UNIVERSITI PUTRA MALAYSIA

INFECTION OF CHILLI (CAPSICUM ANNUUM L.) SEEDS BY COLLETOTRICHUM GLOEOSPORIOIDES (PENZ.) SACC. AND ITS TRANSMISSION

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FP 1996 17

Infection of Chilli (*Capsicum annuum* L.) Seeds by *Colletotrichum gloeosporioides* (Penz.) Sacc. and Its Transmission

By

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Dissertation Submitted in Fulfilment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Agriculture, Universiti Pertanian Malaysia.

July 1996

ACKNOWLEDGEMENTS

In the name of ALLAH the most Benevolent and Merciful.

All praise be for ALLAH for giving me the opportunity, patience, help, and guidance in completing this thesis. My deepest gratitude and love to my mother, my wife and other family members who constantly pray for my success.

I am indebted to the chairman of my supervisory committee, Assoc. Prof. Dr. Sariah Meon for her dedicated efforts, invaluable advice and intellectual guidance throughout the study. I am also thankful to Assoc. Prof. Dr. Hor Yue Luan and Dr. Zainal Abidin Mior Ahmad, members of the supervisory committee for their constructive comments, in the preparation of the final manuscript and to Prof. Ayama from the Department of Agronomy, Faculty of Agriculture for the statistical analysis.

The financial support from the University of Garyounis Benghazi-Libya and the research grant from the National Council for Scientific Research and Development-Malaysia is gratefully acknowledged.

Special thanks to my friends, Omar Mussa El-Sanousi, Salim A.Mohammed and Mohammed Abdel Malik for their understanding, cooperation and moral support. Acknowledgement is also due to Mr.Ahmed Sharkawi in the Photography section and to the staff at the laboratory of plant pathology, Faculty of Agriculture for their cooperation.

Last but not least, I would like to extend my appreciation to all those who have contributed to the success of this study. May ALLAH bless them all. Amin.

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July 1996

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This study aimed to detect the external and internal seed-borne infection of *C.gloeosporioides* in chilli seeds and to determine its site of infection in the seeds. The study also proposed to investigate the mechanism of seed infection and its role in the development of anthracnose fruit rot in the field.

The fungus was isolated frequently from naturally infected seeds by moist blotter and on PDA suggesting its presence on the seed surface as well as inside the seed. Based on the cultural, morphological and microscopic characteristics the single spore isolate of the pathogen was identified as *Colletotrichum gloeosporioides* (Penz) Sacc. Optimum temperature for growth and sporulation was between 25^{o}C and 30^{o}C. The growth and sporulation were favoured by high relative humidity.

In the nonincubated seeds, *C.gloeosporioides* was detected in the seed coat but not in the endosperm. The infection in the endosperm was detected only in the infected seeds when incubated on moist blotter. The embryo was free of infection in incubated and non-incubated seeds. Tissues of the infected nonincubated seeds were well differentiated, suggesting that infection might have occurred late during the seed development.

C.gloeosporioides was seed transmitted from seeds to seedlings causing pre and post-emergence damping-off. The severely infected seeds failed to germinate as a result of seed rot. The survived seedlings were of poor growth as compared to the seedlings from healthy seeds.

To study the mechanism of seed infection, different parts of the chilli plant were artificially inoculated with the conidia of the fungus. The chilli flowers were very susceptible to infection resulting in flower abortion. Only the inoculation of the green and red fruits resulted in *C.gloeosporioides* seed infection. The fungus penetrated both the green and red fruits by penetration pegs from the appressoria. Direct penetration by the hyphal strands was observed in the red fruits. This is a new finding for an anthracnose fungus to penetrate the chilli fruit tissue directly without the development of appressoria. The hyphae of the pathogen penetrated the seeds through the naturally occurring cracks and pores of the seed coat, grew over the seed and penetrated into the inner layer of the seed coat through the micropyle. At later stages of infection penetration also occurred through the funiculus.

Results showed that seed-borne infection has no role in the development of anthracnose fruit rot in the field. However, the pathogenic effect of the seed - borne infection on the growth of the seedling was significant during the early stages of growth. These plants showed poor growth, delayed flowering and consequently resulted in poor yield as compared to the

control. Parallel experiment conducted in the glasshouse showed that the seeds were not infected through systemic infection from the mother plant. This further supports the findings of the field experiment that seed-borne infection has no role in the incidence of anthracnose fruit rot in chilli.

Abstrak disertasi yang dikemukakan kepada Senat Universiti Pertanian Malaysia sebaga syarat keperluan untuk Ijazah Doktor Falsafah

JANGKITAN BIJI BENIH CILI (*Capsicum annuum* L.) OLEH *Colletotrichum gloeosporioides* (Penz.) Sacc. DAN PENYEBARANNYA

Oleh

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Kajian ini bertujuan untuk mengesan jangkitan bawaan biji benih luaran dan dalaman disebabkan oleh *C.gloeosporioides* dalam biji benih cili dan menentukan tapak jangkitannya di dalam bijibenih.Ia juga bercadang untuk memahami mekanisme jangkitan biji benih dan peranannya dalam pembentukan reput buah antraknos di lapangan.

Kulat telah dipencilkan daripada biji benih yang dijangkiti secara semulajadi menggunakan kaedah kertas serap lembap dan di atas PDA, mencadangkan kehadirannya pada permukaan dan juga di dalam biji benih. Berdasarkan kepada ciri-ciri kultura, morfologi dan mikroskop pencilan spora tunggal patogen dikenalpasti sebagai *Colletotrichum gloeosporioides* (Penz.) Sacc. Suhu optima untuk tumbesaran dan pensporulan adalah diantara 25-30°C. Tumbesaran dan pensporulan disokong oleh kelembapan bandingan yang tinggi.

Dalam biji benih tanpa eraman, *C.gloeosporioides* dikesan di dalam kulit biji benih tetapi tidak di dalam endosperma. Jangkitan dalam endosperma dikesan hanya dalam biji benih yang dieramkan di atas kertas serap lembap. Embrio bebas dari jangkitan dalam kedua-dua biji benih yang dieramkan atau tanpa eraman. Tisu-tisu biji benih yang dijangkiti dalam sampel tanpa eraman didapati membeza dengan baik mencadangkan bahawa jangkitan mungkin telah berlaku pada peringkat lewat semasa pembentukan biji benih.

C.gloeosporioides disebarkan melalui biji benih daripada biji benih kepada anak benih menyebabkan pelecuhan pra dan pos - cambahan. Biji benih yang dijangkiti dengan teruk gagal untuk bercambah akibat

pereputan biji. Anak benih yang hidup mempunyai pertumbuhan yang lambat dibandingkan dengan anak benih dari biji yang sihat.

Untuk mengkaji mekanisme jangkitan biji benih, bahagian-bahagian tertentu pokok cili diinokulat secara tiruan menggunakan konidia kulat. Bunga cili didapati sangat rentan kepada jangkitan menyebabkan keguguran bunga. Hanya penginokulatan buah hijau dan merah mengakibatkan jangkitan *C.gloeosporioides* pada biji benih. Kulat menembusi kedua - dua buah hijau dan merah melalui peg-peg penembusan daripada apresoria. Penembusan secara terus oleh bebenang hifa diperhatikan berlaku dalam buah yang merah. Ini merupakan penemuan baru untuk kulat antraknos menembusi tisu-tisu perumah secara terus tanpa pembentukan apresoria. Hifa patogen menembusi biji benih melalui rekahan dan liang-liang semulajadi pada kulit biji benih, hidup di atas biji benih dan menembusi kedalam lapisan dalam kulit biji melalui mikropil. Pada peringkat lewat jangkitan penembusan berlaku melalui funikulus.

Keputusan menunjukkan bahawa jangkitan bawaan biji benih tidak mempunyai peranan dalam pembentukan reput buah antraknos di lapangan. Walau bagaimanapun, kesan kepatogenan jangkitan bawaan biji benih ke atas

pertumbesaran anak benih bererti semasa peringkat awal pertumbuhan. Pokok-pokok ini menunjukkan pertumbesaran rendah, pembungaan lambat dan seterusnya mengakibatkan penurunan hasil dibandingkan dengan kawalan. Eksperimen selari yang dijalankan di rumah kaca menunjukkan biji benih tidak dijangkiti secara jangkitan sistemik daripada pokok induk. Ini menyokong penemuan eksperimen lapangan di mana jangkitan bawaan biji benih tidak berperanan dalam kejadian reput buah antraknos cili.

CHAPTER I

INTRODUCTION

Capsicum annuum L. is commonly known as chilli, or chilli pepper. It is a solanaeceous vegetable native to tropical America and is the most common and important species among the five species recognized in *Capsicum* (Salunkhe and Desai, 1984). *C.annuum* has white flowers, blue to purple anthers, a toothed calyx and typically single fruited node. It includes large number of horticultural varieties that can be differentiated according to fruit shape, size, colour, texture, flavour and pungency as well as uses. The seeds are white or straw in colour, flat, and kidney in shape and are attached to fleshy placenta. The *Capsicum* fruit is either sweet or pungent (hot) depending on the amount of a non volatile compound called capsaicin (Purseglove, 1968).

According to FAO production year book (1993) the world production of green chilli increased from 7.313 million metric tonnes (MT) in 1981 to 10.630 million

metric tonnes(MT) in 1993 during which the production in Asia continued to be the largest from 2.932 million MT to 5.287 respectively.

C.annuum or locally known as chilli is the most commonly cultivated species in Malaysia. Among the many cultivated varieties of chilli in Malaysia, the local varieties *Kulai* and *Langkap* are the most popular (Leong et al.,1985). Chilli is traditionally cultivated in Malaysia as a garden vegetable in small farms. During the 1970's large chilli cultivation was established by the Federal Land Development Authority (FELDA) in Malaysia in an attempt to meet demands for fresh chilli for the local sauce manufacturing industry (Federal Agricultural Marketing Authority, 1984). In Peninsular Malaysia the hot chilli is cultivated in low land and occupies the largest area (12.3%) among the 14 main cultivated vegetables (Hashim and Anang, 1988). The area under chilli cultivation is approximately 1,153 hectares with production of about 17,237 tonnes annually. However, the volume produced locally is insufficient to meet the demand and RM 27 million ringgit worth of chilli is imported every year (Syed et al., 1992). According to the FAO Trade Year Book (1990), the amount of chilli imported by Malaysia was 21685 MT, 16000 MT and 14000 MT for the years 1988, 1989 and 1990 respectively. Although there

was a decrease in chilli being imported yearly, Malaysia is still among the main countries in the world importing chilli ranking third after USA and Singapore. A 1991 survey by Federal Agricultural Marketing Authority (FAMA) in Malaysia found that the per capita consumption of fresh chilli was 1.784 kg per annum and the total utilization of chilli by Malaysians was valued over RM 90 million (Narayanan and Abdullah, 1992).

The production of vegetables in Malaysia faces many problems among which are pests and diseases. Chilli is no exception. Its major diseases are viral diseases, anthracnose fruit rot and bacterial wilt (Syed et al., 1992). Diseases including anthracnose are among the main constraints to fresh chilli production not only in Malaysia but also in other tropical areas (Poulos, 1992). In Malaysia, anthracnoses on chilli result in high yield loss. Observations showed that the reduction in yield of marketable fruits due to anthracnoses generally ranged from 10% to 60% (Mah, 1987). Such losses increase the market price which goes to the consumers apart from the direct effect on the growers and processors.

Fungal pathogens associated with anthracnoses are known to be seed-borne. The importance of seed-borne

pathogens can be realized from the fact that 90% of the world food crops are sown through seeds and majority of the pathogens attacking them are seed-borne and many of them are seed transmitted. The annual loss in crop yield due to these seed-borne diseases runs into billions of dollars a year. Seed-borne diseases may cause seed decay and/or pre or post emergence damping off. Plants that grow and survive from the infected seeds may have weak stand, stunted growth and poor yield. Besides the role that the infected seed may play in the survival and perpetuation of the pathogen, it can also be a source of inoculum for the next crop (Agarwal and Sinclair, 1987).

Presently, anthracnose on chilli in Malaysia is caused by *Colletotrichum capsici* (Syd.) Butler and Bisby and *C.gloeosporioides* (Penz.) Sacc. *C.capsici* was first shown to be the causal agent by Burnett (1947). Since then numerous research have been attempted. However, *C.gloeosporioides* was recognized in Malaysia only recently causing great losses in several chilli cultivated areas in Peninsular Malaysia (Sariah, 1991). The establishment of the seed-borne nature and seed transmission of *C.capsici* in chilli was well documented (Sariah and Nik, 1988; Sariah, 1992). There is however, no information on the seed - borne nature of *C. gloeosporioides* in chilli. Since chilli is propagated