
UNIVERSITI PUTRA MALAYSIA

# COMPARATIVE STUDIES OF PHYTOPHTHORA PALMIVORA FROM COCOA AND DURIAN AND THEIR CONTROL

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# COMPARATIVE STUDIES OF *PHYTOPHTHORA PALMIVORA* FROM COCOA AND DURIAN AND THEIR CONTROL

by

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A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in the Faculty of Agriculture, University Pertanian Malaysia

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TABLE OF CONTENTS

LIST OF TABLES

# LIST OF FIGURES

# LIST OF PLATES

An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia in partial fulfilment of the requirements for the Degree of Master of Agricultural Science.

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Phytophthora palmivora (Butl.) Butler is known to be a variable organism and is considered to be one of the most destructive pathogens of cocoa and durian which are being intercropped in Malaysia. Studies were undertaken to compare the cultural characteristics, sexuality, and pathogenicity of six Phytophthora isolates from these crops as well as the in-vitro and in-vivo efficacy of fungicides against the pathogen. Preliminary studies on the use of a bio-control agent against the organism were also looked into.

Cultural studies established that the Phytophthora isolates belonged to MF1 P. palmivora. They produced stellate and striate

colonies on carrot, cornmeal, and V-8 juice agar media. Mycelial growth was optimum at 28 ± 1.5C. No growth occurred at 36C. Sporangia were caducous, with length:breadth ratios from 1.22 to 1.8 and possessed short, occluded pedicels. Abundant chlamydospores were produced in culture.

The isolates required another compatible isolate to form oospores. Exposure to Trichoderma did not result in sexual reproduction. When paired with standard testers, the durian and cocoa isolates were identified as A1 and A2 compatibility types, respectively.

The isolates were host specific. Inoculation of roots, stems, leaves and fruits of their respective host plants resulted in infection.

In-vitro efficacy studies of the chemicals indicated that metalaxyl, etridiazole, and captafol were inhibitory on mycelia, sporangium and chlamydospore formation, and sporangium germination of the isolates. Captafol was most inhibitory on zoospore germination. Cyprofuram and benalaxyl had moderate effects whereas poor activity was observed for phosethyl-Al and propamocarb.

Metalaxyl-mancozeb mixture (Ridomil-MZ) maintained its superiority as a protectant and therapeutant on cocoa and durian seedlings when sprayed or drenched. Phosethyl-Al and cyprofuram also exhibited good activity. With the exception of propamocarb which had negligible effects, the rest of the fungicides could be considered as an alternative. Captafol was effective only as a protectant.

*Gliocladium roseum* was found parasitizing *P. palmivora* from durian fruit. Scanning and transmission electron microscopy revealed that sporangia and chlamydospores were parasitized. The use of this mycoparasite as a bio-control agent, however, requires further investigations.

Abstrak tesis yang diserahkan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi sebahagian dari syarat yang diperlukan untuk mendapatkan Ijazah Master Sains Pertanian.

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*Phytophthora palmivora* (Butl.) Butler diketahui sebagai organisma yang mempunyai variasi dan dianggap sebagai salah satu patogen yang amat merbahaya pada tanaman koko dan durian yang ada ditanam sebagai tanaman selangan di Malaysia. Kajian-kajian di jalankan untuk membandingkan ciri-ciri kultura, seksualiti dan patogenisiti enam asingan-asingan *Phytophthora* dari tanaman-tanaman ini, termasuk keberkesanan in-vitro dan in-vivo beberapa racun kulat terhadap patogen ini. Kajian-kajian awal mengenai penggunaan kawalan biologi terhadap organisma ini juga telah dibuat. Kajian-kajian kultura menunjukan bahawa asingan-asingan

Phytophthora terdiri daripada MFI P. palmivora. Ia mengeluarkan koloni-koloni 'stellate' dan 'striate' diatas medium-medium agar carrot, corn mean dan jus V-8. Pertumbuhan maiselium adalah optima pada suhu 28 ± 1.5C. Tiada pertumbuhan berlaku pada 36C. Sporangia adalah 'caducous' dengan nisbah panjang:lebar daripada 1.22 hingga 1.8 dan mempunyai pedisel yang pendek dan 'occluded'.

Asingan-asingan memerlukan asingan lain yang berserasi untuk membentuk oospora. Pendedahan kepada Trichoderma tidak menyebabkan pembiakan seksual. Apabila dipasangkan dengan penguji-penguji yang piawai, asingan durian dan koko dikenalpasti sebagai jenis-jenis serasi iaitu A1 bagi durian dan A2 bagi koko.

Asingan-asingan adalah khusus kepada satu-satu perumah. Suntikan akar, batang, daun dan buah pada perumah masing-masing menyebabkan penjangkitan.

Kajian-kajian keberkesanan in-vitro racun kulat menunjukkan metalaxyl, etridiazole dan captafol merencatkan maiselium, sporangium, pembentukan klamidospora dan percambahan sporangium asingan-asingan. Captafol adalah paling baik bagi merencatkan percambahan zoospora. Cyprofuram dan benalaxyl memberi kesan yang lebih sederhana sementara aktiviti yang lemah dilihat pada phosetyl-Al dan propamocarb.

Campuran metalaxyl-mancozeb (Ridomil-MZ 58 WP) mengekalkan sifatnya sebagai bahan pelindung dan 'therapeutant' pada anak benih koko dan durian yang terbaik sekali apabila disembur atau disiram. Phosethyl-Al dan cyprofuram juga menunjukkan aktiviti yang baik. Melainkan propamocarb yang mempunyai kesan yang

sedikit sahaja terhadap asingan-asingan, kesemua racun-racun kulat yang diuji boleh dianggap sebagai pengganti yang mungkin. Captafol berkesan hanya sebagai racun kulat pelindung.

Gliocladium roseum telah didapati sebagai parasit pada P. palmivora pada buah durian. Pengimbasan dan 'transmission' mikroskop elektron menunjukkan sporangia dan klamidospora adalah diserang terus oleh parasit ini. Penggunaan mikoparasit ini untuk kawalan biologi walau bagaimanapun memerlukan kajian-kajian yang lebih mendalam.

# CHAPTER I

# INTRODUCTION

*Phytophthora palmivora* (Butl.) Butler, an Oomycetous fungus is an extremely variable species which encompasses a wide range of cultural types and isolates (Zentmeyer, 1974). Its adaptability and ability to exhibit a high degree of variation makes it an extremely interesting microorganism but a difficult pathogen to understand creating considerable confusion in its nomenclature.

During its life cycle, *P. palmivora* differentiates a number of organs which play a vital role in its survival during adverse environmental conditions or in the attack of organs of the host plant (Tarjot, 1974). It produces four distinct spore stages: zoosporangium, zoospore, chlamydospore, and oospore. With some exceptions, oospore is formed by pairing of A1 and A2 mating types (Zentmeyer, 1974; Zentmeyer et al, 1973). This mating phenomenon provides the opportunity for hybridization and development of new strains of the pathogen (Zentmeyer, 1974; Brasier et al, 1981).

*P. palmivora* incites various disease responses on many important agricultural crops (Chee, 1969; 1974; Zentmeyer, 1974) which vary in severity from place to place (Gregory, 1983). It attacks more than a hundred different species of plants, many of which are considered of prime economic importance in Malaysia. *P. palmivora* causes collar rot as well as fruit rot of papaya (Singh,

1980). A number of orchid species are also affected: Vanda spp (Thompson, 1959), Vanda hybrids (Lim, 1980), Aranda varieties (Lim, 1980; Singh, 1980) varieties of Cattleya, Laeliocattleya and Ridleya (Singh, 1980), and species of Aranthera and Arachnis (Lim, 1980). The same pathogen had been associated with diseases known as black stripe (Chee, 1969) and stem or patch canker (Chee, 1968) of Hevea brasiliensis Muell. Arg. Both diseases caused which caused severe infection in areas where susceptible clones like RRIM 600, PB 86, and PR 107 were planted (Lim, 1982). On black pepper, annual losses due to foot rot disease incited by P. palmivora were estimated to be about 10% or 5 to 6 million ringgit of pepper export in Sarawak alone (Kueh, 1977).

The most recent (1982) statistical estimate of the cocoa (Theobroma cacao L.) hectarage in Malaysia hovers around 195,455 hectares which represent close to a hundred-fold increase from 2,000 hectares in 1961 (Anon, 1984). Malaysia has a production output of 1,000 to 1,500 kilograms of cocoa beans per hectare compared to Brazil's 745 kg,Ivory Coast's 575 kg, Nigeria's 395 kg, and Ghana's 365 kg (Anon, 1983). However, although Malaysia has the highest cocoa yield on per hectare basis among other producing countries, quality wise, it is inferior and thus, suffers a 10% to 15% discount in the international cocoa bean price (Anon, 1983). In addition, Malaysia's cocoa industry is beset with problems of pests and diseases which constantly threaten its cocoa production. For instance, although an annual loss of approximately 1% to 4% of the country's total crop due to

P. palmivora black pod disease (Turner and Shepherd, 1978) is common, without proper cultural practices employed, this figure can go as high as 10% (Mainstone, 1978) or over 20% as observed in Kuala Selangor and Jerangau (T.K.Lim, pers. comm.). Heavy losses ascribed to the same pathogen were also reported on budgrafted, selected hand-pollinated crosses, and hybrid cocoa seedlings in several estates in Selangor, Perak, and Sabah (Lim and Ang, 1980; T.K.Lim, pers. comm.). Similarly, the introduction of the Upper Amazon cocoa hybrids in the country was accompanied by outbreaks of stem canker due to P. palmivora (Chan et al, 1977). Losses due to this disease were however difficult to appraise (Anon, 1978). The very severely cankered trees may actually die, but more usually, there will be fewer branches on which to produce the pods.

Cultivation of durian (Durio zibethinus Murr.) on the other hand is confined to countries like Thailand, Burma and Indo-China, Malaysia, the Indonesian archipelago, and the Philippines (Valmayor et al, 1965). Recently, durian cultivation has attracted farmers in North Queensland, Australia (Anon, 1984). However, although production is limited as it may be, durian is regarded as the most popular, most fascinating, and most lucrative especially in Thailand and Malaysia (Lim, 1983). It brings 26 to 37 million ringgit in revenue to farmers in Perak alone, Malaysia's leading durian producing state (Anon, 1983). At the farms, a large durian fruit is sold at about 4 to 5 ringgit and retail price in the town is almost doubled (Kam, 1983). Like

cocoa, durian is similarly confronted with pest and disease problems, foremost of which is the durian fruit borer Plagideicta magniplaga (Nga and Mohd. Nor, 1980) and P. palmivora. Records of patch canker disease caused by the said fungus on durian trees in Malaysia dates back to 1934 (Thompson, 1934) and is considered by Navaratnam (1966) and Tai (1970; 1973) to be serious and increasingly widespread throughout the durian growing areas in the country. Later studies and field observations revealed that P. palmivora also infects the roots (Navaratnam, 1966; Tai, 1970), leaves and fruits (T.K.Lim, pers. comm.) of the durian plant. Durian fruit rot in particular was observed to be severe in Dengkil, Muar and Kluang areas where the affected fruits completely lose their marketable value (T.K.Lim, pers. comm.).

Despite the fact that P. palmivora causes destruction to both cocoa and durian plants, some estates in Malaysia are trying out durian trees as shade plant for cocoa trees (T.K.Lim pers. comm.). And so far, there have been no attempts to investigate whether this is a safe practice despite knowing that both are hosts of P. palmivora.

This lack of knowledge is further heightened by the fact that control measures geared towards the organism are mainly cultural and fungicides used which are protective in nature and possess no systemic and curative activity. This deficiency is particularly significant in epidemic situations where these fungicides are rendered useless. Furthermore, this type of fungicides force the farmers to make use of it in high dosages, a practice that is not

only expensive (Schwinn, 1983) but also harmful to the ecosystem and only yields short term, inconsistent, and unsatisfactory disease control (T.K.Lim, pers. comm.).

In view of the great importance of P. palmivora and the tremendous threat it poses to agriculture particularly on cocoa and durian which are being intercropped here in Malaysia, studies were undertaken with the following objectives:

(1) to compare the cultural characteristics of different isolates of Phytophthora from durian and cocoa.

(2) to study the pathogenicity of the various Phytophthora isolates from durian and cocoa utilizing different methods of artificial inoculation.

(3) to develop a rationale approach in the control of P. palmivora by :

3.1 investigating the in-vitro effects of some selected new systemic fungicides on germination, growth, and sporulation of the organism.

3.2 investigating the prophylactic and therapeutic activity of these fungicides against P. palmivora infection on seedlings.

3.3 investigating other possible measures (e.g. biological control) which could complement chemical control.

# CHAPTER 2

# CULTURAL AND PATHOGENICITY STUDIES

## REVIEW OF LITERATURE

### Taxonomy of Phytophthora palmivora

The taxonomic concept of the species Phytophthora palmivora has long been in dispute, a situation which had been exacerbated by the absence of type cultures and adequate herbarium material of Butler's (1907; 1910) original fungus (Brasier and Griffin, 1979). Detailed taxonomic studies of the fungus had been made by Waterhouse in 1956, 1963, and 1974. In tracing the historical development in the naming of the species, Waterhouse (1974a) re-emphasized the need not to discount nor regard morphological differences within a taxon rather than over-rely on compatibility types.

Within the species, there had been several attempts to place isolates of P. palmivora into broad categories. Ashby (1929) found that cultures of P. palmivora could be separated culturally ('typical' and 'atypical') and sexually ('cacao' and 'rubber'). Brasier and Sansome (1977) used the size of chromosomes ('large' and 'small') while Zentmeyer et al (1977) designated groups (Group I, Group II, Group III and Group IV) based on sporangium caducity and pedicel length. In a cocoa Phytophthora workshop at