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EVALUATION OF MALAYSIAN ISOLATES OF TRICHODERMA HARZIANUM RIFAI AND GLIOCLADIUM VIRENS MILLER, GIDDENS AND FOSTER FOR THE BIOLOGICAL CONTROL OF SCLEROTIUM FOOT ROT OF CHILLI

JINANTANA JOMDUANG

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By

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To my mother and my husband
for their love which nourishes my inspiration

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Abstract of dissertation submitted to the Senate of Universiti Pertanian Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy.

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Chairman : Assoc. Prof. Dr. Sariah Meon
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Sclerotium foot rot of chilli (*Capsicum annuum* L.) caused by *Sclerotium rolfsii* Sacc. is commonly found to cause damage in almost all the chilli growing areas in Malaysia. Conventional methods to control the disease, for example, fungicide drenching and crop rotation, seem to be uneconomical and impractical. Recent years, integrated pest management strategy is being adopted for plant disease control which reduces or have lesser impact on the environment. The present study was carried out to evaluate the Malaysian isolates of *Trichoderma harzianum* Rifai and *Gliocladium virens* Miller, Giddens and Foster as biocontrol agents for Sclerotium foot rot of chilli both in glasshouse and small scale field trials. Infection mechanism of *S. rolfsii* on chilli plants and the antagonistic effects of *T. harzianum* and *G. virens* on *S. rolfsii* were also studied.

S. rolfsii was isolated from naturally infected chilli plants collected from Slim River Estate, Perak, Malaysia. Infection mechanism of *S. rolfsii* was observed on both stem segments and the 2-month-old chilli plants, local variety "Langkap". Mycelial aggregates, formed on stem surface along with the ramifying hyphal strands, showed a distinct role in facilitating penetration of *S. rolfsii* into the host cells. Success in penetration through the epidermis was due to mechanical pressure induced by penetration hyphae produced from mycelial aggregates, and cell wall degrading enzyme activity. There was no specific site on the chilli stem for initiation of mycelial aggregates and majority of them appeared as flat masses of hyphae. Few were dome-shaped structures.

The target sites of infection by *S. rolfsii* were found to be the stem collar and underground stem although primary ingression could either be on the stem collar or roots depending on the placement of the inoculum. Further, the 2-month-old chilli plants had shown to be more susceptible to *S. rolfsii* than the 4-month-old ones. However, high concentration of *S. rolfsii* propagules was needed to cause foot rot disease in the older plants.

Nine isolates of *T. harzianum* and one isolate of *G. virens* were isolated from soils and *S. rolfsii* mycelia found on infected chilli plants, respectively, collected from Slim River and Trolak Estates, Perak, Malaysia. Screening for potential *T. harzianum* and *G. virens* antagonistic as biocontrol agents to *S. rolfsii* was

carried out using dual culture and colony degradation test. *T. harzianum* IMI No. 353529 and *G. virens* IMI No. 353523 were the most effective isolates and were used throughout further studies. Antagonism mechanism of these two isolates on *S. rolfsii* was observed under light and scanning electron microscopes. *T. harzianum* and *G. virens* were capable of parasitising both hyphae and sclerotia of *S. rolfsii*. These two antagonistic isolates also produced volatile and non-volatile inhibitors that could retard radial growth of *S. rolfsii* on potato dextrose agar (PDA). Age of antagonists was found to have a significant effect ($p<.01$) on their ability to produce both volatile and non-volatile inhibitors.

Predisposing factors affecting growth and proliferation of *T. harzianum* and *G. virens* were studied. *T. harzianum* grew and proliferated well over a temperature range of 20-30°C, a pH range of 3.8-5.8 and a soil moisture content of 10-25%. *G. virens* was found to grow and proliferate well over the same temperature range, the pH range of 3.8-7.2 and the same soil moisture content.

Inhibition between *T. harzianum* and *G. virens* was not observed on plate culture. In addition, the antagonistic effect of the two antagonists against *S. rolfsii* on paired culture assay could occur simultaneously.

Shelf-life of antagonists (*T. harzianum*, *G. virens* and *T. harzianum* + *G. virens*) incorporated to three types of commercially available organic fertilizer (Amina, Green Supergro and Avanti Green) kept at room temperature were evaluated. It was found

that all antagonists incorporated to Amina and Avanti Green had a significantly ($p<.01$) higher shelf-life period when compared to those incorporated to Green Supergro.

Glasshouse trial to evaluate the efficacy of the antagonists (*T. harzianum, G. virens*, and *T. harzianum* + *G. virens*) either alone or incorporated to three types of organic fertilizer (Amina, Green Supergro and Avanti Green) in controlling Sclerotium foot rot of chilli, local variety "Kulai", was carried out. The result showed that soil amended with antagonists and Amina had the lowest number of viable *S. rolfsii* propagules per 100 g air-dried soil. In addition, populations of the antagonists in soils around the chilli plants (within 5 cm radius from stem and 10 cm depth) and on roots were found to be higher and subsequently the percentages of surviving plants.

Small scale field trial was carried out to evaluate the efficacy of antagonists (*T. harzianum, G. virens*, and *T. harzianum* + *G. virens*) either alone or incorporated to Amina, which was selected based on its effectiveness from the shelf-life study and glasshouse trial, compared to PCNB in controlling Sclerotium foot rot of chilli. The result showed that *G. virens* incorporated to Amina was superior to all other treatments. This antagonist preparation effectively decreased *S. rolfsii* propagules in soils around the chilli plants (within 5 cm radius from stem and 10 cm depth). It also gave higher percentage of surviving plants and number of antagonist population in soils around the chilli plants.

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PENILAIAN PENCILAN-PENCILAN *TRICHODERMA HARZIANUM* RIFAI DAN *GLIOCLADIUM VIRENS* MILLER, GIDDENS DAN FOSTER DARI MALAYSIA UNTUK KAWALAN BIOLOGI REPUT PANGKAL SCLEROTIUM CILI

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Reput pangkal cili (*Capsicum annuum* L.) yang disebabkan oleh *Sclerotium rolfsii* Sacc. didapati menyebabkan kerosakan di kebanyakan kawasan penanaman cili di Malaysia. Kaedah pengawalan penyakit tradisional seperti penyiraman racun kulat dan penggiliran tanaman, menunjukkan tidak ekonomik dan praktik. Kebelakangan ini strategi pengurusan perosak bersepadu telah diamalkan untuk pengawalan penyakit tanaman di mana ia kurang memberi kesan terhadap persekitaran. Kajian berikut dijalankan untuk menilai pencilan-pencilan *Trichoderma harzianum* Rifai dan *Gliocladium virens* Miller, Giddens dan Foster dari Malaysia sebagai agen kawalan biologi untuk reput pangkal *S. rolfsii* pada tanaman cili di dalam rumah kaca dan percubaan-percubaan ladang secara kecil-kecilan. Mekanisme jangkitan *S. rolfsii* ke atas pokok cili dan kesan antagonis *T. harzianum* dan *G. virens* ke atas *S. rolfsii* juga dikaji.

S. rolfsii telah dipencilkan daripada tanaman cili yang dijangkiti secara semula jadi dari Slim River Estate, Perak, Malaysia. Mekanisme jangkitan *S. rolfsii* diperhatikan pada kedua-dua segmen batang dan pokok cili, varieti tempatan "Langkap", berumur dua bulan. Agregat miselium didapati terbentuk di atas permukaan batang bersama-sama dengan helaian hifa yang bercabang-cabang. Struktur ini menunjukkan peranan khusus dalam memudahkan penembusan *S. rolfsii* ke dalam sel-sel perumah. Kejayaan dalam penembusan melalui epidermis adalah disebabkan oleh tekanan mekanikal yang diaruh oleh hifa penembusan yang terhasil daripada agregat-agregat miselium dan aktiviti enzim penghancur dinding sel. Penembusan seterusnya ke dalam sel-sel perumah berlaku secara inter- dan intra-sel. Tidak terdapat tapak yang khusus pada batang cili untuk inisiasi agregat miselium dan kebanyakan daripada mereka kelihatan sebagai massa hifa yang rata. Ada juga yang kelihatan sebagai struktur berbentuk kubah.

Tapak sasaran jangkitan oleh *S. rolfsii* telah didapati pada pangkal batang dan bahagian batang bawah tanah, walaupun penembusan prima mungkin boleh berlaku pada pangkal batang atau akar bergantung kepada penempatan inokulum. Tanaman cili yang berumur dua bulan telah menunjukkan kerentanan yang lebih kepada *S. rolfsii* daripada tanaman yang berumur empat bulan. Walau bagaimanapun, kepekatan propagul *S. rolfsii* yang tinggi diperlukan untuk menyebabkan penyakit reput pangkal pada pokok-pokok cili yang lebih tua.

Sembilan pencilan *T. harzianum* dan satu pencilan *G. virens* yang telah diasingkan daripada tanah dan miselium *S. rolfsii* yang didapati pada tanaman cili yang berpenyakit, masing-masing telah dikutip daripada Estet Slim River dan Estet Trolak, Perak, Malaysia. Penyaringan untuk ciri antagonis *T. harzianum* dan *G. virens* yang berpotensi sebagai agen kawalan biologi terhadap *S. rolfsii* dijalankan dengan menggunakan kultur kembar dan ujian degradasi koloni. *T. harzianum* IMI No. 353529 dan *G. virens* IMI No. 353523 merupakan pencilan yang paling berkesan dan akan digunakan untuk kajian-kajian seterusnya. Mekanisme antagonisme kedua-dua pencilan terhadap *S. rolfsii* diperhatikan di bawah mikroskop cahaya dan mikroskop elektron "scanning". *T. harzianum* dan *G. virens* berupaya memparasit kedua-dua hifa dan sklerotia *S. rolfsii*. Kedua-dua pencilan antagonis ini juga mengeluarkan perencat meruap dan tak meruap yang boleh merencat pertumbuhan radial *S. rolfsii* di atas PDA. Umur antagonis didapati mempunyai kesan berertI ($p<.01$) ke atas keupayaannya untuk mengeluarkan perencat meruap dan tak meruap.

Faktor-faktor pradedah yang mempengaruhi pertumbuhan dan proliferasi *T. harzianum* telah dikaji. *T. harzianum* hidup dan proliferat dengan baik pada julat suhu 20-30°C, julat pH 3.8-5.8 dan kandungan kelembapan tanah 10-25%. *G. virens* didapati hidup dan proliferat dengan baik juga pada julat suhu yang sama, julat pH 3.8-7.2 dan kandungan kelembapan tanah yang sama.

Ujikaji di ladang dijalankan untuk menilai keberkesanan antagonis-antagonis (*T. harzianum*, *G. virens* dan *T. harzianum* + *G. virens*) sama ada sendirian atau digabungkan dengan Amina, yang dipilih berdasarkan kepada keberkesanannya daripada kajian jangka hayat rak dan rumah kaca, dibandingkan dengan PCNB untuk pengawalan reput pangkal Sclerotium tanaman cili. Keputusan menunjukkan bahawa *G. virens* yang digabungkan dengan Amina adalah yang terbaik sekali di antara kesemua rawatan. Penyediaan antagonis berupaya menurunkan bilangan propagul *S. rolfsii* dalam tanah di sekitar pokok cili (di sekitar jejari 5 cm daripada batang dan kedalaman 10 cm). Ia juga memberi peratusan yang tinggi bagi pokok yang hidup dan populasi antagonis yang tinggi dalam tanah di sekitar pokok cili.

CHAPTER I

INTRODUCTION

Chilli has been an important food crop for an extensive period of time. It is native to tropical America and was grown in North and South America for over 2,000 years ago (Splittstoesser, 1979). Chilli belongs to the genus *Capsicum* of the Solanaceae family. Two popular cultivated species are *Capsicum annuum* L. and *Capsicum frutescens* L. The first species constitutes a large number of cultivated varieties and is by far the most important species (Salunkhe and Desai, 1984). The pungency in chilli is dependant on the amount of a substance called capsaicin which is found in high concentration in the placenta of the fruit. Accordingly, chilli can be divided into two groups: i) those producing hot or pungent fruits, and ii) those bearing mild or sweet fruits.

Choudhury (1984) stated that chilli is a good source of vitamins A and C. These two vitamin constituents amount to 1,000 IU and 110 mg per 100 g fresh weight, respectively, which are evidently higher than those found in tomato, snap bean, yellow vegetables, whole wheat and rice.

Chilli is one of the main fruit vegetables in Malaysia. It is grown in an area of approximately 1,153 hectares with an annual production of about 17,237 tonnes (Syed et al., 1992). It is also