UNIVERSITI PUTRA MALAYSIA

STUDIES ON CORYNESPORA CASSIICOLA (BERK. & CURT.) WEI ON RUBBER

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STUDIES ON *Corynespora cassiicola* (Berk. & Curt.) Wei ON RUBBER

By
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LIST OF ABBREVIATIONS

a.i.	Active Ingredient
I. M. I.	International Mycological Institute
IRSG	International Rubber Study Group
nuv	Near ultra violet
PDA	Potato Dextrose Agar
PSA	Potato Sucrose Agar
RLEA	Rubber Leaf Extract Agar
RRIC	Rubber Research Institute of Ceylon (Sri Lanka)
RRIM	Rubber Research Institute of Malaysia
SC	Soluble Concentrate
uv	Ultra violet
WA	Water Agar
WP	Wettable Powder
YE	Yeast Extract

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Corynespora cassiicola (Berk. & Curt.) Wei from infected rubber leaves in Sungai Buloh (two isolates) and Serdang (two isolates) were cultured on Potato Dextrose Agar (PDA). All four isolates exhibited variability in colony colour and growth rates on artificial media (PDA, PSA, RLEA and WA). The optimum temperature for growth on PDA was 28°C.

Significant increase in sporulation was recorded on Potato Sucrose Agar (PSA) when exposed to uv light for 2 h daily for three days. Exposure

to nuv light also increased sporulation significantly but maximum sporulation occurred only with 4 h daily treatment.

Conidial size, hilum width and septal number of isolates cultured on PSA were variable and were not affected by uv light treatment. The size and shape of conidia on PSA differed from those on naturally infected rubber leaves. On rubber leaves, the average conidial and hilum width were longer, and the average septal number was higher. Conidia were long, slender and stick-like in culture, but were curved, with a broader based on rubber leaves.

Maximum sporulation was achieved when cultures on PSA were scraped once, and maintained for three days under continuous fluorescent light with 2 h exposure to uv light daily. By this method, variation of uv light exposure of between 15 min to 4 h daily was found to have no negative effect on conidial germination and germ tube growth. PSA was the best medium for sporulation compared to PDA and RLEA. The addition of yeast extract in these media did not improve sporulation.

The leaf disc inoculation method confirmed the pathogenicity of all four rubber isolates on rubber leaf discs but not on chilli, papaya and tomato leaf discs. The papaya isolate infected only papaya leaf discs but not rubber, chilli and tomato leaf discs. The seedling inoculation method, similarly, confirmed the pathogenicity of all four rubber isolates on young leaves of budded rubber, but not on one month old papaya, chilli and tomato seedlings. On the other hand, the papaya isolate could only infect papaya seedlings, but

not budded rubber, chilli and tomato seedlings. The papaya isolates differed in size compared to rubber isolates. The conidium of papaya isolate, on average, was shorter in length but longer in width, had a longer hilum width and higher septal number than rubber isolates.

In-vitro screening of fungicides by the Poison Agar Technique provided data of percentage inhibition of linear growth from which ED50 values (in ascending order) were determined as follows: 0.26 ppm (hexaconazole), 0.46 ppm (difenoconazole), 1.19 ppm (tebuconazole), 3.84 ppm (bitertanol), 5.09 ppm (cyproconazole), 52.34 ppm (prochloraz) and 89.73 ppm (chlorothalonil). The ED50 values determined from the percentage inhibition of conidial germination in ascending order were: 5.27 ppm (difenoconazole), 15.20 ppm (hexaconazole), 24.12 ppm (chlorothalonil), 24.23 ppm (cyproconazole), 34.66 ppm (tebuconazole), 37.30 ppm (prochloraz) and 108.81 ppm (bitertanol). Total inhibition of germ tube growth was recorded at 1000 ppm of cyproconazole, hexaconazole, difenoconazole and chlorothalonil.

Preliminary studies on the potential use of *Gliocladium virens* as a biocontrol agent showed that it significantly inhibited the growth of all four *C. cassiicola* rubber isolates in cultures.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi syarat-syarat keperluan bagi mendapatkan ijzah Master Sains Pertanian

KAJIAN KE ATAS *Corynespora cassiicola* (Berk. & Curt.) Wei PADA TANAMAN GETAH

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Corynespora cassiicola (Berk. & Curt.) Wei dari daun-daun getah berpenyakit di Sungai Buloh (dua pencilan) dan Serdang (dua pencilan) telah dikultur di atas Potato Dextrose Agar (PDA). Keempat-empat pencilan menunjukkan perbezaan warna koloni dan kadar pertumbuhan pada medium

tiruan (PDA, PSA, RLEA dan WA). Suhu optimum untuk pertumbuhan di atas PDA ialah 28°C.

Peningkatan pensporulan yang bererti telah direkod di atas Potato Sucrose Agar (PSA) selepas didedahkan kepada cahaya uv selama 2 jam sehari untuk 3 hari. Pendedahan kepada cahaya nuv juga meningkatkan pensporulan dengan bererti tetapi pensporulan tertinggi berlaku hanya dengan rawatan 4 jam sehari.

Saiz konidium, lebar hilum dan bilangan septum pencilan-pencilan di atas PSA adalah berbeza dan tidak dipengaruhi oleh rawatan cahaya uv. Saiz dan bentuk konidia di atas PSA adalah berbeza daripada daun-daun getah berpenyakit. Pada daun-daun getah, purata lebar konidium dan hilum adalah lebih panjang, dan purata bilangan septum adalah lebih tinggi. Konidium berbentuk panjang, landai dan seperti kayu di dalam kultur tetapi adalah lengkung dan bertapak lebih besar pada daun-daun getah.

Pensporulan tertinggi dicapai apabila kultur di atas PSA digores sekali, dan disimpan selama 3 hari di bawah cahaya pendafluor berterusan dengan pendedahan kepada 2 jam cahaya uv sehari. Dengan cara ini, pendedahan kepada cahaya uv di antara 15 min hingga 4 jam sehari didapati tidak memberi sebarang kesan negatif terhadap percambahan konidium dan pertumbuhan tiub germa. PSA adalah medium terbaik untuk pensporulan berbanding dengan PDA dan RLEA. Penambahan ekstrak yis pada media-media ini tidak menambah pensporulan.

Kaedah inokulasi cakera daun mengesahkan kepatogenan keempat-empat pencilan getah pada cakera daun getah tetapi tidak pada cakera daun betik, cili dan tomato. Pencilan betik menjangkiti hanya cakera daun betik tetapi tidak pada cakera daun getah, cili dan tomato. Kaedah inokulasi anak benih telah memberi pengesahan kepatogenan yang sama bagi keempat-empat pencilan getah pada daun-daun muda getah cantuman tetapi tidak pada anak-anak benih betik, cili dan tomato yang berumur satu bulan. Sebaliknya, pencilan betik hanya boleh menjangkiti anak benih betik tetapi tidak pada getah cantuman, anak benih cili dan tomato. Pencilan betik berbeza dari segi saiz dengan pencilan getah. Konidium pencilan betik pada puratanya lebih pendik tetapi lebih lebar, mempunyai hilum yang lebih lebar dan bilangan septum yang lebih banyak dari pencilan getah.

Saringan *in vitro* racun kulat menggunakan Teknik Agar Beracun memberi data peratus perencatan pertumbuhan liner di mana nilai-nilai ED 50 (mengikut susunan meningkat) telah ditentukan seperti berikut: 0.26 bsj (hexaconazole), 0.46 bsj (difenoconazole), 1.19 bsj (tebuconazole), 3.84 bsj (bitertanol), 5.09 bsj (cyproconazole), 52.34 bsj (prochloraz) dan 89.73 (chlorothalonil). Nilai-nilai ED 50 yang ditentukan daripada peratus perencatan percambahan konidium mengikut susunan meningkat ialah: 5.27 bsj (difenoconazole), 15.20 bsj (hexaconazole), 24.12 bsj (chlorothalonil), 24.23 bsj (cyproconazole), 34.66 bsj (tebuconazole), 37.30 bsj (prochloraz) dan 108.81 bsj (bitertanol). Perencatan pertumbuhan tiub germa sepenuhnya telah dicatat pada 1000 bsj cyproconazole, hexaconazole, difenoconazole dan chlorothalonil.

Kajian awal mengenai potensi penggunaan *Gliocladium virens* sebagai agen kawalan biologi menunjukkan ia menyebabkan perencatan yang bererti pada pertumbuhan keempat-empat pencilan *C. cassiicola* pada getah yang dikultur.

CHAPTER I

INTRODUCTION

Ninety-nine percent of the world's natural rubber is derived from tapping the rubber tree (*Hevea brasiliensis* Muell. Agr.) which originated from the tropical rain forest of South America (George et al., 1980). At the end of the nineteenth century, rubber planting was introduced from its native habitat to other countries in Asia and Africa. Today, over 90 % of the total of nine million hectares of rubber planted in the world is located in Asia (International Rubber Study Group, 1993; - See Table 1) contributing significantly to export earnings and agriculture employment.

The vast area of rubber replanting and new planting were grown with modern clones selected primarily for yield and girth but not for disease resistance (Tan & John, 1985). Many were susceptible to diseases resulting in significant losses in productivity (Peries & Liyanage, 1985).

Table 1

Natural Rubber Area and Production in Asia

Country	Area (`000 ha)	Production (`000 ton)
Indonesia	3,040.0	1,387.0
Malaysia	1,874.6	1,217.5
Thailand	1,844.0	1,531.0
China	603.2	310.0
India	451.0	383.0
Other	509.6	445.0
Asia total	8,321.8	5,273.5
World total	9,035.4	5,629.6

Source: International Rubber Study Group (1993)

Chee (1976) reported 550 microorganisms associated with rubber diseases of which 24 were considered economically important. Most were caused by indigenous fungal pathogens and four categories identified were: root diseases, panel diseases, stem diseases and leaf diseases.

A very serious leaf spot disease of rubber in Asia is caused by *Corynespora cassiicola* (Berk. & Curt.) Wei (Liyanage et al., 1986; Pongthep, 1987; Chee, 1988; Liyanage, 1988; Soekirman, 1988; Liyanage et al., 1989; Liyanage & Jacob, 1992). It occurs in all stages of growth of rubber from nursery to the mature tree. The fungus attacks both young and old leaves causing severe defoliation or death of trees. Many thousand hectares of rubber trees were removed due to susceptibility in Sri Lanka in the absence of effective control measures (Liyanage et al., 1989).

A serious outbreak of *Corynespora* leaf spot on rubber was recorded in Malaysia in 1985 (Chee, 1987; 1988). The disease has since been detected throughout the country and attacking larger areas of rubber (Tan et al., 1992). However, very little information is available on the characters and behaviour of the Malaysian isolates. There is difficulty in obtaining spores in culture and factors influencing production are not well defined. There is also a need to look for alternative or more effective control measures.