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Application of biofungicides against *Rigidoporus microporus* causing white root disease of rubber trees

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Ten species of fungi were tested for their abilities to control the growth of *Rigidoporus microporus* causing white root disease of rubber trees. Among them, five species were screened as follows:- *Aspergillus niger* SN72, *Chaetomium bostrychodes* BN08, *Ch. cupreum* RY202, *Trichoderma hamatum* STN07, and *T. harzianum* STN01 to inhibit the growth of pathogen over 50%. *Trichoderma hamatum* STN07 and *T. harzianum* STN01 were rapidly grown over the colony of pathogen whereas *Ch. bostrychodes* BN08 and *Ch. cupreum* RY202 could grow over the colony of pathogen within 30 days. The crude extracts with hexane, ethyl acetate, and methanol from these antagonistic fungi were determined by plate assay and found that crude extracts from *Ch. cupreum* RY202 gave the best results to inhibit the growth of *R. microporus* with ED_{50} value of 170, 402, and 1,220 µg/l, respectively. Moreover, rotiorinol; a bioactive compound produced from *Ch. cupreum* could inhibit the growth of pathogen with ED_{50} value of 26 µg/l that implies, a control mechanism. The formulation of *Ch. cupreum* RY202 in the powder and oil form could significantly inhibit the pathogen to infect the root of the rubber trees.

Key words: biofungicides, *Rigodoporus microporus*, *Chaetomium cupreum*

Introduction

Rigidoporus microporus the causing agent of white root disease is well known destructive agent to several crops and fruit trees especially rubber trees (*Hevea brasiliensis)* (Jayasuriya and Thennakoon, 2007). It is considered to be one of the main pathogen in rubber plantation. White root disease is found in all rubber planting areas throughout the world, for example India, Indonesia, Malaysia, Sri Lanka, Thailand, West and Central Africa. In some countries, it causes greater losses than those caused by all other diseases and pests. It can result in substantial death of trees and sometimes losses of a whole stand

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(Guyot and Flori, 2002). Normally, this disease is controlled by using an integration of cultural methods and chemical fungicides but chemical fungicides have been known to have a negative effect on human health, cause environmental pollution and leave residues in the agricultural soil (Soytong *et al*., 2005; Haggag and Moamed, 2007). Moreover, several plant pathogenic fungi have developed resistance to chemical fungicides (Benítez *et al*., 2004; Kim and Hwang, 2007). To avoid the negative or harmful effect of chemical use, biological control would therefore be an alternative method to save and sound measure for controlling disease by reducing the inoculum sources, as well as inhibiting the disease spread.

Numerous kinds of fungal species have been found in soil and reported to be biological control agent against plant diseases, e.g. *Aspergillus niger*, *Chaetomium globosum*, *Ch. cochlioides*, *Ch. cupreum*, *Gliocladium catenulatum*, *Trichoderma hamatum*, *T. harzianum*, *T. virens*, *T. viride* (Butt *et al.*, 1999; Soytong *et al.*, 2001; Fravel, 2005). *Aspergillus* species are effective against the white-rot basidiomycetes (Bruce and Highley, 1991). *Chaetomium globosum*, *Ch. cochliodes*, *Ch. cupreum* are reported to be antagonistic fungi which are able to suppress plant pathogens such as *Curvularia lunata*, *Pyricularia oryzae*, *Rhizoctonia oryzae*, *Fusarium oxysporum* f.sp. *lycopersici* (Soytong *et al*., 2001). *G. catenulatum* has been reported to reduce the incidence of damping-off disease caused by *Pythium ultimum* and *Rhizoctonia slolani* (Panja and Utkhede, 2004). *Trichoderma* species have been used as biological control agent against a wide range of plant pathogenic fungi including; *Botrytis cineria*, *Fusarium*, *Pythium*, *Rhizoctonia* in many crops such as; corn, soybeans, potatoes, tomatoes, beans, cotton, peanuts, and trees, (Khetan, 2001; Paulitz and Belanger, 2001). Some *Trichoderma* species were reported to control white root disease for example *T. harzianum*, *T. virens* and *T. viride* (Bruce and Highley, 1991; Hightley, 1997; Jayasuriya and Thennakoon, 2007). *Trichoderma harzianum* is reported to be most widely used as an effective biological control agent (Vizcaino *et al.*, 2005; Abdel-Fattah *et al*., 2007).

Biological control may result from direct or indirect interactions between biological control agent and pathogen (Viterbo *et al*., 2007) such as physical contact and synthesis of hydrolytic enzyme, toxic compound or antibiotic, competition, and induce resistance in plant host (Benítez *et al*., 2004; Pal and Gardener, 2006). Many antagonistic fungi produce toxic compound or antibiotic. Some antibiotics have been shown to play role in impede spore germination or kill the cells (Handelsman and Stabb, 1996; Benítez *et al*., 2004; Haggag and Mohamed, 2007). The interest in the secondary metabolites produced by biological control agents has been substantial studying. An

increasing number of metabolites from biological control agents have been discovered due to the application of biochemical assays that are used to identify metabolites (Vizcaino *et al.*, 2005).

In this study, the effective antagonistic fungi were investigated to control the growth of *R. microporus* causing agent of white root disease of rubber trees.

Materials and methods

Pathogen and pathogenicity test

Rigidoporus microporus was isolated from infected root of rubber trees by tissue transplanting technique. The culture was maintained in potato dextrose agar (PDA) medium and deposited at Biocontrol Research Unit, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand. The isolate was also proved for pathogenicity by modified technique from Rodesuchit (1998). The isolate was cultured on sterilized inoculum medium (100 g sawdust, 3 g rice bran, and 2 g sugar, moisten with water) contained in plastic bag and incubated at room temperature (28-30 ^{o}C) for 30 days. Then the 5 months rubber trees were planted in pots containing sterilized mixed soil (soil : sand : compost; 8 : 8 : 2) and inoculum was placed into planting pot next to the root system as seen in Fig. 1. The inoculated rubber trees were maintained in nursery and observed for disease incidence.

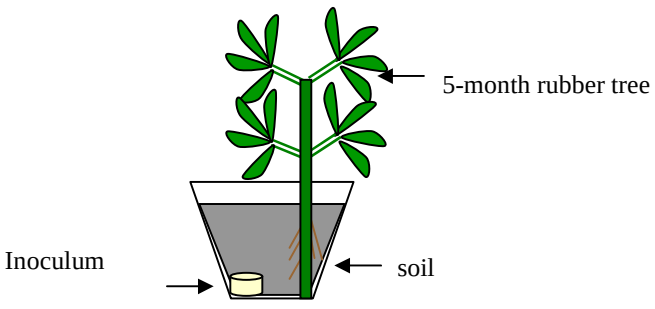


Fig. 1. Inoculation technique for pathogenicity test.

Biological control agents

The fungal isolates used in this study were isolated from rhizosphere soil from rubber plantation by soil plate and baiting techniques as described by Soytong and Quimio (1989). All fungal isolates were tested for their abilities to control *R. microporus* by dual culture. Then the fungal isolates which could inhibit the colony of *R. microporus* over 50% were taken to study their crude extracts to inhibit the colony of *R. microporus* by plate essay.

Effect of biological control agents by dual culture

The fungal isolates were separately evaluated for their abilities to inhibit the mycelial growth of *R. microporus* causing white root disease of rubber trees by dual culture technique. The experiment was conducted by Completely Randomized Design (CRD) with four replications. Biological control agents were separately grown on PDA for 6 days at room temperature (28-30 ^{o}C). The agar plug from the growing edges of colony of biological control agents was removed with a sterile cork borer (3 mm. diameter) and placed on one side of Petri dishes (9-cm diameter). The agar plug of *R. microporus* was taken at the age of 6 days colony and placed on the opposite sides of Petri dish. The dual plates were incubated at room temperature (28-30 ^{o}C) for 10-30 days. Data were recorded as colony diameter (cm) at 10 days. Percent inhibition of mycelial growth (PI) was computed as [(Dc-Dd)/Dc] x 100, where Dc is growth diameter of pathogen in control plates and Dd is growth diameter of pathogen in the dual culture plates. The experiment was repeated twice.

Effect of crude extracts of biological control agents

***Crude extraction method*:** The crude extracts were extracted from mycelial mats of biological control agents using solvent as described by Kanokmedahkul *et al*. (2006) The biological control agents were separately cultured in potato dextrose broth (PDB) for 30 days, filtered to yield mycelial mats. Air dried mycelial mats were ground and extracted successively with hexane, ethyl acetate, and methanol. The solvents were evaporated using rotary vacuum evaporator to yield crude hexane, ethyl acetate, and methanol extracts, respectively. Each crude extract was kept in the refrigerator until used.

***Testing method*:** The experiment was conducted by 3 x 6 factorials in CRD with 4 replications. Factor A was crude extracts from antagonistic fungus as follows:-crude hexane, crude ethyl acetate, and crude methanol extracts. Factor B was the concentrations of crude extracts, varies from 0 (control), 10,

50, 100, 500, and 1,000 µg/l. Each crude extract in each concentration was dissolved in dimethyl sulfoxide (DMSO), and then mixed in PDA before sterilization. The agar plug from growing edges of colony of pathogen were placed at the centre of petri dish containing PDA incorporating with each concentration of crude extract and incubated at room temperature (28-30 ^{o}C) for 6 days. The mycelial growth of pathogen was measured as colony diameter (cm). Effective dose (ED_{50}) of each crude extract was also computed by probit analysis. Treatment means were compared using Duncan's Multiple Range Test (DMRT) at $P = 0.01$. The experiment was repeated twice.

Effect of rotiorinol, a bioactive compound from Chaetomium cupreum

The bioactive compound named rotiorinol produced by *C. cupreum* was provided by Somdej Kanokmedhakul, Faculty of Chemistry, Khon Kaen University, Khon Kaen, Thailand. It was tested for ability to inhibit mycelial growth of *R. microporus*. The experiment was conducted by CRD with 5 treatments and 4 replications. The treatments were different concentrations of rotiorinol as follows:- 0 (control), 10, 50, 100 and 250 µg/l. Rotiorinol was dissolved in dimethyl sulfoxide (DMSO) after that mixed with PDB in different concentrations and sterile. The agar plug of *R. microporus* at 6 days was transferred to the flask containing PDB which mixed with different concentrations of rotiorinol and shaked at 100 rpm for 10 days. The fresh weight and dry weight of mycelium of pathogen were recorded and computed as percent of fresh weight and dry weight inhibition. The effective dose (ED_{50}) was also computed. The experiment was repeated twice.

Effect of Chaetomium cupreum RY202 formulated as biofungicide to control pathogen in vivo

The experiment was set up to study the effect of *Ch. cupreum* RY202 as biofungicide in greenhouse condition. The experiment was conducted by CRD with 7 treatments and 4 replications. The treatments were as follows:- non-treated one, inoculated with pathogen, treated with antagonistic fungus in powder form, treated with antagonistic fungus in the oil form, treated with antagonistic fungus in the powder form and pathogen, treated with antagonistic fungus in the oil form and pathogen and treated with fungicide (sulfur). *Chaetomium cupreum* RY202 was culture in PDB for a month and after that formulated according to the methods of Dr. Kasem Soytong, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand (unpublished data), in the powder and oil form at the standard concentrations of 1 x 10^6 cfu/g and 1 x 10^6 cfu/ml, respectively. The most virulent isolate of *R.*

microporus was cultured as pathogen inoculum in medium mixed substrate as described above. The 5 months rubber trees (variety RRIM600) was planted in the pot containing sterilized mixed soil (soil : sand : compost; 8 : 2 : 2). Then the pathogen inoculum was inoculated in the pot near the root of rubber trees. The biofungicide was mixed with the planting sterilized soil according into each treatment as stated at the rate of 100 g or 100 ml/plot. After that each biofungicide was applied by spraying every 2 weeks in the treatments at the rate of 1 g or 1 ml/tree. Data collection as disease index (DI) was recorded at 120 days after treatment. The disease index was categorized as follows: level 1 = healthy, green leaves, level 2 = 1-25% yellow leaves, level 3 = 26-50% yellow leaves, level 4 = 51-75% yellow leaves and level 5 = 76-100% yellow leaves. Infected root colonized was observed and recorded. The percentage of disease reduction was also calculated. The experiment was repeated twice.

Results

Pathogen and pathogenicity test

The characteristic of *R. microporus* which isolated from infected root of rubber trees by tissue transplanting technique were showed in Fig. 2. The colony on PDA at 6 days showed white and flattened mycelium. The hypha showed hyaline, septate, and possession many branches but no clamp connection. The fruiting body showed broad, thin, and orange-red. Basidiospores showed globose, colorless, thin-walled, and smooth**.**

Pathogenicity tests of *R. microporus* on rubber trees variety RRIM600 showed symptom of yellowing leaves at 70 days. The root of the dead tree was possessed with rhizomorph of the pathogen and it produced fruiting body at the collar of the dead stem (Fig. 3).

Biological control agents

The fungal isolates used in this study are listed in Table 1. They were isolated from Narathiwat and Surat Thani provinces which located in the south of Thailand. Four species of *Chaetomium* were isolated by baiting technique. One species of *Aspergillus*, one species of *Penicillium*, and two species of *Trichoderma* were isolated by soil plate technique. *Chaetomium cupreum* RY202 and *Ch. cocchliodes* RY301 were kindly provided by Dr. Chaninan Pronsuriya, International College, King Mongkut's Institute of Technology Ladkrabang, Bangkok, Thailand.

Table 1. The fungal isolates used in this study.

Isolate code	Species	Isolation method	Geographical origin
BN01	*Chaetomium aureum*	Baiting technique	Narathiwat province
BN08	*Chaetomium bostrychodes*	Baiting technique	Surat Thani province
BS31	*Chaetomium fusiforme*	Baiting technique	Surat Thani province
BN05	*Chaetomium indicum*	Baiting technique	Narathiwat province
SN72	*Aspergillus niger*	Soil plate technique	Narathiwat province
SN03	*Penicillium canescens*	Soil plate technique	Narathiwat province
STN07	*Trichoderma hamatum*	Soil plate technique	Surat Thani province
STN01	*Trichoderma harzianum*	Soil plate technique	Surat Thani province
RY301	*Chaetomium cochliodes*	Baiting technique	Rayong province
RY202	*Chaetomium cupreum*	Baiting technique	Rayong province

a b c d

Fig. 2. Characteristic of *Rigidoporus microporus*: colony on PDA at 6 days (a), hypha (b) fruiting body (c), and basidiospores (d).

a b c

Fig. 3. The symptom of white root disease of rubber trees; yellowing leaves (a), rhizomorph at the root (b) and fruiting body at the collar of the dead stem (c)

Effect of biological control agents by dual culture

Based on the results, five tested fungal isolates could inhibit the mycelial growth of *R. microporus* over 50% as follows:- *A. niger* SN72, *Ch. bostrychodes* BN08 *Ch. cupreum* RY202, *T. hamatum* STN07 and *T. harzianum* STN01. *Aspergillus niger* SN72, *Ch. bostrychodes* BN08 and *Ch. cupreum* RY202 which inhibited the mycelial growth for 10 days as 75.8, 58.1, and 57.5%, respectively. The mycelium of pathogen could not grow when it came to contact with *A. niger* SN72. Moreover, *Ch. bostrychodes* BN08 and *Ch. cupreum* RY202 could produce the fruiting bodies over the colony of *R.*

microporus after leaving them at room temperature (28-30 ^{o}C) for 30 days. The results showed that *T. hamatum* STN07 and *T. harzianum* STN01 were highly antagonistic against *R. microporus* on agar plate with inhibition of mycelial growth at 89.5% and 85.3%, respectively and both of them rapidly grown over *R. microporus* colony.

Effect of crude extracts of biological control agents

Five tested biological control agents inhibited the mycelial growth of *R. microporus* over 50% as follows:- *A. niger* SN72, *Ch. bostrychodes* BN08 *Ch. cupreum* RY202, *T. hamatum* STN07 and *T. harzianum* STN01 as previous experiment. They were then cultured and extracted for their metabolites. Results showed significant differed in percentage of colony inhibition at the concentration of 10, 50, 100, 500, and 1,000 µg/l. All crude extract at the concentration of 1,000 µg/l, which extracted from *Ch. cupreum* RY202 inhibited colony of *R. microporus* as 82.0, 78.0, and 50.0%, respectively. Whereas, the methanol crude extract which extracted from *T. hamatum* STN07 and *T. harzianum* STN01 inhibited colony of pathogen at 1,000 µg/l as 80.0 and 61.5%, respectively. All crude extracts which extracted from *Ch. bostrychodes* BN08 and *A. niger* SN72 could not inhibit the mycelial growth of *R. mocroporus* (Table 2).

The effective dose (ED_{50}) of each crude extract which extracted from biological control agents was showed in Table 3. Crude hexane extract from *Ch. cupreum* RY202 gave the best inhibition to mycelial growth of *R. microporus* with ED_{50} of 170 µg/l follow by crude methanol extract from *T. hamatum* STN07 and crude ethyl acetate extract from *Ch. cupreum* RY202 with ED_{50} of 187 and 402 µg/l, respectively.

Effect of rotiorinol, a bioactive compound from Chaetomium cupreum

Rotiorinol is charaterized as red and amorphous powder and its structure was showed in Fig. 4. Rotiorinol gave significantly inhibit the growth of *R. microporus*. The percent of fresh weight inhibition of mycelium after treated with rotiorinol at concentrations of 10, 50, 100 and 250 µg/l were 29.0, 49.0, 97.8 and 99.3%, respectively and percent of dry weight inhibition were 31.4, 42.0, 97.2 and 99.1%, respectively (Table 4). The effective dose (ED_{50}) of this bioactive compound was 26 µg/l. Based on the results, the mycelium of *R. microporus* could not grow at concentrations 100 and 250 µg/l (Fig. 5). This research finding is implies antibiosis, a control mechanism of *Ch. Cupreum* against *R. microporus*.

Table 2. Percent growth inhibition after treated with crude extracts for 6 days.

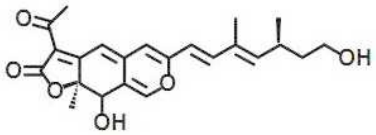
Biological control agents	Crude extracts	% growth inhibition				
		10	50	100	500	1,000
Aspergillus niger SN72	hexane	0.0c	0.0d	0.0e	0.0i	0.0h
	ethyl acetate	0.0c	19.5a	24.0b	28.5d	33.0d
	methanol	0.0c	0.0d	0.0e	20.0f	25.5e
Chaetoium bostrychodes BN08	hexane	0.0c	0.0d	0.0e	0.0i	9.0g
	ethyl acetate	0.0c	0.0d	0.0e	0.0i	0.0h
	methanol	0.0c	0.0d	0.0e	0.0i	0.0h
Chaetomium cupreum RY202	hexane	11.0a	13.0b	21.0c	80.5a	82.0a
	ethyl acetate	9.5b	13.5b	20.5c	42.5c	78.0a
	methanol	0.0c	9.0c	19.0d	24.5e	50.0c
Trichoderma hamatum STN07	hexane	0.0c	0.0d	0.0e	0.0i	4.5gh
	ethyl acetate	0.0c	0.0d	0.0e	15.0g	17.5f
	methanol	0.0c	0.0d	53.0a	80.0a	80.0a
Trichoderma harzianum STN01	hexane	0.0c	0.0d	0.0e	0.0i	0.0h
	ethyl acetate	0.0c	0.0d	0.0e	10.0h	27.5e
	methanol	0.0c	0.0d	0.0e	59.0b	61.5b
C.V. (%)		42.2	25.1	10.2	6.2	8.4

*Mean of four replications. Mean followed by a common letter are not significantly different when compared by Duncan's Multiple Range Test (DMRT) at $P = 0.01$.

Table 3. Effective dose (ED_{50}) of crude extracts from antagonistic fungi.

Biological control agents	Crude extracts	ED_{50} (µg/l)
Aspergillus niger BN72	hexane	NF
	Ethyl acetate	1,981
	Methanol	2,949
Chaetomium bostrychodes BN08	hexane	NF
	Ethyl acetate	NF
	Methanol	NF
Chaetomium cupreum RY202	hexane	170
	Ethyl acetate	402
	Methanol	1,220
Trichoderma hamatum STN07	hexane	NF
	Ethyl acetate	2,495
	Methanol	187
Trichoderma harzianum STN01	hexane	NF
	Ethyl acetate	1,635
	Methanol	556

NF = No effect



Source: Kanokmedhakul *et al*. (2006)

Fig. 4. Chemical structure of rotiorinol.

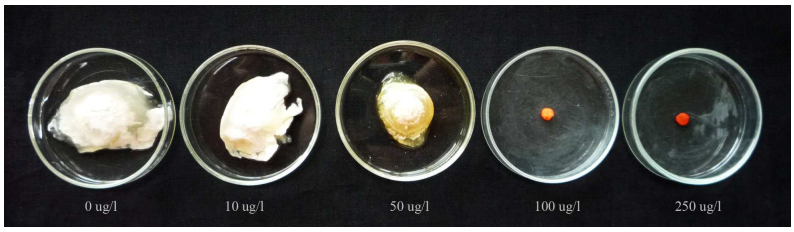


Fig. 5. Mycelial growth of *Rigidoporus microporus* after treated with rotiorinol at different concentrations**.**

Table 4. Fresh and dry weights of mycelia of *Rigidoporus microporus* after treated with rotiorinol.

Concentrations (µg/l)	% Fresh weight inhibition	% Dry weight inhibition
0	0.0d*	0.0d
10	29.0c	31.4c
50	49.0b	42.0b
100	97.8a	97.2a
250	99.3a	99.1a
cv. (%)	10.34	5.14

*Mean of four replications. Mean followed by a common letter are not significantly different when compared by Duncan's Multiple Range Test (DMRT) at $P = 0.01$.

Effect of Chaetomium cupreum RY202 formulated as biofungicide to control pathogen in vivo

The results showed that the disease symptom occurred in the inoculated with *R. microporus* (DI = 4) and inoculated with *Ch. cupreum* in the powder form and *R. microporus* (DI=2). The percentage of disease reduction in the treatment of inoculated with *R. microporus* and *Ch. cupreum* RY202 in the powder form, oil form and sulfur were 50, 75, and 75%, respectively (Table 5).

Table 5. Disease index (DI) and disease reduction (DR) after treated with *Rigidoporus microporus* and biofungicide for 120 days.

Treatments	DI*	DR** (%)
Non-treated	1***	-
R. microporus	4	-
Ch. Cupreum in powder form	1	75
Ch. Cupreum in the oil form	1	75
Ch. Cupreum in the powder form and *R. microporus*	2	50
Ch. Cupreum in the oil form and *R. microporus*	1	75
Sulfur and *R. microporus*	1	75

*Disease index was categorized as follows: level 1 = healthy, green leaves, level 2 = 1-25% yellow leaves, level 3 = 26-50% yellow leaves, level 4 = 51-75% yellow leaves and level 5 = 76-100% yellow leaves.
** Disease reduction
***Mean of four replications.

Discussion

*Rigidoporus mciroporu*s was isolated from infected root of rubber tree and causes white root disease. This disease is an important disease of rubber trees which causing economically important loses in rubber plantation in Thailand and many countries. Nandris *et al.* (1987) reported that this fungus infects the roots by free rhizomorphs growing from the stumps or infected woody debris remaining in the ground and by contacting with the infected root. The visible symptom is changed in color of the leaves from green to yellow (Guyot and Flori, 2002). The decrement or inhibition the source of inoculum by using effective biological control agents is the best way to control this disease. This research tried to find out the biological control agents that possess the antagonistic properties to control *R. microporus* by dual culture and their bioactive compound. The results of dual culture showed that *A. niger*, *Ch. bostrychodes*, *Ch. cupreum*, *T. hamatum* and *T. harzianum* could inhibit the mycelial growth of *R. microporus* over 50%, especially *T. hamatum* STN07 and *T. harzianum* STN01 which rapidly grown and could grow over the colony of pathogen within a few days. These results were similar to those reported by Jayasuriya and Thennakoon (2007) who reported that *T. harzianum* was highly antagonistic against *R. microporus* and rapidly overgrown on *R. microporus* colonies *in vitro*. Bruce (1991) also reported that *Trichoderma* strains quickly overgrown and killed Basidiomyctes causing wood decay fungi. The other species of *Trichoderma* which reported to be an effective antagonist for pretreatment to protect white rot fungi was *T. virens* (Hightley, 1997; Bruce 1991). Nowadays, there are several reports on the use of *T. hamatum* and *T. harzianum* as biological agents against plant pathogens, especially *T.*

harzianum (Harman *et al*., 2004). This taxa used as biocontrol in a wide range of pathogenic fungi such as *Botrytis cinerea*, *Fusarium* spp., *Phytopthora palmivora*, *P. parasitica*, *Pythium* spp. and *Rhizoctonia* spp. (Soytong *et al*., 2001; Benítez *et al*., 2004). Some strain of *T. harzianum* was formulated as mycofungicide such as *T. harzianum* T-22 and strain T-39 (Etebarian *et al*., 2000; Khetan, 2001; Paulitz and Belanger, 2001). *Trichoderma* species successfully used as an biological control agent because they are fast growing, high productivity, diversity of control mechanisms, excellent competitors in the rhizosphere, tolerant or resistance to fungicides, strong aggressiveness against phytopathogenic fungi, and promote plant growth (Tang *et al*., 2001; Benítez *et al*., 2004; Szekeres *et al*., 2004). *Chaetomium bostrychodes* BN08 and *Ch. cupreum* RY202 were isolated from soil and inhibited the mycelial growth of *R. microporus* over 50% in dual culture and produced the fruiting bodies on the colonies. This is the first report using *Ch. bostrychodes* and *Ch. cupreum* to control the colony of white root pathogen *in vitro*. These fungi may be one of the antagonists which can be applied to decrease the source of inoculum or inhibit the spread of disease. In this study, *Ch. cupreum* RY202 was selected to be used as the most effective antagonist against R. microporus due to the results from crude extract test and rotiorinol an antibiotic substance from *Ch. cupreum*. Then, *Ch. cupreum* RY202 was formulated as powder and oil forms. These formulations were applied to inhibit *R. microporus* to protect the root of the rubber trees *in vivo*. The results showed that formulated biofungicide produced from *Ch. cupreum* RY202 could inhibit the pathogen to infect the root of the rubber trees. However, several species of *Chaetomium* can act as an antagonists such as *Ch. globosum* and *Ch. cupreum* (Soytong *et al*., 2001, Soytong, 2003; Tomilova and Shternshis, 2006), *Ch. spirale* (Xin and Shang, 2005). *Ch. cupreum* can antagonizes a wide range of plant pathogens such as *Curvularia lunata*, *Pyricular*ia *oryzae* and *Rhizoctonia solani* (Soytong, 2003). Moreover, *Ch. cupreum* and *Ch. globosum* were formulated as mycofungicide to control several diseases such as Fusarium wilt of tomato and Phytophthora root rot (Soytong *et al*., 2001).

Based on this research, *A. niger* could inhibit the growth of *R. microporus* in dual culture. The result was similar to the report of Bruce and Hightley (1991) who found that *Aspergillus* species were effective against the white-rot basidiomycetes. Moreover, Bruce (1991) reported that *Aspergillus* could lysis the mycelium of white rot fungi at the point of contact but did not overgrow the colony of pathogen.

Our reports provide evidence of control mechanism as antibiosis from *Ch. cupreum* that produces antifungal metabolites for inhibition of growth of *R. microporus*. The crude extracts and rotiorinol from *Ch. cupreum* could inhibit

the growth of *R. microporus*. There are many reports using crude extracts produced from *Ch. cupreum* to control the plant pathogens such as *Colletotrichum gloeosporioides* causing anthracnose disease and *Phytophthora parasitica* causing root rot *Pythium* sp. causing Pummelo root rot (Soytong *et al*., 2005; Meepeung and Soytong, 2004). Moreover, Kanokmedhakul *et al*. (2006) extracted bioactive compound from *Ch. cupreum* CC3003 named rotiorinol and tested their antagonistic properties against pathogenic fungi and found that this compound could inhibit the growth of *P. parasitica*, *P. palmivora*, *C. gloeosporioides*.

In conclusion *Ch. cupreum* and its metabolite could inhibit the growth of *R. microporus*. The formulation of *Ch. cupreum* in the powder and oil form could inhibit *R. microporus* to infect the root of the rubber trees *in vivo*. This study was the first report using *Chaetomium* species and their metabolites to control *R. microporus.* However, these biological control agents need to prove their ability to control white root disease in the field by using their formulations or metabolites.

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