ORIGINAL ARTICLE

Effect of *Trichoderma harzianum* biomass and *Bradyrhizobium* sp. strain NC 92 to control leaf blight disease of bambara groundnut *(Vigna subterranea)* caused by *Rhizoctonia solani* in the field

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Abstract

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Four hundred and sixty two strains of *Trichoderma* spp. were isolated from 23 soil samples in which groundnut (*Arachis hypogaea* L.) and bambara groundnut (*Vigna subterranea* L.) had been planted in Songkhla, Phattalung, Nakhon Si Thammarat, Narathiwat and Yala provinces. These fungi were tested against *Rhizoctonia*

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***solani*, a causal agent of leaf blight of bambara groundnut, using dual culture technique on PDA medium. Among 462 isolates tested, 226 isolates had an ability to overgrow *R. solani* completely. Further testing found 13 isolates having the ability to parasitize mycelia of *R. solani.* Among these isolates, ThB-1-54 produced a cellulolytic enzyme on congo-red agar. This isolate was later identified as *T. harzianum* Rifai. In the field test, applying biomass of the isolate ThB-1-54 cultured on ground mesocarp fiber of oil palm, the combination of the isolate ThB-1-54 on ground mesocarp fiber of oil palm and *Bradyrhizobium* sp. (strain NC 92), or fungicide (iprodione) had no effect on disease severity, yield, or the amount of total nitrogen content in stems or seeds of bambara groundnut plant.**

Key words : biological control, *Bradyrhizobium* sp. *Rhizoctonia solani*,*Trichoderma harzianum*, *Vigna subterranea*

บทคัดย่อ

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ผลของมวลชีวภาพเชื้อรา *Trichoderma harzianum* Rifai และเชื้อ *Bradyrhizobium* sp. สายพันธุ์ NC-92 ต่อการควบคุมโรคใบไหม้ของถั่วหรั่ง *(Vigna subterranea)* ที่เกิดจากเชื้อรา *Rhizoctonia solani* ในสภาพแปลงทดลอง

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เชื้อรา *Trichoderma* spp. ซึ่งแยกได้จากดินปลูกถั่วลิสง (*Arachis hypogaea* L.) และถั่วหรั่ง (*Vigna subterranean* L.) จำนวน 23 แปลง ในพื้นที่จังหวัดสงขลา พัทลุง นครศรีธรรมราช นราธิวาส และยะลา จำนวนทั้งสิ้น 462 สายพันธุ์ ถูกนำมาทดสอบการเป็นเชื้อราปฏิปักษ์ต่อเชื้อรา *Rhizoctonia solani* ซึ่งเป็นเชื้อสาเหตุโรคใบไหม้ของถั่วหรั่งโดยวิธี dual culture บนอาหาร PDA พบว่า เชื้อรา *Trichoderma* spp. จำนวน 226 สายพันธุ์ สามารถเจริญเข้าครอบครองเชื้อรา *R. solani* โดยสมบูรณ์ โดยในจำนวนนี้ มี 13 สายพันธุ์ สามารถเป็นปรสิตต่อเส้นใยของเชื้อรา *R. solani* และสายพันธุ์ ThB-1-54 สามารถผลิตเอนไซม์เซลลูเลสได้ดีที่สุดบนอาหารแข็ง congo red ซึ่งเชื้อราสายพันธุ์นี้ได้จำแนกเป็น *Trichoderma harzianum* Rifai จากการทดสอบในแปลง พบว่าการใช้มวลชีวภาพ ThB-1-54 ของกากใยปาล์มน้ำมัน มวลชีวภาพ ThB-I-54 ของกากใยปาล์มน้ำมันร่วมกับเชื้อ *Bradyrhizobium* สายพันธุ์ NC-92 และสารกำจัดเชื้อรา iprodione ไม่มีผลต่อน้ำหนักสดและแห้งของเมล็ด น้ำหนักซากสด รวมทั้งปริมาณไนโตรเจนทั้งหมดในลำต้นและเมล็ดของถั่วหรั่ง

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The bambara groundnut (*Vigna subterranea* (L.) Verdc.) is an African indigenous pulse crop which can be grown in many countries such as Cameroon, Congo, Madagascar and Malawi (Herklots, 1972).In Namibia,this plant has played a crucial role as a food crop and helped to improve the protein supply of the rural population (Wolbling, 1998). In Asia and the Pacific region, bambara groundnut has been introduced and grown in India, Malaysia, The Philippines and Fiji (Herklots, 1972), and Thailand (Sansanee, 2000).

In Thailand, *V. subterranea* has been planted mostly in the South of Thailand, particularly in Nakhon Si Thammarat,Narathiwat,Pattani,Surat Thani and Songkhla provinces. The total bambara groundnut planting area was 1,093.5 hectares, producing approximately 1,072 tons of bambara groundnut, during the 1998-1999 growing season (Sansanee,

2000). Most people usually plant a local variety of bambara groundnut in a small area and gain extra income by selling this groundnut in the local market. Recently,Songkhla 1 (a new variety of *V.subterranean*)was developed by the Songkhla Field Crop Research Center, Department of Agriculture, Ministry of A griculture and Cooperatives,and approved for release for farmer use in 1998 after many years of field trials. The improved characteristic of this variety is that it produces a higher yield than the local varieties. Currently, the Songkhla1 bambara groundnut has been recommended to the farmers for intercropping in the young coconut,oil palm and rubber plantations. It has also been recommended for planting in the infertile sandy soils in some areas in the South of Thailand because it requires comparatively less inputs such as fertilizer application and irrigation comparing to other plants. However, this bambara groundnut has been ravaged with leaf blight disease caused by *Rhizoctonia solani* Kuhn, a soilborne fungal pathogen.Field observations in Pattani province have found that the Songkhla 1 variety has been heavily infected with *R.solani*.The disease infected plants produced substantially less yield than healthy plants (Sansanee, 2000). Iprodione (a chemical fungicide) has been recommended to control this leaf blight disease. However, farmers producing bambara groundnut rarely take action to control this disease because it is uneconomical. In some circumstances, the farmers are not aware that leaf blight disease caused by *R.solani* is one of the limiting factors for bambara groundnut production.

In terms of plant disease epidemiology, mycelia and sclerotia of *R. solani* on the diseased tissue on the soil surface are the inocula which initiate disease development. In the field, leaves of *V. subterranea,* particularly at the flowering stage, become infected by contact with diseased tissue or sclerotia on the soil surface.These infected and diseased leaves later become a source of inoculum for spreading the disease both within and between plants. One control strategy is to apply biomass of a fungal biological control agent both within and between plants to prevent the disease from spreading, particularly at the beginning of the flowering stage when bambara groundnut canopies become thick and the environmental conditions are conducive for disease development. A basic protocol for producing biomass of *Trichoderma* sp. has been devised (Pengnoo *et al.,* 2000). The efficacy of biomass of *Trichoderma* sp. to control leaf blight of bambara groundnut was evaluated in the greenhouse (Sawangsri *et al.,*2002). The biomass of *Trichoderma* sp. has potential for controlling leaf blight of bambara groundnut but further tests in field conditions are required.

The aims of this research were (1) to devise a basic protocol for producing biomass of *T. harzianum* on some agricultural wastes, and (2) to test its effectiveness in controlling bambara groundnut leaf blight disease in a field.

Materials and Methods

Sites of soil sample collection

Soil samples from 23 fields in 5 provinces (Table 1) in the South of Thailand, planted with either bambara groundnut (*V. subterranea* L.) or groundnut (*Arachis hypogaea* L.), were collected for isolating *Trichoderma* spp. These fields were chosen for soil sampling on the basis that leaf blight disease of bambara groundnut caused by *R. solani* had not been found before. In each field, soil samples from five locations, within an area of 30 x 30 m^2, were taken and put together in plastic bags. The soil sample, of approximately 500 g, was taken from each location to a depth of 15 cm. These soil samples were later used for isolating the *Trichoderma* spp.

Isolation, screening, and selection isolate of *Trichoderma sp.*

Trichoderma spp. were isolated from the soil samples using the soil plate method on *Trichoderma* Selective Medium (TSM) (Elad and Chet,1983). The plates were incubated at room temperature (26 -32^{o}C) in the cabinet for 7 days. The growing mycelium of *Trichoderma* spp. was aseptically transferred onto PDA slant. These pure cultures of *Trichoderma* spp. were used to test against *R. solani* using a dual culture technique on PDA medium. An isolate of *Trichoderma* sp. was selected for

Table 1. Sites where soil samples were collected and number of *Trichoderma* sp. isolates obtained from each site

Site	Field code	Crop	No.of *Trichoderma* spp. isolated
Rataphum[1]	ThA-1	*V. subterranea*	19
	ThA-2	*V. subterranea*	28
	ThA-3	*V. subterranea*	13
	ThA-4	*V. subterranea*	19
	ThA-5	*V. subterranea*	23
	ThA-6	*V. subterranea*	28
	ThA-7	*V. subterranea*	3
	ThA-8	*V. subterranea*	16
Hatyai[1]	ThB-1	*A. hypogaea*	66
	ThB-2	*V. subterranea*	7
	ThB-3	*A. hypogaea*	5
	ThB-4	*V. subterranea*	15
Chana[1]	ThC-1	*A. hypogaea*	20
Khong-Ra[2]	ThD-1	*A. hypogaea*	26
Thung-Song[3]	ThE-1	*A. hypogaea*	16
Rae-Sea[4]	ThF-1	*V. subterranea*	13
	ThF-2	*A. hypogaea*	9
Yaha[5]	ThG-1	*A. hypogaea*	29
	ThG-2	*V. subterranea*	7
	ThG-3	*A. hypogaea*	3
	ThG-4	*V. subterranea*	72
	ThG-5	*V. subterranea*	1
	ThG-6	*V. subterranea*	24
Total			462

[1]A district in Songkhla province, South of Thailand.
[2]A district in Phattalung province, South of Thailand.
[3]A district in Nakorn Si Thammarat province, South of Thailand.
[4]A district in Narathiwat province, South of Thailand.
[5]A district in Yala province, South of Thailand.

biomass preparation based upon its ability to overgrow *R.solani* aggressively (Bell *et al*.,1982),to parasitize *R.solani* (coil around the mycelium of *R.solani*) (Dennis and Webster, 1971; Chet *et al*.,1981) and to create a clear zone in media containing carboxymethyl cellulose (CMC) (Beguin,1983). An isolate of *Trichoderma* sp., which could suppress growth of and parasitize *R. solani* and create clear zone,was later identified based upon its morphological characteristics using the keys by Rifai (1969) and von Arx (1970).

Preparation of biomass of *Trichoderma* spp.

One isolate of *Trichoderma* sp. was selected for the production of biomass to test its ability to control leaf blight disease of bambara groundnut caused by *R. solani*. The biomass was produced by culturing the fungus on 100 g of ground mesocarp fibre of oil palm in a plastic bag.The mesocarp fibre of oil palm was ground and passed through a sieve (1mm pore size),and was then moistened with water at 2:1 (w/v) and put in a plastic bag.The material in plastic bag was then sterilized at 121°C for 20 minutes before being inoculated with an agar plug of *Trichoderma* sp. The plastic bags containing ground oil palm mesocarp fiber were then incubated at room temperature (26-32°C) for 10 days before being used in the experiment.

Isolation of *R. solani*

V. subterranea with symptoms of leaf blight was obtained from the Songkhla Field Crop Re-

search Center,Songkhla province.*R.solani* was isolated from leaves of *V. subterranea* using a tissue transplanting technique on Water Agar (WA). The growing mycelia were later transferred onto PDA. The pure culture of the fungus was maintained on PDA slant and identified as *R. solani* (Sneh *et al.,* 1991).

Preparation of *R. solani* inoculum

The inoculum of *R. solani* was produced by transferring the hypha tip of the fungus onto sterile sorghum grains in plastic bags. These plastic bags were incubated on a bench at room temperature (26 -32°C) for 7 days.These sorghum grains,which were heavily colonized by *R.solani*, were later used for inoculation in the field test.

Preparation of bambara groundnut plants for the field test

V.subterranea (Songkhla 1 variety developed by the Songkhla Field Crop Research Center, Department of Agriculture, Songkhla province, Thailand) was used in this experiment. In the field test, bambara seeds were sown in soil at the Songkhla Field Crop Research Center, Rataphum, Songkhla province, Thailand. These seeds were sown in a plot of 3.6 x 4.8 m^2. Six rows with 8 plants/row of bambara groundnuts were planted in this plot using 60 x 60 cm spacing (for one replication). The soil had a sandy loam texture (18.6% clay, 19.2% silt, 62.1% sand), 0.05% total N, 27.6 mg/kg available P, 0.15 mgq/100g K, pH 5.5 and 0.8% organic matter. Twenty days after sowing, the 16-20-0 (N-P_2O_5-K_2O) fertilizer was applied and these bambara plants were grown for 60 days before use in the field experiment.

Experimental design

In the field test, a randomized complete block design (RCBD) with 3 replications and 7 treatments was employed. Seven treatments were (1) bambara groundnut plants with an application of *Trichoderma* sp. growing on ground mesocarp fiber of oil palm, (2) bambara groundnut plants in which their seeds were treated with fresh cells of *Bradyrhizobium* sp.and applied with *Trichoderma* spp. growing on ground mesocarp fiber of oil palm, (3) bambara groundnut plants sprayed with fungicide (Iprodione), (4) bambara groundnut plants in which their seeds were inoculated with fresh cells of *Bradyrhizobium* sp. (3.2 x 10^{11} cfu/mL at 1:1 w/v), (5) bambara groundnut plants inoculated with only *R. solani,* (6) bambara groundnut plants in which their seeds were inoculated with only *Bradyrhizobium* sp.,and (7) un-inoculated bambara groundnut plants as a control treatment. All treatments were inoculated with *R.solani* except two treatments (6 and 7).

Pathogen inoculation

Bambara groundnut plants were inoculated with *R. solani* after 60 days of planting in the field tests. Each bambara groundnut plant (the inner 6 rows and 4 columns) was inoculated with 20 g of the *R. solani* inoculum, while the border rows and border plants adjacent to the walkway were used as guard rows.

Disease assessment

Disease severity of leaf blight of bambara groundnut was assessed, classified according to the degree of leaf blight symptom occurring on the individual canopies of the bambara groundnut plants. Disease severity was classified into 6 levels: (1) level 0 = no symptom, (2) level 1 = 1-20% blight symptom,(3) level 2 = 21-40% blight symptom, (4) level 3 = 41-60% blight symptom, (5) level 4 = 61-80% blight symptom, and (6) level 5 = 81-100% blight symptom. This system of assessing disease severity was arbitrarily employed in this experiment because disease severity index of leaf blight of bambara groundnut has not been previously studied. Yield (as fresh and dry weights of bambara groundnut seeds) and total nitrogen content in both stem and seed of bambara groundnut plants were also assessed after the experiment.

Statistical analysis

The degree of leaf blight severity on the whole canopy of each bambara groundnut plant, fresh and dry weights of bambara seeds, and total nitrogen content in both stem and seed of bambara groundnut plants were assessed in the field test. The

significance of means among treatments were evaluated at the 1% level by DMRT.

Results

Isolation, screening and selection of *Trichoderma* spp.

Four hundred and sixty two strains of *Trichoderma* spp.were obtained in this study. *Trichoderma* spp. were most abundant in the field from Yaha district. In the location in Yala province (field code name: ThG-4), 72 isolates of *Trichoderma* spp. were isolated from the field planted with *V. subterranea,* followed by 66 isolates of *Trichoderma* spp. from the field planted with *A. hypogaea* at Hatyai district, Songkhla province (field code: ThB-1) (Table 1).

Trichoderma spp. were least abundant in another field from Yaha district, Yala province (field code: ThG-5), in which only 1 isolate of *Trichoderma* spp. was isolated from the field planted with *V. subterranea,* followed by 3 isolates from the field planted with *V. subterranea* at Rataphum district, Songkhla province (field code: ThA-7) (Table 1).

Trichoderma spp. from fields, in which more than 25 isolates, were obtained were chosen to test their aggressiveness against *R. solani* on agar (Table 2). Among 226 isolates of *Trichoderma* spp. tested, 66 isolates of *Trichoderma* spp. from Hatyai district, Songkhla province (field code: ThB-1) had the capability to overgrow *R. solani* completely on agar (classified as "class 1 of aggressiveness") (Table2). Among 72 isolates of *Trichoderma* spp. from Yaha district, Yala province (field code:ThG-4) tested,61 isolates had the ability to overgrow *R.solani* completely on agar (class 1 aggressiveness), and 11 isolates encountered *R.solani* half way on agar in a petri dish in which neither *Trichoderma* spp.nor *R. solani* had the capacity to overgrow each other(class 3 aggressiveness) (Table 2).

Trichoderma spp. which showed class 1 aggressiveness were screened and detected for their capability to parasitize *R. solani* on agar. Among 226 isolates of aggressive *Trichoderma* spp. tested, 13 isolates were found to be able to parasitize mycelia of *R. solani*. Parasitization was detected by using a microscope to observe the coiling of *Trichoderma* spp. around the mycelia of *R. solani* and penetration of *Trichoderma* spp.into those of *R. solani*.

Among 13 isolates, the culture filtrate of *Trichoderma* spp. isolate ThB-1-54 produced the largest clear zone using the congo red agar technique (Table 3).This, however,was not significantly different from some other *Trichoderma* isolates(Table 3).

From these screening tests, isolate ThB-1-54 was chosen to produce biomass of *Trichoderma* sp. on sorghum seed and ground mesocarp fiber for the field test.

Field test

Biomass of the *Trichoderma* isolate ThB-1-

Table 2. Dual culture test to determine the aggressiveness of *Trichoderma* spp. against *R. solani*

Isolate	Crop	Degree of Aggressiveness of *Trichoderma* spp.*				
		1	2	3	4	5
ThA-2	*V. subterranea*	28**	-	-	-	-
ThA-6	*V. subterranea*	28	-	-	-	-
ThB-1	*A. hypogaea*	66	-	-	-	-
ThD-1	*A. hypogaea*	14	-	12	-	-
ThG-1	*A. hypogaea*	29	-	-	-	-
ThG-4	*V. subterranea*	61	-	11	-	-
Total		226	-	23	-	-

*Degree of aggressiveness of *Trichoderma* spp. against *R. solani* is based upon Bell *et al.* (1982).
**Number of isolates of *Trichoderma* spp. which has a particular aggressiveness against *R. solani* on agar test.

54 cultured on ground mesocarp of oil palm was best in controlling leaf blight. It was, however, not significantly different from the other treatments, such as the application of the combination of fungal biomass and seed bacterization with *Bradyrhizobium* sp. (strain NC 92), fungicide (Iprodione), seed bacterization only with *Bradyrhizobium* sp. (strain NC 92) or the control treatment (inoculated only with *R. solani*) (Table 4). All treatments had no effect on bambara groundnut plants with respect to yield and nitrogen content in both stems and seeds (Table 4).

Table 3. Mean of clear zones generated by culture filtrate of some selected isolates of *Trichoderma* spp. using congo red agar technique

Isolate	Mean of clear zones (mm)
ThA-2-05	0.0[d*]
ThA-2-13	17.5[ab]
ThA-6-04	17.0[b]
ThA-6-23	17.0[b]
ThB-1-15	16.5[b]
ThB-1-23	17.5[ab]
ThB-1-26	16.5[b]
ThB-1-32	17.5[ab]
ThB-1-54	18.0[a]
ThB-1-59	17.5[ab]
ThB-1-60	17.5[ab]
ThB-1-65	17.0[b]
ThD-1-02	15.0[c]
Aspergillus flavus	0.0[d]
A. niger	14.0[c]
Sterile water (control)	0.0[d]

[*] **Means followed by a common letter are not significantly different at the 1% level by Duncan's Multiple Range Test.**

Table 4. Mean degree of leaf blight severity, fresh and dry weights of bambara seeds, total nitrogen content in plant tissues and seeds of bambara groundnut plants in the field test

Treatment	Severity	Yield (kg/rai*)		Total nitrogen(g/kg)	
		Fresh	Dry	Stem	Seed
ThB-1-54	0.8[ab**]	172.3[ab]	40.6[a]	27.7[a]	27.3[a]
ThB-1-54+NC 92	1.1[a]	162.2[ab]	39.2[a]	27.4[a]	28.2[a]
Iprodione	1.3[a]	115.2[b]	20.2[a]	25.9[a]	27.5[a]
NC 92	1.1[a]	120.3[ab]	26.6[a]	26.9[a]	27.6[a]
Only R. solani	1.0[a]	125.8[ab]	22.2[a]	26.1[a]	27.3[a]
Only NC 92	0.1[c]	152.6[ab]	34.2[a]	26.7[a]	26.8[a]
Un-inoculated plant	0.3[bc]	183.8[a]	39.7[a]	27.9[a]	26.9[a]

[*] **A unit area in which one rai is an area equivalent to 1,600 m^2.**

[**] **Means in the same column followed by a common letter are not significantly different at the 1% level by Duncan's Multiple Range Test.**

Discussion

Although *T. harzianum* had been extensively reported to suppress other soil-borne plant pathogens in other crops (Elad *et al.* 1982 a; Elad *et al.* 1982b; Paulitz *et al.* 1990; Bin *et al.* 1991; Singh, 1991;Chet, 1987; Tronsmo, 1996), this fungal biological control agent was only undergone preliminary testing for assessment of its possible role to control *R. solani,* a causal agent of leaf blight of bambara groundnut in the greenhouse tests (Sawangsri *et al.,* 2002). Mesocarp fiber of oil palm was chosen as a substrate to support growth of *T. harzianum* because it had been satisfactorily used to culture *T.harzianum* (Kanjanamaneesathian *et al.,*2003).Other cheap substrates such as rice bran, rice chaffy grain, farmyard manure, banana pseudostem, and dried banana leaf were also tested to produce *T. harzianum* for managing banana wilt disease caused by *Fusarium oxysporum* f. sp. *cubense* (Thangavelu *et al.,* 2004).

It was apparent that the abundance of *Trichoderma* spp. varied greatly from field to field (Table 1). Soil samples from fields, in which a comparatively high number of *Trichoderma* spp. isolates were obtained, might have characteristics suitable for *Trichoderma* spp. colonization. Askew and Laing (1994) reported that *Trichoderma* spp. from different environmental sites possessed different degrees of aggressiveness against *R.solani*. It was reported that cultivated soils were a better source of potential antagonistic *Trichoderma* spp. compared to forest soils because cultivated soils harbored more isolates of *Trichoderma* spp. which were more aggressive against soil borne plant pathogens, such as *Phytophthora palmivora*, *R. solani* and *Sclerotium rolfsii* (Kanjanamaneesathian *et al.,* 2000). The characteristics of cultivated soils which harbor more aggressive isolates of *Trichoderma* spp. should be identified so that soil sampling for isolating potential *Trichoderma* spp. can be carried out objectively and systematically.

Soil sample (field code: ThB-1),planting with *A. hypogaea,* was found to harbor more isolates of aggressive *Trichoderma* spp. (Table 2). *Trichoderma* sp. (isolate ThB-1-54) was selected from 66 isolates of *Trichoderma* spp. based upon its ability to parasitize the mycelia of *R. solani* and create a clear zone in media amended with cellulose (Table 3). This selection process may give a better chance to obtain potential isolates of *Trichoderma* sp., although there may be no correlation between the test for antagonism in dual culture on a synthetic agar medium and the antagonist effect under greenhouse and field conditions (Tronsmo, 1996). The selection of isolate ThB-1-54 was also compatible with the aim of using ground mesocarp fiber of oil palm as a substrate for biomass production. This characteristic is important because isolate ThB-1-54 will be able to grow very well and utilize ground mesocarp fiber of oil palm.

Treating bambara groundnut seeds with *Bradyrhizobium* sp. (strain NC 92) did not increase the yield of the crop (Table 4), although this *Bradyrhizobium* strain had been recommended for use with bambara groundnut seeds in Thailand.This may be due to soil conditions in the field trial, such as the acidity (pH 5.5) and low level of available P (at 27.6 mg/kg), were not conducive for infection and colonization of bambara groundnut roots by the strain NC 92. These poor conditions of the soil can be rectified by the application of lime and P-fertilizer before strain NC 92 is used in bambara groundnut production. Alternatively,other strains of *Bradyrhizobium* sp. should be tested and evaluated for their efficacy in increasing the yield of bambara groundnut because variation in nitrogen fixation and yield of bambara groundnut plants treated with different strains of *Bradyrhizobium* sp.had been reported (Somasegaran *et al.,*1990; Kishinevsky *et al.,* 1996).

As mesocarp fiber of oil palm was abundant and the isolate ThB-1-54 could grow very fast on this substrate, a large quantity of biomass of the antagonist fungus could be produced. As a result, a higher load of the isolate ThB-1-54 cultured on this inexpensive substrate could be used to obtain better control efficacy. Ground mesocarp fiber was found to support growth of T. *harzianum* relatively well and the fungus cultured on this substrate did not decline very rapidly during 7 months storage (Kanjanamaneesathian *et al*., 2003). Using biomass of

fungus cultured on this substrate could achieve the aim of developing a commercial biological control agent, which was to select for organisms that grow fast on cheap nutrients and to produce propagules with an acceptable shelf life (Tronsmo,1996).

Another option is that biomass of this antagonist fungus can be produced and used by individual farmers providing that farmers are supplied with pure culture of *T. harzianum* and know-how in basic sterile technique to produce biomass of *T. harzianum* by themselves.This alternative will broaden the possibility for the farmers to adopt the application of biological control agent.

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