



**UNIVERSITI PUTRA MALAYSIA**

**ANTHRACNOSE FRUIT ROT OF CHILLI (*CAPSICUM ANNUM L.*):  
SOME ASPECTS OF ITS ETIOLOGY, EPIDEMIOLOGY AND  
CONTROL IN PENINSULAR MALAYSIA**

**MAH SHOOK YING**

**FP 1987 9**

ANTHRACNOSE FRUIT ROT OF CHILLI (CAPSICUM ANNUUM L.):  
SOME ASPECTS OF ITS ETIOLOGY, EPIDEMIOLOGY  
AND CONTROL IN PENINSULAR MALAYSIA

by

MAH SHOOK YING

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

March 1987



## ACKNOWLEDGEMENTS

I am greatly indebted to my supervisor, Professor George Varghese and co-supervisor Dr. Sariah Meon, Department of Plant Protection, Faculty of Agriculture, University of Agriculture Malaysia, for their invaluable advice and guidance throughout the course of this study and for critically reading through this manuscript.

I wish to acknowledge my gratitude to the Malaysian Agricultural Research and Development Institute (MARDI) for financial support and for providing the necessary research facilities at the Institute.

My thanks also go to Dr. J.E.M. Mordue and Dr. B.C. Sutton of the Commonwealth Mycological Institute, Kew, England who confirmed my identifications of the Colletotrichum isolates; Dr. Lee Chong Soon, Puan Salbiah Hussin and Puan Zaharah Haji Taib of the Statistical Branch in MARDI who provided statistical advice and assistance; and to Miss H.F. Wee, Encik Mohamad Razali Mohamad Adnan and Puan Sariah Jonid for technical assistance in the laboratory and in the field.

I wish to thank my husband and children for their help, encouragement and most of all, for their patience and support throughout the project.

Finally, I wish to thank all my colleagues and friends in MARDI and in the University of Agriculture Malaysia who have helped me in various ways.



## TABLE OF CONTENTS

	PAGE
ACKNOWLEDGEMENTS .....	iii
TABLE OF CONTENTS .....	v
LIST OF TABLES .....	ix
LIST OF FIGURES .....	xi
LIST OF ABBREVIATIONS .....	xiv
ABSTRACT .....	xv
CHAPTER 1 – INTRODUCTION	
1.1    The chilli plant .....	1
1.2    Chilli cultivation in Peninsular Malaysia .....	3
1.3    Anthracnose of chilli .....	7
1.4    Objectives of present study .....	8
CHAPTER 2 – LITERATURE REVIEW	
2.1    The genus <u>Colletotrichum</u> .....	11
2.2    Anthracnose disease of chilli .....	12
2.3 <u>Colletotrichum capsici</u> – host range and pathogenicity .....	13
2.4    Control of anthracnose .....	15
CHAPTER 3 – ETIOLOGICAL INVESTIGATIONS	
3.1    Introduction .....	18

	PAGE
3.2 Materials and methods .....	19
3.2.1 Survey and collection of disease samples .....	19
3.2.2 Isolation of the pathogen .....	22
3.2.3 Cultural characteristics of the isolates.....	23
3.2.4 Germination of conidia on chilli agar .....	24
3.2.5 Effect of environmental factors on growth of <u>C. capsici</u> .....	25
a) Hydrogen-ion concentration .....	25
b) Temperature .....	26
3.2.6 Development of rapid in vitro inoculation technique for evaluating resistance of chilli fruits to <u>C. capsici</u> .....	26
a) Spraying detached chilli fruits with spore suspension .....	27
b) Dip inoculation of mechanically injured fruits .....	29
c) Dip inoculation of uninjured fruits .....	29
d) Injection of fruits with hypodermic needle .....	29
e) Pin prick method .....	31
f) Fungal plug method .....	31
3.2.7 Interaction between varieties, growth stages of chilli and incidence of anthracnose disease .....	33

	PAGE
3.3      Results .....	35
3.3.1 Collection and isolation of <u>C.</u> <u>capsici</u> isolates .....	35
3.3.2 Cultural characteristics of the isolates .....	41
3.3.3 Germination of conidia on chilli agar .....	44
3.3.4 Effect of environmental factors on growth of <u>C. capsici</u> .....	50
a) Hydrogen-ion concentration .....	50
b) Temperature .....	50
3.3.5 Development of rapid in vitro inoculation technique for evaluating resistance of chilli fruits to <u>C. capsici</u> infection .....	52
3.3.6 Interaction between varieties, growth stages of chilli and incidence of anthracnose disease .....	56
3.4      Discussion .....	63

#### CHAPTER 4 - EPIDEMIOLOGICAL ASPECTS

4.1      Seed transmission .....	71
4.1.1 Introduction .....	71
4.1.2 Materials and methods .....	72
4.1.3 Results and discussion .....	73
4.2      Disease spread .....	80
4.2.1 Introduction.....	80
4.2.2 Materials and methods .....	82

	PAGE
4.2.3 Results .....	83
4.2.4 Discussion .....	87
 CHAPTER 5 – CHEMICAL CONTROL	
5.1 Introduction .....	89
5.2 Materials and methods .....	90
5.2.1 In vitro screening of fungicides .....	90
5.2.2 Field screening of fungicides .....	93
5.3 Results .....	94
5.3.1 In vitro screening .....	94
5.3.2 Field screening .....	100
5.4 Discussion .....	105
 CHAPTER 6 – GENERAL DISCUSSION .....	109
REFERENCES .....	114
APPENDICES .....	123

LIST OF TABLES

TABLE		Page
I	Average nutritive value (ANV) of sweet and hot peppers (chillies), per 100 grams edible product .....	4
II	Total net import and production of chillies in Peninsular Malaysia, 1967-1981 .....	6
III	<u>Colletotrichum capsici</u> isolates: origin and locations in Peninsular Malaysia .....	24
IV	Cultural and morphological characteristics of <u>C. capsici</u> isolates obtained from various localities in Peninsular Malaysia .....	45
V	Growth and development pattern of germ tubes of conidia of <u>C. capsici</u> on solidified chilli agar medium (CA) .....	48
VI	Linear growth of <u>C. capsici</u> in culture under a range of hydrogen-ion concentrations. Five replicates were used at each pH value .....	51
VII	The effect of temperature on the linear growth of five <u>C. capsici</u> isolates .....	52
VIII	The effect of variety and host growth stage on the incidence of anthracnose caused by <u>C. capsici</u> on chilli fruits - 1st Experiment .....	58
IX	Analysis of variance of <u>C. capsici</u> infection on 5 different varieties of chilli inoculated at 5 stages of growth .....	59
X	The effect of variety and host growth stage on the incidence of anthracnose caused by <u>C. capsici</u> on chilli fruits - 2nd Experiment .....	60

TABLE	PAGE
XI      Mean percentage infection of 6 different varieties of chilli fruits by <u>C. capsici</u> .....	61
XII     Effect of <u>C. capsici</u> on emergence, survival and growth of chilli, <u>Capsicum annuum</u> .....	75
XIII    Inhibition of mycelial growth of <u>C. capsici</u> at various concentrations of fungicides .....	92
XIV     Effectiveness of different fungicides against mycelial growth of <u>C. capsici</u> .....	95
XV      Effect of fungicides on yield and incidence of anthracnose fruit rot on chilli (Trial I) .....	101
XVI     Effect of fungicides on yield and incidence of anthracnose fruit rot on chilli (Trial II) .....	102
XVII    Effect of fungicides on yield and incidence of anthracnose fruit rot on chilli (Trial III) .....	103
XVIII   Effect of fungicides on yield and incidence of anthracnose fruit rot on chilli (Trial IV) .....	104

## LIST OF FIGURES

FIGURE		PAGE
1	Map of Peninsular Malaysia showing localities where samples were collected .....	20
2	Map showing the average monthly rainfall distribution for selected stations in Peninsular Malaysia .....	21
3	Potato dextrose agar blocks for determining viability of <u>Colletotrichum capsici</u> spores .....	28
4	Incubation of inoculated chilli fruits in moist chamber .....	30
5	Inoculating chilli fruit with conidia of <u>Colletotrichum capsici</u> by the pin-prick method .....	32
6	Greenhouse experiment to study the influence of growth stage and varieties of chilli on severity of anthracnose fruit rot disease .....	34
7	Chilli fruit showing typical anthracnose lesion. Black acervuli are arranged in concentric rings giving the lesion a target-board appearance .....	36
8	Acervulus of <u>Colletotrichum capsici</u> developed on PDA, showing stroma and hyaline, non-septate conidia. Note the absence of setae .....	38
9	Five-day old cultures of <u>Colletotrichum capsici</u> (isolate IMI 256699) on potato dextrose agar .....	39
10	Ten-day old colony of <u>Colletotrichum capsici</u> cultured on PDA showing presence and distribution of acervuli .....	42

FIGURE		PAGE
11	Changes in cultural characteristics of <u>Colletotrichum capsici</u> isolate with repeated sub-culturing on PDA. Note white, appressed mycelial mat and lack of acervuli development on 10-day old culture .....	43
12	A. Conidia of <u>C. capsici</u>  B. Germ tube development 4 hours after plating on chilli agar medium  C. Germ tube development 22 hours after plating on chilli agar medium .....	47
13	Percentage germination of <u>C. capsici</u> conidia 4, 8, 16 and 22 hours after plating on chilli agar medium .....	49
14	The effect of temperature on five isolates of <u>C. capsici</u> - colony diameter on PDA after 7 days of incubation in darkness .....	53
15	Pecentage infection of 5 varieties of chilli fruits when inoculated at 5 different growth stages with <u>C. capsici</u> .....	57
16	Percentage infection of 6 varieties of chilli fruits when inoculated at 5 different growth stages with <u>C. capsici</u> .....	62
17	<u>C. capsici</u> infection on cotyledon, cotyledonary leaves and hypocotyl of chilli seedlings .....	74
18	Germination response of non-infected chilli seeds and infected chilli seeds (with <u>C. capsici</u> ) over time .....	76

FIGURE		PAGE
19	Survival of seedlings from non-infected chilli seeds and infected chilli seeds ( <u><i>C. capsici</i></u> ) over a period of 45 days .....	77
20	Response of chilli seedlings from non-infected and <u><i>C. capsici</i></u> -infected seeds in terms of increase in height over a period of 45 days .....	78
21	Spread of anthracnose fruit rot in a chilli plot .....	84
22	Relationship between rainfall and anthracnose disease incidence in a chilli crop .....	86
23	Inhibition of mycelial growth of <u><i>C. capsici</i></u> at different concentrations of Maneb, Dichlofuanid, Benomyl and Bitertanol .....	96
24	Inhibition of mycelial growth of <u><i>C. capsici</i></u> at different concentrations of Propineb, Carbendazim and Chlorothalonil .....	97
25	Inhibition of mycelial growth of <u><i>C. capsici</i></u> at different concentrations of Maneb + Fentin acetate .....	98
26	Inhibition of mycelial growth of <u><i>C. capsici</i></u> at different concentrations of Mancozeb, Anilazine and Maneb + Zineb + Ferbam .....	99

## LIST OF ABBREVIATIONS

g	gram
hr	hour
ml	millilitre
mg	milligram
mm	millimetre
pH	hydrogen ion exponent
$\mu$	micrometre
MT	metric ton
CWT	hundredweight
%	percent
kcal	kilo calories
kg	kilogram
ha	hectare
ppm	parts per million
PDA	potato dextrose agar
CA	chilli agar
°C	degree (s) Celsius
viz.,	namely
ft	foot; feet
in	inch(es)
m	metre
Mo	Molybdenum
Mn	Manganese
Zn	Zinc
Fe	Ferrum, iron

An abstract of the thesis presented to the Senate of Universiti Pertanian Malaysia as partial fulfilment of the requirements for the degree of Master of Agricultural Science

ANTHRACNOSE FRUIT ROT OF CHILLI (CAPSICUM ANNUUM L.):  
SOME ASPECTS OF ITS ETIOLOGY, EPIDEMIOLOGY  
AND CONTROL IN PENINSULAR MALAYSIA

by

MAH SHOOK YING

March 1987

Supervisor : Professor George Varghese, Ph. D.  
Co-supervisor : Sariah Meon, Ph. D.  
Faculty : Agriculture

Anthracnose fruit rot is a serious limiting factor in the production and yield of fresh chillies (Capsicum annuum L.) in Peninsular Malaysia. Isolates obtained from diseased chilli fruits collected in various localities in the country yielded the fungus, Colletotrichum capsici (Syd.) Butler & Bisby.

Pathogenicity studies on mature chilli fruits confirmed that C. capsici could produce typical elliptical or oblong straw-coloured lesions on the fruit surface with subsequent development of black acervuli in concentric rings. Comparison of isolates on potato dextrose agar showed that all the isolates were relatively similar in cultural and morphological

characteristics. Germination of conidia occurred 3 - 4 hours after plating on chilli agar medium. Branching of germtubes occurred between 16-24 hours after germination. The fungus grew over a wide range of hydrogen-ion concentrations (between pH 3.0 and pH 10.0) with optimum between pH 7.0 and pH 9.5. Acervuli formation and development was more evident on alkaline medium than on acidic medium. Optimum temperature for mycelial growth was between 25°C and 32°C. The minimum and maximum temperatures for visible growth were 10°C and 45°C, respectively.

Studies on several inoculation methods under laboratory conditions showed that dipping non-blemished chilli fruits into a spore suspension of  $1 \times 10^6$  spores/ml, followed by incubation in a moist chamber gave good lesion development. This method was therefore adopted as a rapid in-vitro inoculation technique for mass screening tests. Screening of local and introduced varieties of chilli at various growth stages indicated differential resistance of chilli varieties to C. capsici, although growth stage had little influence on the incidence of anthracnose ripe rot disease.

Studies also showed that chilli seeds infected with C. capsici had a higher percentage of germination than non-infected seeds though subsequent seedling growth was retarded. The spread of the disease in the field was greatly influenced

by the direction of prevailing winds, indicating the importance of wind-borne spores to disease spread. Rainfall influenced disease depending on the amount, duration, intensity and pattern of rainfall during a crop cycle.

In-vitro screening of fungicides indicated that Brestan 10 (maneb + fentin acetate) was highly inhibitory to the growth of the fungus. However, field evaluation of 14 test fungicides indicated that none of the chemicals tested was very effective in controlling anthracnose ripe rot disease of chilli due to various limitations.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebagai memenuhi sebahagian daripada syarat-syarat keperluan ijazah Master Sains Pertanian

PENYAKIT ANTHRAXNOS BUAH CILI (CAPSICUM ANNUUM L.):  
BEBERAPA ASPEK ETIOLOGI, EPIDEMIOLOGI SERTA  
PENGAWALAN DI SEMENANJUNG MALAYSIA

oleh

MAH SHOOK YING

Mac 1987

Penyelia : Professor George Varghese, Ph. D.

Penyelia Bersama : Sariah Meon, Ph. D.

Fakulti : Pertanian

Penyakit bintik berpusar adalah satu faktor pembatasan yang serius dalam produksi dan penghasilan cili-cili segar (Capsicum annuum L.) di Semenanjung Malaysia. Buah-buah cili berpenyakit yang diambil dari beberapa tempat dalam negara, dan telah diasingkan, mengandungi kulat, Colletotrichum capsici (Syd.) Butler & Bisby.

Kajian-kajian patogenisiti ke atas buah-buah cili yang masak mengesahkan bahawa C. capsici boleh menyebabkan lesion-lesion berbentuk bulat - panjang atau bujur yang biasa, berwarna jerami, di permukaan buah, diikuti oleh tumbuhan acervulus hitam berbentuk bulat. Perbandingan terhadap

dektros kentang (PDA) yang telah diasinkan menunjukkan semuanya adalah seakan-akan bersifat terpelihara dan morfologi. Percambahan conidia berlaku 3-4 jam selepas saduran ke atas agar cili. "Germtubes" atau tiub germa merebak di antara 16-24 jam selepas percambahan. Kulat-kulat tumbuh dalam lingkungan kepekatan hydrogen ion yang luas (di antara pH 3 dan pH 10) dengan optima di antara pH 7 dan pH 9.5. Pembentukan dan perkembangan acervulus lebih nyata pada penimbang alkali dibandingkan dengan penimbang asid. Suhu optima untuk pertumbuhan kulat adalah di antara  $25^{\circ}\text{C}$  dan  $32^{\circ}\text{C}$ . Suhu minima pertumbuhan nyata ialah  $10^{\circ}\text{C}$  dan maksimanya  $45^{\circ}\text{C}$ .

Kajian-kajian ke atas beberapa kaedah suntikan di makmal menunjukkan bahawa mencelup buah-buah cili yang tidak cedera ke dalam ampaian spora dengan kepekatan air  $1 \times 10^6$  spora/ml, diikuti dengan kaedah pengeraman dalam tempat lembab menghasilkan calar-calar yang baik. Cara ini telah dipilih sebagai kaedah suntikan in vitro yang sesuai untuk ujian-ujian penyiasatan berkumpul. Siasatan terhadap cili-cili tempatan dan jenis-jenis yang dicadangkan pada peringkat-peringkat pertumbuhan tertentu, menunjukkan beberapa penahanan yang berlainan pada C. capsici, walaupun di peringkat pertumbuhan, kecil pengaruhnya terhadap berlakunya penyakit anthracnose.

Kajian-kajian menunjukkan juga bahawa biji-biji cili yang dijangkiti C. capsici mempunyai peratus percambahan yang lebih

tinggi daripada biji-biji yang tidak dijangkiti, walaupun pertumbuhan anak-anak pokok yang seterusnya tergencat. Arah tiupan angin telah mempengaruhi penyebaran penyakit ini di ladang menunjukkan pentingnya spora yang dibawa angin dalam merebakkan penyakit. Hujan boleh mempengaruhi penyakit, bergantung kepada kekerapan, lama, lebat dan pola-pola hujan semasa sesuatu giliran tanaman.

Siasatan in vitro terhadap racun kulat menunjukkan bahawa Brestan 10 (maneb + fentin acetate) adalah peka besar ke atas pertumbuhan kulat itu. Walau bagaimanapun penyaringan di ladang terhadap 14 racun kulat yang diuji, menunjukkan bahawa tiada bahan kimia yang didapati paling berkesan untuk mengawal penyakit anthraknos cili, disebabkan terdapatnya beberapa pembatasan.

## CHAPTER 1

### INTRODUCTION

#### 1.1 The chilli plant

Capsicum annuum L., commonly known as capsicum, chilli or chilli pepper, includes a large number of horticultural varieties and is by far the most economically important of all the cultivated species of Capsicum. It belongs to the family Solanaceae and is a native of Central and South America (Purseglove, 1968; Ware and McCollum, 1975; Thompson and Kelly, 1978; Anon., 1983). Under cultivation, the chilli plant is an annual although several cultivars may be perennials in warmer climates. Flowers are small, white, 5-partite and borne singly on the axils of leaves. Different cultivars have berries or pods or seed-vessels that vary in shape and size. When ripe, the colour of the berries ranges from blood-red to yellow, orange, dark violet to even green or black. Seeds are white or straw-coloured, flat and kidney-shaped and are attached to a fleshy placenta. Pungency is due to a non-volatile compound called capsaicin, the vanillyl amide of isodecylanic acid (Burkhill, 1935; Purseglove, 1968; Anon., 1983). The amount of capsaicin is controlled by a single dominant gene and varies enormously in various cultivars. Matthew et al. (1971) reported that generally the amount of capsaicin in chilli

ranges from 0.1 percent to 1 percent, while the Indian variety has capsaicin content in the range of 0.2 percent to 0.5 percent.

Domestic and wild species of Capsicum were found in the New World as early as prehistoric times. Columbus carried the seed to Spain in 1493 (Thompson and Kelly, 1978) and from there, it was introduced to Europe and Asia. Purseglove (1968) and Heiser (1976) reported that by 1542 there were already three races of Capsicum being cultivated in India. In 1931 Ochse, Bakhuizen and van den Brink reported that C. annuum was "cultivated everywhere in the Dutch East Indies for its pungent fruits". C. annuum can be grown from sea-level to 6,000 feet or more. At present in Java, chilli is planted between rice-crops and in Malaysia it is cultivated as a garden crop or on small farms. In India, chilli is widely grown in areas around Madras and in the plains of upper India. During the period from 1969 - 1971, the world production of fresh capsicum was given as 5,027,000 metric tons(MT) per year and production increased to 7,055,000 MT per year during the period from 1979 - 1981 (FAO, 1982). India is now the biggest producer and exporter of chillies with annual exports exceeding 200,000 cwt. (9,072 MT) per year in some years, followed by Thailand with about 100,000 cwt (4,536 MT) per year (Purseglove, 1968). Purseglove (1968) also reported that Sri Lanka imports the largest quantities of chillies, followed by the United States and Malaysia.