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Potential for Biological Control of *Sclerotium* Foot Rot of Chilli by *Trichoderma* spp.

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ABSTRAK

Penyediaan kering udara Trichoderma harzianum *dan* T. virens *telah dinilai kemandirian danpotensinya sebagai calon kawalan biologi terhadap reput pangkal Sclerotium cili (*Capsicum annuum L*) sendirian atau sebagai campuran dengan baja organik Amina, Avanti Greeen dan Greeen Supergrow sebagai pembawa.* T. harzianum *dan* T. virens *apabila dicampurkan sendirian dengan Amina dan Avanti Green masih kekal kebernasannya sehingga 180 hari tetapi apabila di campurkan dengan Green Supergrow hilang kebernasannya dengan cepat kepada sifar selepas 30 hari dalam simpanan. Kegiatan calon-calon kawalan biologi juga signifikan dengan Amina apabila diuji dalam tanah yang diinoklat secara tiruan. Di ladang, penambahan pembawa organik hanya meningkatkan penetapan awal calon-calon kawalan biologi. Kejadian reput pangkal* Sclerotium *berkurangan dengan bererti dengan pengurangan sclerotia pathogen yang bernas dan pengasingan semula calon-calon kawalan biologi dalam rizosfera cli dan akar adalah tinggi* T. Virens *adalah calon yang lebih baik dengan memberi 100% dan 92.1% pengawalan terhadap reput pankal* Sclerotium *dalam tanah yang diinokulat secara tiruan dan tanah yang dijangkiti semulajadi berurutan.*

ABSTRACT

Air-dried preparations of Trichoderma harzianum *and* T. virens *were evaluated for their survival and potential as biocontrol candidates against* Sclerotium *foot rot of chilli* (Capsicum annuum, *L) singly or as mixtures incorporated into organic fertilizers Amina, Avanti Green and Green Supergro as carriers.* T. harzianum *and* T. virens *incorporated singly into Amina and Avanti Green remained viable for 180 days but when incorporated into Green Supergro lost viability rapidly to zero after 30 days' storage. The performance of the biocontrol candidates was also significant with Amina when tested in the artificially-inoculated soils. In the field, the incorporation of an organic carrier only improved initial establishment of the biocontrol candidates. The incidence of* Sclerotium *foot rot was significantly reduced with the reduction in the viable sclerotia of the pathogen and recovery of the biocontrol candidates in the chilli rhizosphere and roots was high.* T. virens *was a better candidate, giving 100 and 92.1% control of* Sclerotium *foot rot in the artificially-inoculated and naturally - infested soils respectively.*

INTRODUCTION

Sclerotium rolfsii Sacc. is one of the most destructive pathogens of chilli (*Capsicum annuum L*). Various strategies for controlling *S. rolfsii* have been introduced over the years including soil disinfestation, cultural practices and fungicide treatments but losses still occur, largely because the effectiveness of these approaches is variable and short lived. In addition, chilli cultivars with strong resistance to *S. rolfsii* are not commercially available. As a result, research efforts are directed toward developing effective and environmentally safe means of combating

foot rot of chilli. Indigenous isolates of *Trichoderma harzianum* and *T. virens* (= *Gliocladium virens*) have been identified as potential biocontrol agents for use against *S. rolfsii* (Jinantana and Sariah 1993, 1997). However, applications of these biocontrol agents have been difficult, mainly because their efficacy not only requires an excessively large amount of inoculum but also varies with soil type and environmental conditions.

Recent advances in our understanding of the infection mechanism of *S. rolfsii* on chilli have led to the conclusion that the target sites for infection are the stem collar and underground stem (Jinantana and Sariah 1994). Accordingly, the biocontrol agent should be placed near the collar region of the chilli plant in order to achieve satisfactory control. Application of the biocontrol agent to soil mixture in nursery bags may assist in the early establishment of the biocontrol agent around the collar region before plants are transferred to the field.

The present study attempted to evaluate the shelf-life of air-dried *T. harzianum* and *T. virens* individually and as mixtures incorporated into 3 types of commercially available organic fertilizers widely used by chilli growers. The potential of the biocontrol preparations was also evaluated for the control of *Sclerotium* foot rot of chilli.

MATERIALS AND METHODS

Fungal Cultures

T. harzianum (IMI 378843) and *T. virens* (IMI 378842) isolated from parasitized sclerotia and confirmed to have antagonistic properties against *S. rolfsii* (Jinantana and Sariah 1993, 1997) were maintained on potato dextrose agar (PDA) as the biocontrol candidates tested in this study.

Preparation of Inoculum

The inoculum of the biocontrol candidates consisted of hyphae, conidia and chlamydospores grown on wheat grains. Three hundred grams of commercial wheat grains were soaked in water for 3 h, rinsed and autoclaved at 103 kPa twice for 30 min in 100 ml of distilled water. After cooling, four 5-mm diameter discs from the margin of a 4-day-old colony of the candidate isolate growing on PDA were transferred to the wheat grain preparation and incubated for 14 days at room temperature (28°C). The preparation was then ground and air-dried at 28°C for 48h. Colony forming units (cfu) per g air-dried preparation were determined by dilution plate technique on *Trichoderma* medium E (TME) (Papavizas 1981).

Preparation of Dried Formulation

The air-dried biocontrol preparations were incorporated into each of the three commercial organic fertilizers (Table 1) in the ratio of 1: 5 w/w (biocontrol candidate: organic fertilizer) and kept in sealed polythene bags. For the mixture, *T. harzianum* and *T. virens* were mixed in equal proportions before adding to each of the organic fertilizers. The biocontrol-fertilizer preparations were stored at room temperature (28 ± 2°C) in a completely randomized design with 3 replications. Samples (1 g) were taken from each replication at 0, 30, 60, 90, 120, 150 and 180 days and the rate of survival and proliferation of the biocontrol candidates were determined by dilution plate technique. The data were expressed as cfu per g air-dried biocontrol-fertilizer preparation. Data were statistically analysed by ANOVA and means compared by using Duncan's multiple range test.

Application of Biocontrol Candidates and Rating of Disease in Sclerotium-chilli System

Glasshouse trial: Potting medium was prepared by mixing sterilized soil mixture (3 : 2 : 1 v/v mixture of soil, sand and organic matter) with the respective biocontrol preparations. For each organic carrier evaluated, treatments consisted of organic fertilizer alone; amended with each air-dried biocontrol candidate (2-weeks-old dried formulation) or mixtures of the biocontrol candidates at 0.08% and 0.05% (w/w) respectively. Four hundred grams of each mixture were placed in pots, sampled and assayed for initial population densities of the biocontrol candidates. Soil mixture alone was also used as the control. One-month-old chilli seedlings were transferred to each of the pots and maintained in the glasshouse for 28 days with normal watering.

These plants were then transferred to sterilized soil mixture which had been artificially inoculated two weeks earlier with 4-week-old *S. rolfsii* inoculum raised on wheat grains at a rate of 3% w/w. There were 24 plants in each treatment. The treatments were arranged in completely randomized design.

TABLE 1
Trade name, formulations and compositions of the commercial organic fertilizers used in the study

Organic fertilizer	Trade name	Formulation	Application rate for chilli	Organic matter[1/]	Total N[2/] (%)	Avail. P[3/] (%)	Avail. K[4/] (%)	pH	Moisture content (%)
Tapioca organic fertilizer	Amina	5.5:5.5:5.5:5.1+TE	Not labelled	10.69	4.70	1.64	0.03	6.80	18.52
Oil palm organic fertilizer	Green Supergro	5 : 5 : 5 : 1 +TE	Not labelled	20.61	4.89	1.81	0.04	3.48	25.08
Chicken dung organic fertilizer	Avanti Green	Not labelled	40 kg/100-200 m^2	21.47	1.41	0.71	0.03	5.97	18.74

[1/] analytical analysis by Walkly and Black Method (Nelson and Sommers 1982)
[2/] analytical analysis by Micro Kjeldahl method (Bremmer and Mulvaney 1982)
[3/] analytical analysis by Bray II method (Bray and Kurtz 1945)
[4/] analytical analysis by using NH4AC (pH 7.0) (Anon. 1996)

The number of infected (wilting with white mycelium at the collar) chilli plants was determined 28 days after transplanting to the *Sclerotium* infested soil. Determination of the populations of the biocontrol candidates in the chilli rhizospheres (within 5-cm radius from the stem and 10-cm depth) and on the roots was carried out 20 and 40 days after transplanting. Soil and roots were sampled, homogenized and placed on a shaker for 20 min in sterilized water. The homogenate was serially diluted with sterile water. One-ml aliquots of the dilution (10^{-4}) was spread with a glass rod over the surface of the agar plate containing TME, and incubated for five days in the dark, after which typical greenish colonies were counted. Similarly, populations of surviving *S. rolfsii* propagules were assessed following the method of Rodriquez-Kabana *et al.* (1980).

Field Trial: The organic fertilizer Amina was chosen as the carrier for the biocontrol candidates based on the results of the survival and glasshouse evaluation trials. The preparation of the seedlings was similar to that used in the glasshouse trial. There were eight treatments (Table 5), arranged in randomized complete block design with three replicates; each replicate consisted of two rows and each row comprised nine chilli plants. The initial population of *Sclerotium* propagules in the experimental plots was determined.

Six-week-old chilli plants previously grown in the nursery bags containing different biocontrol-amended and non-amended (fungicide and control) soil mixtures were transplanted to the naturally infested field. For the fungicide treatment, 500 ml of aqueous Brassicol suspension (200 mg a.i./l H_2O) was applied to each plant by drenching soon after transplanting. Watering, fertilization and pest control were as recommended for chilli cultivation in the area. No fungicide was subsequently used. The number of chilli plants infected was assessed two and four months after transplanting. Determination of the populations of biocontrol candidates and *S. rolfsii* in the soil around the chilli rhizospheres (within 5-cm radius from the stem and 10-cm depth) was determined 1, 2 and 4 months after the plants were transferred to the infested fields.

RESULTS

Survival of Biocontrol Candidates

The survival of air-dried biocontrol propagules differed with the organic fertilizer used as carrier, resulting in different shelf-lives of each biocontrol preparation. *T. harzianum* and *T. virens* singly or as mixtures incorporated into Amina and Avanti Green had a significantly longer ($P < 0.01$) shelf-life than those incorporated into Green Supergro (Table 2). The population levels of both fungi dropped to zero within 30 days of incorporation into Green Supergro while those incorporated into Amina and Avanti Green could be detected for 180 days and 120 days for *T. harzianum* and *T. virens* respectively, after storage at room temperature ($28 \pm 2°C$). Amina and Avanti Green support significantly higher numbers of viable *T. harzianum* propagules than *T. virens* during storage. This was further observed when both biocontrol candidates were used as mixtures, the majority of the biocontrol population recovered was that of *T. harzianum* rather than *T. virens.*

Application of Biocontrol Candidates and Rating of Disease in the Sclerotium-chilli System

The biocontrol candidates parasitized the sclerotia of *S. rolfsii,* resulting in the failure of these sclerotia to germinate when plated on PDA (Jinantana and Sariah 1997). The numbers of viable sclerotia per 100 g air-dried soil sampled from the respective treatments were significantly lower ($P < 0.01$) than those taken from the control at 3 and 40 days after transplanting (Table 3). The lowest number of viable sclerotia was achieved by treatments of biocontrol candidates with Amina and highest when incorporated into Green Supergro at day 3, but all treatments were equally as good at day 40.

The incidence of *Sclerotium* foot rot on chilli plants was evaluated 20 days after transplanting. *T. virens,* either singly or incorporated together with the organic fertilizers, gave 100% control of the disease. However *T. harzianum* supplemented with Amina only gave 83.3% reduction in disease incidence (Table 3). The significant reduction in foot rot of chilli was due to the higher initial populations of the biocontrol candidates in the respective treatments and these populations were maintained throughout the experiments as shown by samplings made after 20 and 40 days after

TABLE 2
Populations of air-dried *T. harzianum*, *T. virens* and *T. harzianum* + *T. virens* culture incorporated into the organic fertilizers and stored at room temperature for 0-180 days

Biocontrol preparations	cfu per g air-dried preparation						
	0 Day	30 Days	60 Days	90 Days	120 Days	150 Days	180 Days
T. harzianum + Amina	$1.6x10^{14}$ a*	$10.7x10^{5}$ a	$4.2x10^{2}$ bc	$3.0x10^{2}$ a	$2.4x10^{2}$ a	$1.5x10^{2}$ b	$10.8x10^{1}$ a
T. virens + Amina	1.4x1014 a	$3.6x10^{5}$ c	$4.2x10^{2}$ bc	$0.9x10^{2}$ d	$0.1x10^{2}$ c	$0.1x10^{2}$ c	$0.9x10^{1}$ b
T. harzianum + *T. virens* + Amina	$1.0x10^{14}$ b	$3.1x10^{5}$ c	4.9x102 a	$1.5x10^{2}$ c	$1.4x10^{2}$ b	$0.1x10^{2}$ c	0.0 b
	Th =$0.6x10^{14}$	Th=$2.0x10^{5}$	Th=2.5 $x10^{2}$	Th=$1.2x10^{2}$	Th=$1.3x10^{2}$	Th=$0.1x10^{2}$	
	Tv =$0.4x10^{14}$	Tv =$1.1x10^{5}$	Tv =$2.4x10^{2}$	Tv =$0.3x10^{2}$	Tv =$0.1x10^{2}$	Tv =0.0	
T. harzianum + Green Supergro	$0.2x10^{14}$ c	0.0 d	0.0 d	0.0 e	0.00 c	0.0 c	0.0 b
T. virens + Green Supergro	$0.1x10^{14}$ c	0.0 d	0.0 d	0.0 e	0.0 c	0.0 c	0.0 b
T. harzianum + *T. virens* + Green Supergro	$0.2x10^{14}$ c	0.0 d	0.0 d	0.0 e	0.00 c	0.0 c	0.0 b
	Th=$0.1x10^{14}$						
	Tv =$0.1x10^{14}$						
T. harzianum + Avanti Green	$1.6x10^{14}$ a	$8.4x10^{5}$ b	$4.0x10^{2}$ c	$2.4x10^{2}$ b	$2.3x10^{2}$ a	$2.2x10^{2}$ a	$8.8x10^{1}$ b
T. virens + Avanti Green	$1.5x10^{14}$ a	$2.9x10^{5}$ c	$4.0x10^{2}$ c	$0.6x10^{2}$ d	$0.1x10^{2}$ c	0.0 c	0.00 b
T. harzianum +*T. virens* + Avanti Green	$0.8x10^{14}$ b	$3.7x10^{5}$ c	$4.6x10^{2}$ ab	$1.5x10^{5}$ c	$0.1x10^{2}$ c	$0.1x10^{2}$ c	0.00 b
	Th=$0.5x10^{14}$	Th=$2.5x10^{5}$	Th=$2.3x10^{2}$	Th=$1.2x10^{2}$	Th=$0.1x10^{2}$	Th=$0.1x10^{2}$	
	Tv =$0.3x10^{14}$	Tv =$1.2x10^{5}$	Tv =$2.3x10^{2}$	Tv =$0.3x10^{2}$	Tv =$0.03x10^{2}$	Tv =0.0	

* Means in the same column with different letters are significantly different ($p<.01$) using DMRT
Th = *T. harzianum* Tv = *T. virens*

TABLE 3
Number of viable Sclerotia and percentage of surviving plants from the artificially-inoculated soil

Treatments	Number of viable *S. rolfsii* propagules per 100 g air-dried soil		% Surviving Plants
	3 Days	40 Days	20 Days
T. harzianum + Amina	5.0 c*	2.3 b	83.3 a
T. harzianum + Green Supergro	18.0 b	4.6 b	61.1 b
T. harzianum + Avanti Green	8.3 c	2.6 b	77.7 ab
T. harzianum	5.6 c	3.0 b	77.7 ab
T. virens + Amina	2.6 c	2.3 b	100
T. virens + Green Supergro	13.0 b	3.3 b	100
T. virens + Avanti Green	11.0 b	3.0 b	100
T. virens	11.0 b	3.0 b	100
T. harzianum + *T. virens* + Amina	5.6 c	2.3 b	100
T. harzianum + *T. virens* + Green Supergro			
T. harzianum + *T. virens*	3.3 c	1.6 b	100
T. harzianum + *T. virens*	3.3 c	1.6 b	100
Control			
Soil mixture alone	73.3 a	58.3 a	0
Amina	72.0 a	60.1 a	0
Green Supergro	70.8 a	59.4 a	0
Avanti Green	72.5 a	60.6 a	0

* Means in the same column of each group of dta with different letters are significantly different ($p<0.1$) using DMRT

transplanting and also on the root rhizosphere (Table 4) . Cfu of *T. harzianum* recovered from the soil was reduced whereas that of *T. virens* increased. Amina enhanced the survival and proliferating ability of *T. virens* in the chilli rhizosphere. The population of biocontrol isolates recovered from roots was higher than soil rhizospheres with respect to the different treatments analysed.

Field trial: T. harzianum and *T. virens* in all preparations with Amina as the carrier or singly, and Brassicol significantly reduced the population of sclerotia in the infested soil as compared to control (Table 5). The symptoms of *Sclerotium* foot rot were observed two months after transplanting. Symptoms appeared as wilting with the production of white rhizomorphs at the base of the stems. *T. virens*-organic carrier and *T. virens* alone gave highest percentages of surviving plants compared to *T. harzianum* or the control (Table 5).

The initial populations of the biocontrol candidates in the soil were assessed in the nursery bags one week after amendment, prior to transplanting and subsequently 1, 2 and 4 months after transplanting. It was found that there were significant differences ($p<0.01$) in the populations among the treatments (Table 6). Results showed that within one month after transplanting, populations of *T. virens* when applied singly had increased from the number found prior to transplanting to the infested field, while the population of *T. harzianum* decreased, suggesting that *T. virens* was sustainable in the *Sclerotium*-infested soil.

DISCUSSION

T. harzianum and *T. virens* isolates evaluated have been proven to effectively parasitize mycelium and sclerotia of *S. rolfsii* (Jinantana and Sariah 1993, 1997) under laboratory conditions, and their potential as biocontrol agents against *Sclerotium* foot rot on chilli has been clarified in this study. Connick *et al.* (1990) have stated that a successful biocontrol formulation is one that is economically produced, safe and stable in the environment, easily applied

TABLE 4
Populations of the biocontrol candidates in the artificially-inoculated soil

Treatments	cfu per g aor-dried soil (x10^4)				cfu per g air-drried root (x10^4)
	In nursery bags	In soil around the chilli plants			
	initial[1/]	on transplanting[2/]	20 Day[3/]	40 Days[3/]	on roots at 40 Days[3/]
T. harzianum + Amina	7.8 a*	5.9 a	0.7 a	0.7 a	2.0 a
T. harzianum + Green Supergro	2.9 b	0.4 c	0.6 a	0.0 c	1.6 b
T. harzianum + Avanti Green	7.1 a	3.2 b	0.7 a	0.3 b	1.5 b
T. harzianum	4.3 b	0.9 c	0.7 a	0.3 b	1.5 b
T. virens + Amina	1.2 a	0.7 a	3.1 a	6.8 a	9.5 a
T. virens + Green supergro	0.3 c	0.5 a	1.6 b	1.4 b	8.9 b
T. virens + Avanti Green	0.8 b	0.6 a	1.9 b	5.8 a	1.3 d
T. virens	1.2 a	0.6 a	1.6 b	1.5 b	6.9 c
T. harzianum + *T. virens* + Amina	3.1 a	1.2 a	2.3 a	3.5 a	7.4 a
	Th=2.2	Th=0.8	Th=0.3	Th=0.1	Th=2.3
	Tv=0.9	Tv=0.4	Tv=2.0	Tv=3.4	Tv=5.1
T. harzianum + *T. virens* + Green Supergro	1.1 b	0.9 b	1.50 b	2.9 ab	6.2 b
	Th=0.6	Th=0.2	Th=0.2	Th=0.1	Th=1.8
	Tv=0.5	Tv=0.4	Tv=1.2	Tv=2.8	Tv=4.4
T. harzianum + *T. virens* + Avanti Green	1.2 b	0.9 b	1.5 b	2.4 b	1.5 d
	Th=0.8	Th=0.5	Th=0.4	Th=0.1	Th=0.9
	Tv=0.4	Tv=0.4	Tv=1.1	Tv=2.3	Tv=0.6
T. harzianum + *T. virens*	3.0 a	0.8 b	1.4 b	2.9 ab	4.0 c
	Th=2.1	Th=0.5	Th=0.2	Th=0.1	Th=1.7
	Tv=0.9	Tv=0.3	Tv=1.2	Tv=2.8	Tv=2.3

* Means in the same column of each group of data with different letters are significantly different (P<01) using DMRT
Th = *T. harzianum* Tv = *T. virens*
[1/] at 1 wk after amendment and prior to transplanting of the chilli into the bags
[2/] on the day the chilli plants were transferred to *S. rolfsii*-infected soil
[3/] days after transplanting the chilli to *S. rolfsii*-infested soil

using conventional agricultural equipment and gives effective and consistent results under a variety of environmental conditions. Three types of commercially available organic fertilizer (Amina, Green Supergro and Avanti Green) were tested as carriers for the biocontrol candidates. Results showed that Amina was superior to the other two organic fertilizers as the delivery system for the biocontrol candidates in the glasshouse. The recovery of biocontrol isolates incorporated into Amina around the plant rhizospheres and roots was high, and viability of *S. rolfsii* propagules in the soil was low, resulting in low incidence of *Sclerotium* foot rot. However, in the field experiment, addition of Amina did not significantly reduce viability of sclerotia compared to biocontrol alone, even though it improved the initial establishment of the biocontrol candidates in the *Sclerotium*-infested soil.

Differences in effectiveness of different organic fertilizers used as carriers may have resulted from the interaction between the biocontrol candidates and chemical and physical characteristics of the carriers (Table 1). The moisture and nutrient content of the carriers possibly affected the effectiveness of the preparations in controlling diseases. Watanabe

TABLE 5
Numbers of viable sclerotia and percentage of surviving chilli plants in the naturally-infested field

Treatments	Number of viable *S. rolfsii* pripagules per 100 g air-dried soil				% Surviving plants
	initial[1/]	1 month[2/]	2 months[2/]	4 months[2/]	4 months[2/]
T. harzianum + Amina	14	5.0 bc*	3.6 bc	2.6 cd	74.7 b
T. virens + Amina	13	3.0 c	2.6 c	1.6 d	92.1 a
T. harzianum + *T. virens* + Amina	15	6.6 b	5.3 b	3.6 bcd	81.4 ab
T. virens	16	7.0 b	5.3 b	4.0 bc	83.3 ab
T. harzianum	14	6.3 b	3.1 b	2.8 bc	73.7 ab
T. harzianum + *T. virens*	12	5.3 bc	5.3 bc	4.3 bc	75.0 b
Brassicol	13	8.0 b	5.6 b	5.3 b	69.9 bc
Control	17	16.0 a	14.3 a	13.7 a	58.3 c

* Means in the same column with different letters are significantly (P<0.1) using DMRT
[1/] 1 day prior to tranplanting of the chilli plants to the field
[2/] after transplanting of the chilli plants to the field

TABLE 6
Populations of the biocontrol candidates sampled from naturally-infested field

Treatment	cfu per g air-dried ($x10^3$)				
	In nursery bags		In soils around the chilli plants		
	initial[1/]	on transplanting[2/] day	1 month[3/]	2 months[3/]	4 months[3/]
T. harzianum + Amina	5.2 a*	3.6 a	1.3 cd	1.1 de	0.6 d
T. virens + Amina	4.9 a	3.7 a	4.6 a	3.1 a	2.3 a
T. harzianum + *T. virens*	3.7 b	2.5 b	2.6 bc	1.8 bc	1.3 bc
+ Amina	Th=2.3	Th=1.3	Th=0.4	Th=0.2	Th0.1
	Tv=1.4	Tv=1.2	Tv=2.2	Tv1.6	Tv=1.2
T. harzianum	4.8 a	2.7 b	0.9 d	0.7 c	0.3 d
T. virens	3.1 c	2.7 b	4.1 ab	2.3 b	1.9 ab
T. harzianum + *T. virens*	3.2 bc	2.2 b	2.0 cd	1.3 cd	0.9 cd
	Th=2.0	Th=1.3	Th=0.5	Th=0.2	Th=0.1
	Tv=1.2	Tv=0.9	Tv=1.5	Tv=1.1	Tv=0.8

Means in the same colimn with different letters are significantly different (P<01) using DMRT
Th = *T. harzianum* Tv = *virens*
[1/] at 1 wk after amendment and prior to transplanting of the chilli into the bags
[2/] on the day the chilli plants were transferred to *S. rolfsii*-infested soil
[3/] months after transplanting the chilli to *S. rolfsii*-infected soil

et al. (1987) found that nitrogen fertilizers stimulated growth and production of conidia of *Trichoderma* in culture and may have a synergistic effect on biocontrol ability. Soil condition is another factor that could affect the availability of nutrients dissolved from organic fertilizers, thus influencing the proliferation and activity of biocontrol candidates. The efficiency of biocontrol agents have been related to rhizosphere competence (Ahmad and Baker 1987a, b). The survival of *Trichoderma* in soils was studied by Papavizas (1981) who concluded that the antagonist did not survive well in the rhizosphere. However, survival of the indigenous *T. harzianum* and *T. virens* isolates in the chilli rhizosphere was significantly improved by incorporating into organic fertilizer in the delivery system for early establishment although effect was diluted with time.

The application of the biocontrol candidates to control soil-borne diseases was mostly through soil amendments in which high quantities of the preparations were needed. Seed treatment and application of the antagonist to rooting or seedling mixtures are alternative methods which could improve establishment and colonization ability in the plant rhizosphere. This will enhance the effectiveness of disease control with the added advantage of using small volumes of biocontrol preparations. Elad *et al.* (1981) applied *T. harzianum* to rooting mixture for carnation and found that disease incidence due to *Rhizoctonia solani* was decreased when the carnations with the whole rooting mixture were transplanted to infested soil. Chilli seedlings are normally prepared in the nursery before being transferred to the field, thus application of the biocontrol formulation to soil mixture in the nursery bags would be the best option. It was found in the present study that this approach provided effective control of *Sclerotium* foot rot of chilli plants in the artificially –inoculated and naturally-infested soils.

Even though *T. harzianum* and *T. virens* possessed no inhibitory effect on each other when used in combinations (Jinantana and Sariah 1993; 1997), the effectiveness of the preparation was mainly due to the activity of *T. virens*. This was confirmed by the results of the recovery (cfu) of *T. virens* from soils with time of application. Although *T. harzianum* survived better in storage, its proliferation in the plant rhizosphere was poor, thereby making *T. virens* a better candidate for the biocontrol of *Sclerotium* foot rot. In addition, biomass of *T. harzianum* and *T. virens* used for incorporation into the carrier in the present study comprised mainly of conidia and mycelia. It has been reported that chlamydospores were more important than conidia in survival and proliferation of the biocontrol candidates (Lewis and Papavizas 1984; Beagle-Ristaino and Papavizas 1985 a, b). Therefore, using biomass comprising abundant chlamydospores of *T. harzianum* and *T. virens* incorporated into Amina may enhance further the effectiveness of the biocontrol candidates in controlling *Sclerotium* foot rot of chilli in Malaysia.

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