fasa pra-penembusan yang melibatkan percambahan spora dan pembentukan apresorium, fasa penembusan yang melibatkan penembusan haifa di antara sel dan di dalam sel dan fasa pos-penembusan yang mengakibatkan penguraian sel dan pembentukan aservulus.

Beberapa racun kulat telah dikaji keberkesanannya terhadap patogen bintik daun tersebut. Patogen didapati peka kepada Benlate(R), Difolatan 4F(R) dan Bayleton(R).

CHAPTER 1

INTRODUCTION

Cocoa (*Theobroma cacao*), like rubber (*Hevea brasiliensis*) and oil palm (*Elaeis guineensis*), is another crop introduced into this country from the West. Cocoa was first established in Malaysia on a 0.2 hectare plot at the Government's Agricultural Research Station at Serdang, Selangor and the first cocoa planting on a commercial scale was undertaken at Jerangau, Terengganu in 1950. With the rapid diversification of the agricultural sector, cocoa had in recent years become one of the very important crops in Malaysia, both in the plantation sector as well as in the small holdings after rubber and oil palm. The area under cocoa cultivation, both as a monocrop and as an intercrop with coconut has increased significantly from slightly over 500 hectares in 1960 to an estimated area of 311,000 hectares in 1987. Of this total, 67.5% amounting to 210,000 hectares are located in Sabah, 22.2% representing 69,000 hectares in Peninsular Malaysia and the remaining 10.3% involving 32,000 hectares are located in Sarawak. Total production of dry cocoa beans in Malaysia is estimated at 150,000 tonnes or 7.7% of world production, thus ranking Malaysia as the fourth largest world producer of cocoa after Ivory Coast, Brazil and Ghana (Economic Report 1987/88).

Among the major limiting factors in the expansion and exploitation of cocoa in Malaysia at present are some serious pest and disease problems. Major disease problems include canker, seedling dieback and black pod, all caused by Phytophthora palmivora and vascular streak dieback caused by Oncobasidium theobromae (Tang, 1986). However, leaf spot disease and pod rot caused by Colletotrichum gloeosporioides have yet to become major disease problem in Malaysia even though they have been recorded to be serious in several cocoa growing countries in the world (Thorold, 1975; Turner, 1974; Dakwa and Danquah, 1978).

In view of the above, an attempt was undertaken to study the leaf spot and pod rot disease problem with the following objectives:

(1) to establish the identity of the causal organism,

(2) to carry out studies on the etiology of Colletotrichum leaf spot and pod rot disease on cocoa, and

(3) to screen suitable fungicides effective against Colletotrichum leaf spot and pod rot disease of cocoa.

CHAPTER 2

REVIEW OF LITERATURE

The Genus *Colletotrichum*

The genus *Colletotrichum* was first described in 1837 by Corda that contained one species, *C. lincola* (Duke, 1928). In later literature, the genus *Colletotrichum* and its species were described by Arx (1957) and Deighton (1972). Based on cultural studies, Arx (1957) recognised only 20 of the over 1000 form species of *Colletotrichum*. In 1970, Arx revised the genus *Gloeosporium* Desm & Mart and placed 734 species in the genus *Colletotrichum*. The presence or absence of setae in the acervuli to distinguish *Gloeosporium* and *Colletotrichum* was rejected since the formation of the setae was greatly influenced by external conditions, particularly the water pressure deficit of the atmosphere (Frost, 1964). The length of the setae, if present, varies, but is rarely more than 200 um long, 4-8 um wide and has 1-4 septa (Mordue, 1971). The acervuli may develop in a subcuticular or subepidermal fashion and usually setose, though sometimes they are sparsely setose or globose and their shape ranges from round to elongated to irregular. Studies on the species of *Colletotrichum* have shown them to be very variable in their morphological characters (Arx, 1970) and cultural characteristics (Stoneman, 1898; Shear and Wood, 1913; Burger, 1921; Arx, 1957), others were able to

distinguish forms within species (Burger, 1921; Simmonds, 1965) on the basis of this variation, while some others were not successful in classifying them into distinct groups (McDonald, 1926; Wastie and Shankar, 1970).

At present, the taxonomy of the genus Colletotrichum is very much in confusion and the taxonomic status of many species is still uncertain. However, it is known that species of Colletotrichum vary widely in infectivity in nature, behaving from saprophytes to specialised parasites in their plant hosts. In recent years, electrophoretically detectable protein and enzyme variation has been used for fungal classification (Burdon and Marshall, 1983). Electrophoretic mobility in gels of low-porosity is one of the most sensitive parameters related to the structure of a protein, since it is determined by charge pattern and by the shape of the protein molecule in solution. The consequent high resolution of mixtures of protein in low-porosity gels has made this the method of preference in biochemical studies of taxonomy. The electrophoretic patterns of soluble enzyme and other proteins in the fungal cell are some of the most direct manifestations of cell's genetic constitution and different enzyme patterns can have different taxonomic weight even in the same organism (Clare et al., 1968). Some patterns have a high degree of intraspecific variability and may be of value at the subspecific level. Some patterns appear to be characteristic of species whilst

others showed interspecific similarities which indicate that they may be characteristic of genera.

Colletotrichum Leaf Spot and Pod Rot of Cocoa

Colletotrichum has a world-wide distribution on many hosts including coffee, tea, rubber, chilli, citrus, mango, papaya, pepper, tomato, vine, yam, banana, avocado, cocoa, apple, pear, cherry and wild plants (Mordue, 1971). *Colletotrichum* leaf spot of cocoa was first reported by Delacroix from Antilles in the French colonies of Africa as early as 1905. According to Sanchez (1953), in the Cauca valley there are no cocoa plants free from this disease. Malaguti and Camero (1960) reported that in Venezuela, nursery plants are often attacked by *Colletotrichum*. In India, its occurrence was reported for the first time in 1976 (Sarma and Nambiar, 1976; Reddy and Mohanan, 1976) where infection occurs on leaves, stems and pods of cocoa. In Central and South American countries, it was reported to cause pod rot and also drying of leaves (Newhall, 1948). In 1974, a nursery in North Sumatra was reported to be severely infected by *Colletotrichum* where the seedlings were raised under mature rubber trees showing foliar infection due to the same pathogen (Turner, 1974). *Colletotrichum* was found associated with cocoa dieback in Bahia, Brazil (Ram *et al.*, 1972) and in Central and South America (Tollenaar, 1959; Newhall *et al.*, 1968). In Malaysia, *Colletotrichum* leaf spot (Lin and Liew, 1975) and rotting of

cherelles and immature pods (Williams and Lin, 1976) were observed in some plantations in Sabah. In Ghana, Dakwa and Danquah (1978) claimed that Colletotrichum causes leaf blight symptom on cocoa leading to heavy defoliation in some of the plantations. Colletotrichum disease of cocoa has also been recorded in several cocoa growing countries like Trinidad, Sri Lanka, Cameroon, Nicaragua, Phillipines, Nigeria, and Costa Rica (Thorold, 1975). Various species of Colletotrichum have been reported to infect cocoa. They are C. theobromae, C. luxificum, C. cradwickii, C. incarnatum (Briton-Jones, 1934); C. fructitheobromae, C. theobromicalum (Thorold, 1975); C. gloeosporioides (Ram et al., 1972; Diaz and Newhall, 1966) and C. crassipes (Ram et al., 1973). In most of the cocoa plantations, it has not been observed to cause very serious problems, but the disease was reported to have attained epiphytotic proportions in Ghana in 1975 (Dakwa and Danquah, 1978).

Symptomatology

Studies conducted by Mohanan (1983), Mohanan and Kaveriappa (1983), Sarma and Nambiar (1976), Dakwa and Danquah (1978) and Tang (1986) on the symptomatology of Colletotrichum disease of cocoa revealed the occurrence of three types of foliar symptoms and a pod rot:

Shot-Hole

The infection occurred anywhere on the leaf lamina and many spots were found on each leaf. They appeared as minute pin-point size, round, sunken, light brown spot with a distinct yellow halo. When such spot attained 4-6 in mm diameter, the centre of the necrotic spot shrivelled and dropped-off forming shot-hole. The spot when enlarge, coalesced with adjacent spot to form bigger spot occupying a considerable area of the leaf lamina. In the advance stage of infection, shrivelling of the leaves could be observed. In very severe cases, defoliation occurred.

Leaf Blight

The infection was found to be initiated anywhere on the leaf lamina, but more usually from the tips or margins. The symptom appeared as round to slightly irregular chlorotic spot of 2-5 mm in diameter which then turned brown with a clear yellow halo around each spot. The spot could increase in size considerably and coalesced with adjacent spot to form large blighted areas with an even margin. Later, defoliation occur when such lesions covered a major portion of the leaf.

Irregular Spot

The infection appeared anywhere on the leaf lamina as small, circular to irregular, black spot with a broad and

bright yellow halo. Later, the spot increase in size longitudinally and irregularly along the veins and was observed to attain a diameter of 10-25 mm. The centre of the spot turned dark brown to black and then greyish.

Pod Rot

Their symptom generally starts from the stalk level, particularly at the point of attachment of the stalk to the pod. The infection then proceed towards the tip of the pod as dark brown discolouration with a diffused yellow halo. The infection also extended to the stalk and reaches the cushion. The infected stalk become highly shrunken and was easily distinguished from a healthy stalk. The internal tissues of the stalk turned brown to dark brown. As the infection progressed, the internal tissue also become discoloured; the discolouration within was faster than that of the outside. Finally, the whole pod shrunk, turned dark brown and black. They remained as mummified fruits.

Epidemiology

Weather plays an important role in the development of Colletotrichum diseases; damp conditions being essential for the spread of the fungus and for infection to take place (Wastie and Shankar, 1970). In wet weather, the spore masses produce on leaves and pods were softened and easily released,