BIOLOGICAL CONTROL OF TOMATO FUSARIUM WILT

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT

OF THE REQUIREMENT FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY (BIOTECHNOLOGY IN PLANT PATHOLOGY)

INTERNATIONAL COLLEGE

KING MONGKUT'S INSTITUTE OF TECHNOLOGY LADKRABANG

2010

KMITL-2010-IC-D-009-004

หัวข้อวิทยานิพนธ์	การควบคุมโรคเหี่ยวจากเชื้อฟิวซาเรียมของมะเขือเทศโดยชีววิธี
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บทคัดย่อ

การแยกเชื้อราจากต้นมะเขือเทศที่เป็นโรคเหี่ยวใน 8 จังหวัดของประเทศไทย พบเชื้อรา *Fusarium* spp. จำนวน 45 ไอโซเลท เมื่อทำการศึกษาลักษณะทางสัณฐานวิทยา และการทำ molecular phylogeny จากไอโซเลทของเชื้อ *Fusarium* พบว่า มีเชื้อราเพียง 12 ไอโซเลท ที่จำแนกได้เป็นเชื้อรา *Fusarium oxysporum* f.sp. *lycopersici* โดยสามารถแบ่งกลุ่มตามดัชนีความรุนแรงของการเกิดโรคได้เป็น 3 กลุ่ม เชื้อรา *F. oxysporum* f.sp. *lycopersici* ไอโซเลท NKSC01 สามารถทำให้เกิดโรคในระดับความรุนแรงสูงสุด มีดัชนีความรุนแรงของการเกิดโรคเท่ากับ 6.00 บนต้นกล้ามะเขือเทศพันธุ์สีดา และเชื้อราไอโซเลทนี้ได้ถูกนำมาใช้เป็นตัวแทนของเชื้อสาเหตุโรคเหี่ยวของมะเขือเทศในการวิจัยครั้งนี้

เชื้อราต่อต้าน 3 ไอโซเลท คือ *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT และ *Trichoderma harzianum* PC01 ได้ถูกนำมาใช้ทดสอบความสามารถในการควบคุมเชื้อรา *F. oxysporum* f.sp. *lycopersici* NKSC01 พบว่า เชื้อราต่อต้านทั้ง 3 ไอโซเลท สามารถควบคุมการสร้างคอนิเดียของเชื้อราสาเหตุโรคได้มากกว่า 90 เปอร์เซ็นต์ในการเลี้ยงเชื้อร่วมบนจานอาหารเลี้ยงเชื้อ PDA สารสกัดหยาบจาก hexane ของเชื้อราต่อต้าน *C. globosum* KMITL-N0802 สามารถควบคุมการสร้างคอนิเดียของเชื้อราสาเหตุโรคได้สูงสุด โดยมีค่า ED_{50} 157 ไมโครกรัมต่อมิลลิลิตร ในขณะที่สารสกัดหยาบจาก hexane ของเชื้อรา *C. lucknowense* CLT และ สารสกัดหยาบจากเมธานอลของเชื้อรา *T. harzianum* PC01 สามารถควบคุมการสร้างคอนิเดียของเชื้อราสาเหตุโรค โดยมีค่า ED_{50} 188 และ 192 ไมโครกรัมต่อมิลลิลิตรตามลำดับ

สารสกัดบริสุทธิ์จากเชื้อราต่อต้าน 3 ชนิด คือ chaetoglobosin C, chaetomanone A และ trichotoxin A50 ได้ถูกนำมาทดสอบประสิทธิภาพในควบคุมการเจริญเติบโตของเชื้อรา *F. oxysporum* f.sp. *lycopersici* NKSC01 ในห้องปฏิบัติการ พบว่า สารสกัดบริสุทธิ์ทั้ง 3 ชนิดที่ความเข้มข้น 100 ไมโครกรัมต่อมิลลิลิตร สามารถควบคุมการเจริญของเส้นใยของเชื้อสาเหตุโรคได้ โดยมีค่า ED_{50} ระหว่าง 68 ถึง 71 ไมโครกรัมต่อมิลลิลิตร และควบคุมการสร้างคอนิเดียของเชื้อสาเหตุโรคได้โดยมีค่า ED_{50} ระหว่าง 17 ถึง 22 ไมโครกรัมต่อมิลลิลิตร

สารสกัดบริสุทธิ์จากเชื้อราต่อต้านได้ถูกนำมาทดสอบการเป็น microbial elicitor เพื่อกระตุ้นให้เกิดการสร้างสาร α-tomatine ในต้นมะเขือเทศ โดยตรวจวัดปริมาณของ α-tomatine โดยเครื่อง High performance liquid chromatography (HPLC) หลังจากการฉีดพ่นต้นมะเขือเทศด้วยสารสกัดบริสุทธิ์จากเชื้อราต่อต้าน จากการฉีดพ่นสาร chaetoglobosin C, chaetomanone A และ trichotoxin A50 บนต้นกล้ามะเขือเทศที่ได้รับการปลูกเชื้อราสาเหตุโรค และทำการตรวจสอบการชักนำให้เกิดภูมิคุ้มกันโรค และตรวจวัดปริมาณของ α-tomatine พบว่า หลังจากฉีดพ่นสารสกัดบริสุทธิ์จากเชื้อราต่อต้าน chaetoglobosin C, chaetomanone A และ trichotoxin A50 ที่ความเข้มข้น 50 ไมโครกรัมต่อมิลลิลิตร เป็นเวลามากกว่า 10 วัน ต้นกล้ามะเขือเทศสามารถสร้างภูมิคุ้มกันโรคได้ 44.97%, 35.18% และ 39.43% ตามลำดับ ในขณะที่การใช้สารเคมีกำจัดรา prochloraz สามารถสร้างภูมิคุ้มกันโรคได้เพียง 29.95% เมื่อนำต้นและใบของมะเขือเทศมาสกัดและทดสอบสารสกัดบนกระดาษ TLC พบว่า สารสกัดจากต้นมะเขือเทศทำให้เกิดจุดสีเขียวบนกระดาษ TLC โดยมีค่า Rf = 0.23 เช่นเดียวกับสาร α-tomatine มาตรฐาน เมื่อทำการฉีดสารสกัดจากต้นมะเขือเทศเข้าไปในเครื่อง HPLC เก็บข้อมูลและคำนวณหาปริมาณของ α-tomatine โดยการใช้ linear regression curve พบว่า ในต้นมะเขือเทศหลังฉีดพ่นสารสกัดบริสุทธิ์จากเชื้อราต่อต้าน chaetoglobosin C, chaetomanone A และ trichotoxin A50 เป็นเวลา 15 วัน มีปริมาณ α-tomatine เท่ากับ 207.87, 254.25, และ 205.04 ไมโครกรัมต่อมิลลิลิตรตามลำดับ ซึ่งมีปริมาณสูงกว่าการฉีดพ่นด้วยสารเคมีกำจัดรา prochloraz และ ต้นมะเขือเทศที่ไม่ได้ฉีดพ่นสารสกัดบริสุทธิ์อย่างมีนัยสำคัญยิ่งทางสถิติ ซึ่งตรวจพบปริมาณ α-tomatine เพียง 131.56 และ 77.46 ไมโครกรัมต่อมิลลิลิตรตามลำดับ จากผลการทดลองแสดงให้เห็นว่าสารสกัดบริสุทธิ์จากเชื้อราต่อต้าน chaetoglobosin C, chaetomanone A และ trichotoxin A50 สามารถกระตุ้นให้เกิดการสร้างสาร phytoalexin ในต้นมะเขือเทศ ซึ่งทำให้เกิดการสร้างภูมิคุ้มกันโรคเหี่ยวของมะเขือเทศพันธุ์สีดาได้ และเมื่อทำการทดสอบประสิทธิภาพของสารสกัดบริสุทธิ์จากเชื้อราต่อต้านเพื่อควบคุมโรคเหี่ยวในต้นมะเขือเทศ พบว่า สารสกัดบริสุทธิ์จากเชื้อราต่อต้านทั้ง 3 ชนิดที่ความเข้มข้น 10, 50 และ 100 ไมโครกรัมต่อมิลลิลิตร สามารถกระตุ้นให้เกิดภูมิคุ้มกันโรคเหี่ยวในมะเขือเทศได้ระหว่าง 53.80% ถึง 65.15% ซึ่งสูงกว่าการใช้สารเคมีกำจัดรา prochloraz ที่สามารถสร้างภูมิคุ้มกันโรคได้เพียง 26.73%

สารควบคุมเชื้อราชีวภาพ N0802, CLT และ PC01 ซึ่งผลิตโดยใช้เชื้อราต่อต้านทั้ง 3 ชนิด สามารถลดการเกิดโรคเหี่ยวมะเขือเทศได้ 43.90%, 41.22% and 41.97 % ตามลำดับ ซึ่งสูงกว่าการใช้สารเคมีกำจัดรา prochloraz อย่างมีนัยสำคัญทางสถิติ (20.39%) การใช้สารควบคุมเชื้อราชีวภาพทั้ง 3 ชนิดยังสามารถทำให้ผลผลิตของมะเขือเทศเพิ่มขึ้นระหว่าง 83.74% ถึง 88.53% ซึ่งมากกว่าการใช้สารเคมีกำจัดรา prochloraz อย่างมีนัยสำคัญทางสถิติ ที่ทำให้ผลผลิตเพิ่มขึ้นเพียง 41.57% จากการทดลองสามารถสรุปได้ว่าสารควบคุมเชื้อราชีวภาพ N0802, CLT และ PC01 ซึ่งเตรียมจากเชื้อราต่อต้าน *C. globosum* KMITL N-0802, *C. lucknowense* CLT และ *T. harzianum* PC01 มีประสิทธิภาพในการควบคุมโรคเหี่ยวของมะเขือเทศได้

Thesis	Biological Control of Tomato Fusarium Wilt
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Year	2010
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ABSTRACT

The forty-five isolates of *Fusarium* spp. were isolated from tomato wilt in the infested field in eight provinces of Thailand. Morphological characters and molecular phylogeny were studied on *Fusarium* isolates. Only twelve isolates were identified as *Fusarium oxysporum* f.sp. *lycopersici* which divided into three virulent groups based on disease severity index. *F. oxysporum* f.sp. *lycopersici* isolate NKSC01 showed the most aggressive with DSI as 6.00 on tomato seedling var. Sida and selected as pathogen in this study.

Three antagonistic fungi, *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT and *Trichoderma harzianum* PC01, were tested for their abilities to control *F. oxysporum* f.sp. *lycopersici*. All antagonistic fungi could inhibit sporulation of pathogen over 90% in dual culture plate. Crude extracts of antagonistic fungi presented a property to inhibit sporulation of pathogen. Crude hexane of *C. globosum* KMITL-N0802 showed the highest property to inhibit sporulation of *F. oxysporum* f.sp. *lycopersici* which ED_{50} value of 157 µg/ml while crude hexane of *C. lucknowense* CLT and crude methanol of *T. harzianum* PC01 gave the ED_{50} values of 188 and 192 µg/ml, respectively.

Bioactive compounds from antagonistic fungi, chaetoglobosin C, chaetomanone A and trichotoxin A50, were tested for their efficacies *in vitro* to inhibit the growth of *F. oxysporum* f.sp. *lycopersici* NKSC01. All tested bioactive compounds at concentration 100 µg/ml exhibited

strongly inhibition on mycelial growth of pathogen which ED_{50} values ranged from 68 to 71 μg/ml and inhibited sporulation of pathogen which ED_{50} values ranged from 17 to 22 μg/ml.

The bioactive compounds were then used as microbial elicitors to elicit α-tomatine in tomato. The α-tomatine was detected by HPLC after treated with bioactive compounds. Chaetoglobosin C, chaetomanone A and trichotoxin A50 were sprayed onto inoculated tomato seedling var. Sida with *F. oxysporum* f.sp. *lycopersici* NKSC01 and detected for plant disease immunity and quantity of α-tomatine. Results revealed that plant disease resistance on treated tomato seedlings with chaetoglobosin C, chaetomanone A and trichotoxin A50 at concentration 50 ug/ml after 10 days were 44.97%, 35.18% and 39.43%, respectively when compared to prochloraz that showed plant disease immunity at 29.95%. The stems and leaves of tomato were extracted and spotted on TLC paper resulted to green spot which showed Rf = 0.23 as same as a spot of standard α-tomatine. Tomato extracts were subjected into vial and analyzed for α-tomatine in HPLC system. The data of α-tomatine quantification were analyzed using linear regression curve. Tomato treated with chaetoglobosin C, chaetomanone A and trichotoxin A50 at 15 days expressed α-tomatine quantity as 207.87, 254.25, and 205.04 ug/g which significantly higher than prochloraz and inoculated treatments which α-tomatine quantity were 131.56 and 77.46 ug/g. It was shown that chaetoglobosin C, chaetomanone A and trichotoxin A50 could induce α-tomatine in tomato plants implies disease resistance against Fusarium wilt of tomato var. Sida through phytoalexin production. The bioactive compounds were tested for their efficacies to control tomato wilt *in vivo*. The results revealed that all bioactive compounds at concentration 10, 50 and 100 μg/ml could induce plant resistance in tomato between 53.80 to 65.15% which higher significantly plant disease resistance than prochloraz which plant disease resistance was 26.73%.

The Bio-agent formulations namely N0802, CLT and PC01 were prepared from antagonistic fungi and gave significantly higher disease reduction of 43.90, 41.22 and 41.97 %, respectively when compared with prochloraz (20.39%). All tested bio-agent formulations could significantly increase in yield of tomato between 83.74 to 88.53% which more than prochloraz (41.57%). It is concluded that *C. globosum*, *C. lucknowense* and *T. harzianum* developed as bio-agent formulations namely N0802, CLT and PC01 were proved for their abilities to control tomato wilt.

ACKNOWLEDGEMENTS

I would like to thank my advisor, Assoc. Prof. Dr. Kasem Soytong, who gave me a huge chance to study in Ph.D. and accepted me as his student. In my memory, he provided me a lot of useful knowledge, facilities for my research and suggestion when I studied in this major.

I would also like to thank my co-advisors, Assoc. Prof. Dr. Somdej Kanokmedhakul, Prof. Dr. Fu-Cheng Lin, and Asst. Prof. Dr. Supattar Poeaim for their kindly providing me the laboratory facilities, best suggestion for better idea and widely vision for my thesis research.

I would like to thank Assoc. Prof. Dr. Somdej Kanokmedhakul and Assoc. Prof. Dr. Kwanjai Kanokmedhakul who supported all facilities for chemical study, well suggestion and new idea for thesis research and offered me for further cooperative work. I would like to thank Department of Chemistry, Faculty of Science, Khon Kaen University, for working in organic chemistry laboratory and providing instruments, chemical and other facilities. And I also thanks for staff of the Department too.

I am grateful Asst. Prof. Dr. Supattra Poeaim who offered me to conduct molecular work in Molecular Laboratory, support materials and facilities. She also supported me for DNA sequencing wage. Furthermore, she gave me warm and valuable suggestion for the experiment of molecular phylogeny and the opportunities to learn molecular techniques. I would like to give thanks for Department of Applied Biology, Faculty of Science, King Mongkut's Institute of Technology Ladkrabang which allowed me to perform the experiment in Molecular Laboratory and supported instruments and other facilities. I also thanks for staff of this Department too.

I would like to acknowledge Nakhon Ratchasima Rajabhat University to permit me for taking leave of absence for studying in Ph.D. program and some financial support.

I would like to thanks Biocontrol Research Unit, Department of Plant Pest Management Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, for instrumental support and all other facilities.

I am thankful for Assoc. Prof. Dr. Weerasak Saksirirat and Miss Manatsawee Chayphad, Department of Plant Pathology, Faculty of Agriculture, Khon Kaen University, who donated *Fusarium oxysporum* f.sp. *lycopersici* isolate KK2 for my thesis experiment.

I would like to thanks for Mr. Petcharat Yowabutra who gave me a chance to collect samples of *Fusarium* wilt of tomato and also thanks for the owner of tomato field in eight provinces who

allowed me to collect samples for my research. I am thankful for my ex-students, Mr. Sowat Mardwangsaeng and Ms. Sukrittaya Dannarong, who helped me a lot in my thesis work. I would like to give thanks for all of my friends at Mushroom Building; Saithong, Kanchalika, Chaninun, Nathacha, Tanapak, Chulaluk, Phoutthasone, Phonesavard, Ohnmar, Ruilin, Sophea, Jessada, Piyada, my friends at Molecular Laboratory; Suvicha, Narumon, Sarun, Puk, and all of my friends at organic chemistry laboratory, Khon Kaen University, and the others who were not mentioned here, for their help and their warmly friendship during my study. I am thankful for Mr. Wimol Chanlee who supported materials in my thesis research. I would like to thanks Ms. Nucharee Bunplaeng who helped me for data analysis and her help from the first day of my student's life. I would like to thanks my friends and my colleague at Nakhon Ratchasima Rajabhat University who always give me the encouragement.

Most of all, I am grateful in member of "Charoenporn Family" who were my inspiration to study Ph.D., my uncle Prasit, who supported some financial payment, my aunt, Jamneuan, my younger sisters, Chayada and Sriwan who always stay back up me. And the last, I would like to dedicate my Ph.D. to my unlived father and mother, who gave me a birth, enless love and education for my life.

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CHAPTER 1

INTRODUCTION

1.1 Statement and Significance of the Problems

Tomato (*Lycopersicon esculentum* Mill.) is one of the world's most cultivated vegetable crop including Thailand. Office of Agricultural Economics (2008) and Department of Agricultural Extension (2008) reported that tomato production in the year of 2006 was 201,000 tons from 50,000 rais of planted areas. Thailand exported tomato products as fresh or chilled tomatoes for 2,243 tons, as the valued of 16,240,000 baht, products from tomatoes e.g. tomato sauce, ketchup and preserved tomatoes for 7,251 tons, as the values of 248,987,000 baht. Fusarium wilt is one of the most serious disease in tomato throughout the world, especially in upland which caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) Snyder & Hansen leading to serious economic losses. It becomes one of the most prevalent and damaging disease wherever tomatoes are grown intensively because the pathogen can persist indefinitely in infested soils. Methods used to control vascular wilt are applied chemical fungicides such as Pentachloronitrobenzene (PCNB), Vitavax, and Etridiazole. Although applications of fungicides have helped control this disease but chemical control is very expensive and environmentally undesirable. In addition, the pathogen has shown resistance against some fungicides (Özgönen *et al.* 2001). Finally *F. oxysporum* f. sp. *lycopersici* become resistant to those chemical fungicides. For this reason, alternative methods of controlling the disease have been studied with emphasis on biological control using antagonistic fungi or bacteria to reduce fungicide application and decrease cost of production. Biological control has potential to manage this disease which occurred through different mechanisms such as antibiosis, competition, suppression, direct parasitism, induced resistance, hypovirulence and predation. The antagonistic activity has often been associated with production of secondary metabolites (Haggag and Mohamed 2007; Larkin and Fravel. 1998). There were many reports of biological control agents to control Fusarium wilt pathogen such as *Trichoderma harzianum*, *Pythium oligandrum*, *Achromobacter xylosoxydans*, *Penicillium oxalicum* and non-pathogenic *Fusarium oxysporum* (Mohamed and Haggag. 2006; Floch *et al.* 2003; De Cal *et al.* 2000; Moretti *et al.* 2008; Silva and Bettiol. 2005). Soytong (1992) and Kanokmedhakul *et al.* (1993) reported that crude extract of *Chaetomium cupreum*

KMITL-N 4320 inhibited spore production of *F. oxysporum* f. sp. *lycopersici* at 85.14%. Moreover, there are many reports on bioactive compounds which extracted from antagonistic fungi to inhibit pathogenic isolates of tomato wilt (Kanokmedhakul *et al.* 2003; Kanokmedhakul *et al.* 2006). The bioactive compounds such as trichotoxin A50 extracted from *Trichoderma harzianum* PC01, and chaetoglobosin C extracted from *Chaetomium globosum* KMITL-N0802 have been reported to elicit the resistant or immunity in plants (Soytong *et al.* 2001). Antifungal activity of the secondary metabolites has importantly become one of disease control strategy. Kanokmedhakul *et al.* (2002) reported that *C. globosum* strain KMITL-0802 produced a novel anthraquinone-chromanone compound namely chaetomanone that exhibited antituberculosis, *Mycobacterium tuberculosis* and it also produced chaetoglobosin C. Thereafter, Soytong *et al.* (2001) reported that chaetoglobosin C exhibited activity against plant pathogens; *Colletotrichum gloeosporiodiodes, C. dematium, Fusarium oxysporum, Phytophthora parasitica, P. palmivora, P. cactorum, Pyricularia oryzae, Rhizoctonia solani* and *Sclerotium rolfsii* and also expressed as an alien substance which induce a localized and sub-systemic oxidative burst in carrot, potato, tomato and tobacco implied plant immunity for disease control. Moreover, *T. harzianum* strain PC01 was reported to produce trichotoxin A50 that because its peptide extract behaved as a potent biological control agent and the peptides could inhibit mycelial growth and sporangial production of *P. palmivora* with ED_{50} values of 2.20 and 0.45 µg/ml respectively. However, these peptide mixture was also effective for promoting plant growth to Chinese cabbage, kale and mungbean (Suwan *et al.* 2000). This research finding was to apply these bioactive compounds against tomato wilt caused by *F. oxysporum* f. sp. *lycopersici* both *in vitro* and *in vivo*. The research finding was to elucidate the known bioactive compounds from antagonistic fungi to be applied as microbial elicitors to induce immunity of tomato against Fusarium wilt.

1.2 Objectives

6.1 To collect, isolate Fusarium wilt pathogen and pathogenicity test.

6.2 To test antagonistic fungi and their abilities to control Fusarium wilt pathogen.

6.3 To elucidate known bioactive compounds as microbial elicitors for possible inducing plant immunity.

6.4 To evaluate the bio-agent formulation for controlling Fusarium wilt of tomato.

1.3 Scope of the Study

1. Collection of diseased plants and rhizospere soil from infested fields of eight provinces in Bangkok, Pathumthani, Nakhon Ratchasima, Burirum, Nong Khai, Sakon Nakhon, Khon Kaen and Mukdahan.

2. Isolation and study for morphological characters and test for their pathogenicity on tomato var. Sida.

3. Study on molecular phylogeny of all isolates of *Fusarium* spp.

4. Test for the abilities of antagonistic fungi to control pathogen both *in vitro* and *in vivo*.

5. Analyze for α-tomatine in tomato plant after being elicited with bioactive compounds.

1.4 Location of Research Work

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Duration of thesis: start from June 2006 until April 2010

CHAPTER 2

LITERATURE REVIEW

2.1 Fusarium wilt of tomato

Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp *lycopersici* (Sacc.) Snyder & Hansen which causes economic losses, especially on susceptible varieties of tomato and under favorable weather condition such as warm climate, sandy soil, low nutrients in nitrogen and phosphorus but high in potassium and low soil pH. It becomes one of the most prevalent and damaging disease wherever tomatoes are grown intensively because the pathogen can persist indefinitely in infested soils. Its symptom also include strong downward bending of petioles, yellowing, wilting and dying of the lower leaves, often on one side of the plant. Root necrosis is often extensive. After a few weeks, browning of the vascular system may be seen by cutting the stem open with a knife. This brown discoloration inside the stem may extend from the roots of the plant to the top (Figure 2.1). Plant growth is stunted and may die (Agrios. 1997).

Figure 2.1 Disease symptom of Tomato Wilt.

a = infected tomato plant, b = browning vessel in disease plant.

F. oxysporum can survive without the host, in the form of thick-walled chlamydospores. Indeed, once an area becomes infected with *F. oxysporum*, it usually remains so indefinitely (Agrios. 1997). After germination, infection hyphae adhere to the host roots and direct penetrate into plant root. The mycelium then advances intercellularly through the root cortex until it reaches the xylem vessels and enters through the pits. At this point, the fungus switches to mode of

infection, during which it remains exclusively within the xylem vessels, using them as avenues to rapidly colonize the host. Fungal mycelia then produce microconidia, which are detached and carried upward in the sap stream. The microconidia eventually germinate and the mycelium penetrates the upper wall of the vessels, producing more microconidia in the next vessel. The characteristic wilt symptoms appear as a result of severe water stress, mainly due to vessel clogging. Wilting is most likely caused by a combination of pathogen activities, such as the accumulation of fungal mycelium and/or toxin production and host defense responses, including production of gels, gums and tyloses and vessel crushing by proliferation of adjacent parenchyma cells (Beckman. 1987). As long as the plant is alive, the vascular wilt fungus remains strictly limited to the xylem tissues and a few surrounding cells. Only when the infected plant is killed by the disease does, the fungus invade the parenchymatous tissue and sporulate profusely on the plant surface. *F. oxysporum* thus occupies a highly specific ecological niche, shared by only a few other fungal plant pathogens (Di Pietro *et al.* 2003). The spores may be disseminated to new plants or areas by wind and water. Sometimes, when the soil moisture is high and the temperature relatively low, infected plants may produce good yields, however, in such cases the fungus may reach the fruit of the plants and penetrate or contaminate seed in fruit. The infected fruits will decay and drop (Agrios. 1997). The disease cycle of tomato Fusarium wilt shows in Figure 2.2.

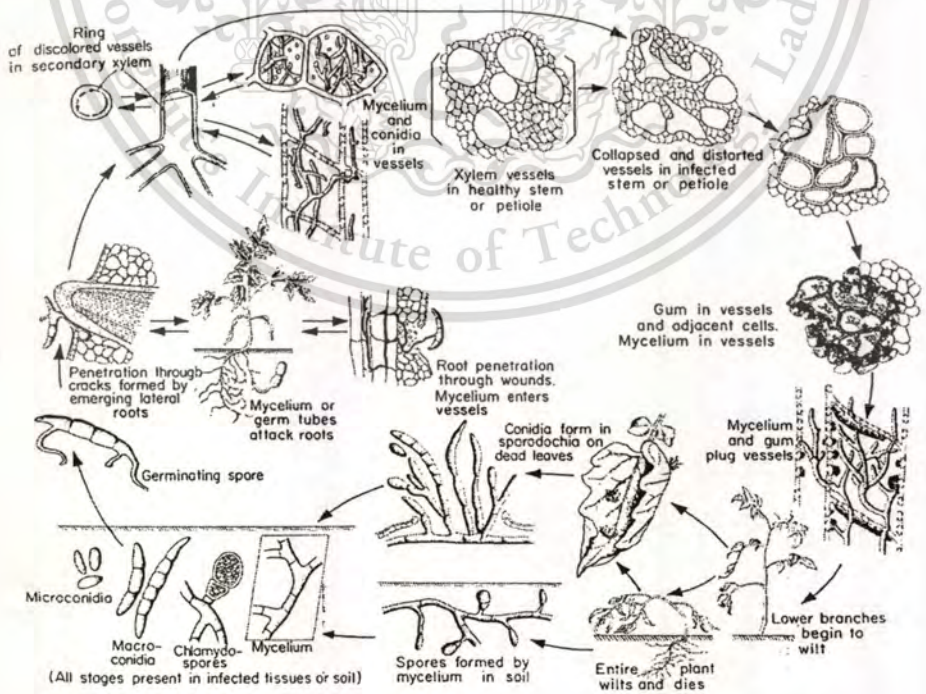


Figure 2.2 Disease cycle of Tomato Fusarium Wilt. (Agrios. 1997)

Successful infection by *F. oxysporum* is a complex phenomenon that requires a series of highly regulated processes: (1) recognition of roots through unknown host signals, (2) root surface attachment and differentiation of penetration hyphae, (3) penetration of the root cortex and degradation of physical host barriers such as the endodermis in order to reach the vascular tissue, (4) adaptation to the hostile plant environment, including tolerance to plant antifungal compounds, (5) hyphal proliferation and production of microconidia within the xylem vessels, and (6) secretion of virulence determinants such as small peptides or phytotoxins. Studies using molecular techniques such as targeted gene knockout have begun to shed light on the mechanisms underlying some of these processes and their role in pathogenesis (Table 2.1). (Di Pietro *et al.* 2003; Roncero *et al.* 2003).

Table 2.1 *Fusarium oxysporum* genes studied by gene knockout.

Gene	Product / function	Effect of gene knockout	References
arg1	argininosuccinate lyase	strongly reduced virulence, arginine auxotroph	Namiki *et al.* (2001)
chsV	class V chitin synthase	strongly reduced virulence, hypersensitive to α-tomatine and H2O2	Madrid *et al.* (2003)
fga1	G protein α subunit	markedly reduced virulence, decreased conidiation	Jain *et al.* (2002)
fgb1	G protein β subunit	markedly reduced virulence, decreased conidiation	Jain *et al.* (2003)
fmk1	MAP kinase	non-pathogenic, impaired in root attachment and invasive growth	Di Pietro *et al.* (2001)
fow1	mitochondrial carrier	strongly reduced virulence, impaired in plant colonization	Inoue *et al.* (2002)
pacC	transcription factor	increase in transcription of acid-expressed genes and virulence	Caracuel *et al.* (2003)
pg1	endopolygalacturonase	fully virulent, reduced saprophytic growth on pectin	Di Pietro and Roncero (1998)
pg5	endopolygalacturonase	fully virulent	García-Maceira *et al.* (2001)
pgx4	exopolygalacturonase	fully virulent	García-Maceira *et al.* (2000)
pl1	pectate lyase	fully virulent	Huertas-González *et al.* (1999)
prt1	serine protease	fully virulent	Di Pietro *et al.* (2001)
xyl3	family 10 endoxylanase	fully virulent	Gómez-Gómez *et al.* (2002)
xyl4	family 11 endoxylanase	fully virulent	Gómez-Gómez *et al.* (2002)
xyl5	family 11 endoxylanase	fully virulent	Gómez-Gómez *et al.* (2001)

2.2 Taxonomy of *Fusarium*

Domsch *et al.* (1993) described species of *Fusarium* as follows: the fungus is usually fast growing, pale or bright colonies with felty aerial mycelium. Conidiophores are usually basitonously branched, if forming complex pustules termed sporodochia, if confluent pionnotes, or consisting in some species only of single phialides. The terminal branches are slender, slightly tapering phialides usually bearing one fertile (in some species polyphialides). Macroconidia form in slimy masses, fusiform to sickle-shaped and one to many septate, mostly differentiated into a beaked apical cell and a pedicellate basal cell. Some species produce one-celled microconidia with smaller than macroconidia. Chlamydospore form terminal or intercalary on hypha and found in some species unless the cultures are very old or grow on poor agar medium.

The taxonomy of *Fusarium* has been influenced by Wollenweber and Reinking whose summarized this fungus into 65 species and 77 subspecific varieties and forms based on 16 sections (Leslie and Summerell. 2006). But in the 1940s, Snyder and Hansen classified species of *Fusarium* spp. into 9 genus by using cultures derived from a single spore which could reliably identify into 9 species. The 9 species of *Fusarium* based on Snyder and Hansen' s concept were *F. oxysporum*, *F. solani*, *F. moniliforme*, *F. roseum*, *F. lateritium*, *F. tricintum*, *F. nivale*, *F. rigidiuscula* and *F. episphaeria* (Snyder and Hansen. 1940). Most species of *Fusarium* are soil fungi and active in decomposed substrates. Some species are plant parasites causing root and stem rot, vascular wilt, fruit rot or ear rot disease. In some species, host-specific pathogenic strains are distinguished as formae speciales. Pathogenicity to man is rare but many species cause storage rots and are important toxin producers (Domsch *et al.* 1993).

Fusarium species are best identified from cultures grown on carnation leaf-piece agar (CLA), oat meal agar (OA) or potato sucrose agar (PSA) where sporulation can occur in less than one week or more than four weeks, depending on the species. Conidial measurements are based on water mounts while conidia in lactic acid or lactophenol appear narrower. Identification into species is difficult with the available dichotomous keys because of the variability between isolates (Booth. 1971; Leslie and Summerell. 2006).

2.3 Morphology of *Fusarium oxysporum*

Booth (1971), Domsch *et al.* (1993) and Leslie and Summerell (2006) reported that colony morphology of *F. oxysporum* on PDA varies widely. Colony grow fast which reaching 4.5-6.5 cm diameter in 4 days at 25°C, aerial mycelium sparse to abundant and floccose,

becoming felted, range in colour from white or peach, but usually with a purple or a tinge of violet. Micro-conidia generally abundant, mostly borne on short, simple, lateral phialides or from sparsely branched conidiophores, never forming chains, mostly 0 septate, ellipsoidal to cylindrical, straight or often curved, 5-12 x 2.3-3.5 μm. Macro-conidia fusiform, moderately curved, pointed at both ends, basal cells pedicellate, 3-5 septate, (20-)27-46(-60) x 3.0-4.5(-5.0) μm. Chlamydospores produces terminal or intercalary in hyphae, often also in conidia, hyaline, smooth or rough walled, 5-15 μm. Sclerotial pustules present in some isolates, pale to green or deep violet.

2.4 Molecular phylogeny of *Fusarium* spp.

The taxonomy of *Fusarium* spp. is based on morphological characters such as morphology of colony, size and shape of macroconidia, the present of microconidia and its shape included conidiophores structure. At present date, molecular approaches have been used to study in phytopathogenic fungi using the analysis of ribosomal DNA (rDNA) sequences (Edel *et al.* 1996 and Konstantinova and Yli-Mattila. 2004). Although the species of *Fusarium* can be identified by their morphological characteristics on selective media. The pathogenic types or formae speciales and races of *F. oxysporum* cannot be identified morphologically. An inoculation assay using tester plants has been shown the most popular strategy to identify the forms (formae speciales) in *F. oxysporum* and races in each form. However, this method is time consuming and a more rapid and accurate method has been urgently needed. Recently, molecular markers have become popular for identifying species and/or subspecies in fungi. Amplified fragment length polymorphisms (AFLP), restriction fragment length polymorphisms (RFLP), random amplified polymorphic DNA (RAPD), and comparison of DNA sequences of ribosomal DNA (rDNA) and internal transcribed spacer (ITS) regions, have been often used to determine species or groups (Yasushi and Tsutomu. 2006). Variation in the intergenic spacer (IGS) of the ribosomal DNA has proven useful for resolving intraspecific relationships within *F. oxysporum*. However, *F. oxysporum* is regarded as a complex of morphologically similar fungi with multiple phylogenetic origins residing in three well-supported clades (Bogale *et al.* 2007). Recently, the PCR technique have been described to resolve genetic variation among isolates within or between formae speciales of *F. oxysporum*. The rDNA regions have been used for taxonomic and phylogenetic studies, because sequence data are available and contain both variable and conserved regions; despite the discrimination at the genus, species, or intraspecific level. The spacer regions,

including the internal transcribed spacer (ITS) and the intergenic spacer (IGS), have been used to examine relationships among closely related taxa by RFLP analysis (Nagarajan *et al.* 2004). The ITS sequences have been preferred over the subunits because they are more variable and thus permit selective detection of closely related fungi. ITS sequences represent a relatively short region (500–800 bp) which are easily amplified by universal primers, require only small amounts of sample DNA, and have been used for the quick generation of species-specific probes. In the heterogeneous genus *Fusarium*, in which species differentiation is based mainly on morphological or biochemical criteria that in some cases are sometimes difficult or even confusing, PCR technology has improved diagnosis (Roncero *et al.* 2003). Kawabe *et al.* (2005) revealed that three evolutionary lineages of the tomato wilt pathogen *F. oxysporum* f. sp. *lycopersici* were found among a worldwide sample of isolates based on phylogenetic analysis of the ribosomal DNA intergenic spacer region. The rDNA region analyses have been used successfully to differentiate other *Fusarium* species such as *F. avenaceum, F. arthroporioides,* and *F. tricinctum* and within the *Fusarium* formae speciales such as *F. oxysporum* f. sp. *cyclaminis*, *F. oxysporum* f. sp. *dianthi*, *F. oxysporum* f. sp. *gladioli* and *F. oxysporum* f. sp. *phaseoli* (Nagarajan *et al.* 2004).

2.5 Plant defense mechanisms in tomato

As the pathogen infected and established itself within the host, plants possess a broad spectrum of basic defense mechanisms, pre-established or induced secondary metabolites with antimicrobial activities, which render them resistant to most potential colonizers. Many of these compounds are present in healthy plants as normal metabolic products, whereas, in other cases, they can be actively synthesized in response to pathogen attack (Roncero, *et al.* 2003). Elicitors are molecules that stimulate any of a number of defense responses in plants, such as synthesis of phytoalexins and pathogenesis-related proteins (PR-proteins). Such responses occurs after the binding of elicitor molecules to receptors normally located on the plant cell surface, promoting a signal transduction pathway that will lead to the activation of one or more defense mechanisms. The first characterized elicitors were oligosaccharide fragments from fungal cell walls, including hepta-β-glucoside, oligochitin and oligochitosan (Hahn. 1996).

VanEtten *et al.* (1994) mentioned on the definition of antibiotic compounds that are produce in the part of plant as "phytoalexins are low molecular weight, antimicrobial compounds that are both synthesized by and accumulated in plants after exposure to microorganisms" and

"phytoanticipins are low molecular weight, antimicrobial compounds that are present in plants before challenge by microorganisms or are produced after infection solely from preexisting constituents". Besides the detoxification of constitutive phytoanticipins, one trait which has also been investigated as to potentially reflect pathogenicity on a specific host is the ability of fungi to tolerate phytoalexins, the so-called antimicrobial compounds synthesized *de novo* by plants in response to microbial infection. Among them, special attention has been paid to the secondary metabolites produced by plants, many of which have antifungal activity. Although preformed inhibitors are present in healthy plants at levels that are anticipated to be antimicrobial, their levels may increase further in response to challenge by pathogens. Some antifungal compounds may be present constitutively in one part of a plant but induced as phytoalexins in other organs. Numerous constitutive plant compounds have been reported to have antifungal activity in vitro and so have been implicated as antimicrobial phytoprotectants. Phytoalexins are a group of structurally diverse molecules that are generally lipophilic, nonspecific in their antifungal activity, and not particularly potent. The mechanisms of antimicrobial action of phytoalexins are not well understood. The accumulation of phytoalexins represents one of an array of induced defense responses associated with plant disease resistance. Although both disease-resistant and susceptible plants may respond to pathogen attack by producing phytoalexins, these compounds generally accumulate more rapidly and to higher levels in resistant plant (Morrissey and Osbourn. 1999). Saponins, which are plant glycosides, have been implicated as preformed chemical barriers against pathogen attacks. Plants have evolved different defense mechanisms to protect themselves against a great variety of invasive pathogens (Roldán-Arjona *et al.* 1999). A possible determinant of resistance of tomato plants to fungi is the presence in the plant of a preformed inhibitor of fungal growth: α-tomatine. α-tomatine is a glycosidal alkaloid consisting of an aglycone moiety (tomatidine) and a tetrasaccharide moiety (β-lycotetraose) which is composed of two molecules of glucose and one each of galactose and xylose; the four monosaccharides form a branched structure which is attached at the C-3 position of the aglycone (Fig. 2.3). It has been calculated as $C_{50}H_{83}NO_{21}$ (Roddick. 1974). It has high molecular weight (MW= 1,033.5458) and Rf = 0.23 (Moco *et al.* 2006; Sandrock and VanEtten. 1998). The current method used for quantitative estimation of tomatine involve spectrophotometry of a chromogen of tomatine (the alkaloid itself having no notable visible/UV absorption spectrum). Coloured products are formed by treating tomatine with strong or conc. H_2SO_4, a lactic acetic solution of silicotungstic acid (Roddick. 1974). α-Tomatine has been found at high concentrations (up to 1 mM) in leaves, stems, roots,

and green fruit, suggesting that it may be important in resistance to potential pathogens. The shoot is recognized as being the main site of tomatine synthesis and accumulation, the former taking place principally in meristematic region. There are some indications that the main sites of tomatine biosynthesis in the root also be the actively growing regions. Flowers of tomato are rich in tomatine as are also, after pollination. Young developing fruits accumulate large amounts of tomatine but as ripening begins alkaloid degradation occurs and concentration decline. Tomatine levels of 0.087, 0.045 and 0.036% have been recorded in green, yellowish and red tomato fruit respectively, and when ripe fruits were left on the plant for further 2-3 days, tomatine almost completely disappeared (Roddick. 1974).

It is sufficient to inhibit the growth of *F. oxysporum* to complex with membrane sterols, with binded to 3β- hydroxy sterols in fungal membranes, causing pore formation and leakage of cells contents (Lairini *et al.* 1996; Sandrock and VanEtten. 1998).

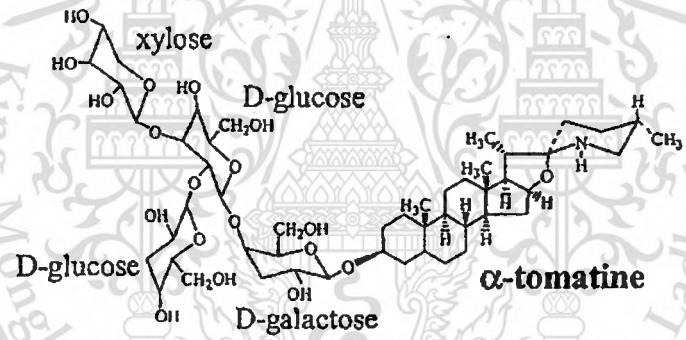


Figure 2.3 Structure of α-tomatine. (Friedman. 2004)

As the antifungal compound α-tomatine, present in tomato plants, has been reported to provide a preformed chemical barrier against phytopathogenic fungi. Some fungi are resistant to α-tomatine because of their membrane composition, while others produce specific tomatine-detoxifying enzymes. *Fusarium oxysporum* f. sp. *lycopersici*, a tomato wilt causing agent, produces an extracellular enzyme inducible by α-tomatine. This enzyme, known as tomatinase, catalyzes the hydrolysis of α-tomatine into its nonfungitoxic forms, tomatidine and β-lycotetraose. Tomatinase remove all four sugars from the steroidal α-tomatine. An inducible enzymatic activity of *F. oxysporum* f. sp. *lycopersici* which was able to detoxify α-tomatine by cleaving the glycoalkaloid into the tetrasaccharide lycotetraose and tomatidine (Lairini *et al.* 1996).

2.6 Detection of α-tomatine by HPLC

Methods used to analyze α-tomatine include GC, GC–MS, and HPLC. The α-tomatine presents in all parts of the tomato plant. Immature green tomatoes contain up to 500 mg/kg of fresh fruit weight. Consumers of green tomatoes, high α-tomatine red tomatoes, and tomato products such as pickled green and green fried tomatoes consume significant amounts of α-tomatine (Friedman. 2004). Friedman *et al.* (1994) developed the technique for α-tomatine determination in tomato by HPLC using pulsed amperometric detection (PAD). The α-tomatine content was recoveried from ripe red fruit and unripe green fruit and the results demonstrated that α-tomatine content from ripe red fruit ranged from 0.03-0.60 mg/100 g of fresh weight while unripe green fruit presented α-tomatine content between 4-17 mg/100 g of fresh weight. In the year 1995 Friedman and Levin detected α-tomatine in different parts of the tomato plant using pulsed amperometric detection HPLC. The results were demonstrated that the quantity of α-tomatine from calyxes, flowers, leaves, roots, and stems of the tomato plant were detected as 14-130 mg/100 g of fresh weight. Red tomatoes contained low-tomatine as 0.03-0.08 mg/100 g. The intermediate stage of tomato fruit contained α-tomatine between 0.1-0.8 mg/100 g plant fresh weight. High quantity α-tomatine was detected from fresh –green fruit as 0.9-55 mg/100 g plant fresh weight (Friedman and Levin. 1995). Tomato seedlings var. Moneymaker *Cf4* and *Cf5* (transformant tomato of two different races of *C. fulvum* expressing the cDNA encoding the *Septoria lycopersici* tomatinase enzyme) at 11 day-old were immersing inoculated with conidial suspension of *Cladosporium fulvum* at concentration as 5×10^5 conidia/ml. The α-tomatine were detected from overground part of tomato seedlings. The contents of α-tomatine in 11-day-old seedlings was determined with ranges of 260 to 380 and 260 to 579 μg per g of fresh weight, respectively while the α-tomatine contents of the leaves of 3-week-old plants were 750 to 1,260 and 730 to 850 μg/g of fresh weight, respectively (Melton *et al.* 1998).

2.7 Biological control of tomato Fusarium wilt

The available control methods for Fusarium wilt of tomato are either inefficient or difficult to apply. Several compounds are effective in vitro against *F. oxysporum*, however, they fail to control disease under field conditions where it is not possible to apply a fungicide directly to roots (Alabouvette *et al.* 1998). The excessive and misuse of a wide range of fungicides has led to harmful to environment and increase resistant pathogen populations (Özgönen *et al.* 2001). *F. oxysporum* f. sp. *lycopersici* becomes resistant to those chemical fungicides. For this reason,

alternative methods to control the disease had been studied with emphasis on biological control involvement of the use of beneficial microorganisms, such as fungi and bacteria to reduce fungicide application and decrease cost of production. Biological control has potential to manage this disease which occurred through different mechanisms such as antibiosis, competition, suppression, direct parasitism, induced resistance, hypovirulence and predation. The antagonistic activity has often been associated with production of secondary metabolites (Haggag and Mohamed. 2007; Larkin and Fravel. 1998). There were many reports of biological control agents to control Fusarium wilt pathogen such as *Trichoderma harzianum*, *Pythium oligandrum*, *Achromobacter xylosoxydans*, *Penicillium oxalicum* and non-pathogenic *Fusarium oxysporum* (Mohamed and Haggag. 2006; Floch *et al.* 2003; De Cal *et al.* 2000; Moretti *et al.* 2008; Silva and Bettiol. 2005). Larkin and Fravel (1998) reported that non-pathogenic *Fusarium* spp. could reduce tomato wilt between 50–100% while *Trichoderma* and *Gliocladium* spp. showed wilt disease reduction between 37–75% and *Pseudomonas* spp. presented disease reduction 30–63% in field trial.

Recently, there have been many reports to bioactive compounds which extracted from different fungi and have been reported to inhibit many plant pathogenic fungi including Fusarium wilt of tomato.

Trichoderma is a genus of fungi which used as biocontrol agents (BCAs) that are successfully used as biopesticides worldwide, and many species are well known producers of secondary metabolites with antibiotic activity. Antibiotic production is often combined with other mechanisms of biocontrol such as mycoparasitism and the production of cell wall-degrading enzymes (CWDEs), competition for nutrients/space, and induced resistance in the plant and thus is considered involved in *Trichoderma*'s interactions with the host plant and the resulting beneficial effects. The production of secondary metabolites in *Trichoderma* spp. is strain-dependent and includes volatile and non-volatile antifungal substances, such as 6-n-pentyl-6H-pyran-2-one (6PP), gliotoxin, viridin, harzianopyridone, harziandione and peptaibols. Synergistic effects between CWDEs and different classes of antibiotics on fungal pathogen growth have been well documented. The overall biocontrol effect of *Trichoderma* spp. was significantly enhanced by the stimulation of plant defense responses to pathogen attack. In addition, the inoculation of the living fungal antagonist produced a growth promotion effect in planta. However, the role that secondary metabolites produced by BCAs such as *Trichoderma* play in the complex three-way

interaction between plant, pathogens and antagonistic fungi has received little attention (Vinale *et al.* 2008).

Chaetomium globosum, an important biocontrol fungus, can inhibit spot blotch of wheat (Aggarwal *et al.* 2004), suppress the development of rice blast and wheat leaf rust (Park *et al.* 2005) and reduce the primary inoculum of *Diaporthe phaseolorum* f. sp. *meridionalis* in soil-surface soybean stubble under field conditions (Dhingra *et al.* 2003). *C. globosum* produces cell wall hydrolases (such as chitinase and glucanase) and antibiotics (such as chaetoglobosin and chaetomanone), which inhibit fungal plant pathogens (Kanokmedhakul *et al.* 2002; Park *et al.* 2005). The CHI46 enzyme which consisted in *C. globosum* can efficiently degrade cell walls of the phytopathogenic *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *S. tritici*, and *Phytophthora sojae.* This study demonstrated that CHI46 enzyme may be involved in the biocontrol mechanism of *C. globosum* (Liu *et al.* 2008). There are many reports indicated bioactive compounds from *C. globosum* gave a good control of plant pathogenic fungi but different found compounds such as chaetoviridins A extracted from *C. globosum* F0142 could inhibit the growth of *Magnaporthe grisea* and *Phytophthora ultimum* (Park *et al.* 2005), chaetomin from liquid culture of *C. globosum* exhibited the activity against damping off of sugar beet caused by *Pythium ultimum* (Di Pietro *et al.* 1992), antifungal metabolite production by *C. globosum* exhibited the ability to control spot blotch of wheat caused by *Cochliobolus sativus* (Aggarwal *et al.* 2004).

T. harzianum, *Chaetomium cupreum* and *C. globosum* are fungi with a world wide distribution. Their potential in the biological control of plant disease are well known. Their bio-control mechanisms include producing antibiotics and ergosterols compounds that can suppress different plant pathogens, especially those soil bone plant pathogenic fungi, stimulate growth of plants and induce resistance to the diseases. They can be used to control many soilborne diseases of vegetable and fruits. However, although they are very effective plant disease bio-control microorganisms, since they are very sensitive to environmental condition, so that they are hardly used in the field where the conditions are not suitable for their survival (Qian *et al.* 2007).

Soytong *et al.* (2001) reported that chaetoglobosin C, the bioactive compound extracted from *Chaetomium globosum*, act as alien substance which induced a localized and sub-systemic oxidative burst in tomato, tobacco, potato, and carrot. This possibility acts as an induction of plant immunity for disease resistance. The report of Suwan *et al.* (2000) mentioned that mycelial extracts of *Trichoderma harzianum* PC01 which produce trichotoxin acted as a potent biological

control agent. The extract could inhibit mycelial growth and sporangial production of *Phytophthora palmivora* with ED_{50} values of 2.2 and 0.45 mg/ml. Soytong (1992) and Kanokmedhakul *et al.* (1993) reported that crude extract of *Chaetomium cupreum* KMITL-N 4320 inhibited spore production of *F. oxysporum* f. sp. *lycopersici* at 85.14%. Moreover, there are many reports on bioactive compounds which extracted from antagonistic fungi to inhibit Fusarium wilt of tomato pathogen (Kanokmedhakul *et al.* 2003; Kanokmedhakul *et al.* 2006). These bioactive compounds such as Trichotoxin A50 extracted from *T. harzianum* PC01, and Chaetoglobosin C extracted from *Chaetomium globosum* KMITL-N0802 have been reported to elicit the resistant or immunity in plant (Soytong *et al.* 2001).

CHAPTER 3

RESEARCH METHODOLOGY

3.1. Sample collection, isolation, identification and pathogenicity test

3.1.1 Sample collection and isolation of *Fusarium* spp.

Disease samples of tomato wilt were collected from infested tomato in the fields in Bangkok, Pathumthani, Nakhon Ratchasima, Burirum, Nongkhai, Sakonnakhon, Khon Kaen, and Mukdahan provinces. Samples were taken from rhizosphere soil and infected tomato plants and fruits. Samples were then separately placed in plastic bags and brought to laboratory. The infected tomato plants, stems and roots were isolated by tissue transplanting method. The roots were surface-disinfected with 10% sodium hypochlorite for 5 minutes, and washed with sterilized distilled water for three times before placing onto water agar (WA), observed and isolated into pure culture. The ripen fruits were surface-disinfected with 10% ethanol and washed in sterilized distilled water for three times before placing into plastic box as moist chamber. Observation was periodically done until the hypha growing and transferred to WA and followed by potato dextrose agar (PDA) to be pure cultures. The soil sample taken from rhizosphere was dried and ground, then random sample of 0.0025 g placed to sterilized Petri dish, poured with isolating medium of glucose-ammonium nitrate agar (GANA) which consist of glucose 10 g, NH_4NO_3 1 g, Difco bacto yeast extract 1 g, K_2HPO_4 0.5 g, $MgSO_4.7\ H_2O$ 0.5 g, agar 20 g, rose bengal 0.06 g, streptomycin 0.03 g, and distilled water 1,000 ml., and incubated in dark condition at room temperature. Observation was done until colonies came out and transferred onto WA and followed by PDA for pure cultures. To study on morphology of fungal isolates, the fungi were cultured on potato dextrose agar (PDA). Fungal colonies were cultured and incubated at room temperature (28-32° C) for 5-10 days. All isolates were purified by single spore isolation and kept in PDA slants for further experiment.

3.1.2 Morphology study of the *Fusarium* spp.

Morphological characters of *Fusarium* spp. isolates obtained from collecting samples were studied for fungal identification by culturing them on potato sucrose agar (PSA) (potato 400 g, sucrose 20 g, agar 20 g, and distilled water 1,000 ml) (Dhingra and Sinclair. 1987). Study on

chlamydospores was done by culturing all fungi on corn meal agar (CMA). The characters of *Fusarium* spp. were determined under binocular compound microscope and the details of fungal morphology were recorded as mycelial growth rate, shape and size of macroconidia, microconidia, conidiophores, as well as chlamydospores.

3.1.3 Pathogenicity tests

The experiment was designed in Completely Randomized Design (CRD) with four replications. Pathogenicity test was conducted in greenhouse to confirm the forma speciales of all isolates using Koch's postulation. The tests were performed using 15-day tomato seedlings variety Sida. The isolates were cultured on PDA for 5-7 days at room temperature (28-32° C) then, the mycelium mats were removed into sterilized distilled water and filtered through two layers cheesecloth to obtain conidial suspension which adjusted to $2x10^6$ conidia/ml using haemacytometer. Seedlings were grown in mix potting soil for 15 days. The root-dipped method was used for inoculation followed the method of Marlatt *et al.* (1996). Dirt and excess soil was removed from the roots of seedlings and washed with tap water. Root tips of seedlings were cut with sterilized scissors of 5 mm and then dipped into conidial suspension for 10 minutes before transplanting into 8 cm diameter plastic pots containing a sterilized soil (soil mixture consists of loam soil: fine coconut shield: sand = 2:1:1) which autoclaved at 121°C 15 lbs/inch^2 for 1 hour. Seedling roots in control were cut and dipped into sterilized distilled water without inoculum. Four replications per isolate (four plants/replication) were used in the experiment. Inoculated plants were maintained in greenhouse at 25-32° C after inoculation. Disease severity index (DSI) was scored at 21 days after inoculation based on the modified disease severity scale of Silva and Bettiol (2005) as follows:- 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting or die 81-100% (as seen in Figure 2.1). DSI was analyzed using analysis of variance (ANOVA). The mean comparison was compared by using Duncan's New Multiple Range Test (DMRT) at P=0.05 and P=0.01. All tested isolates were recorded for non-pathogenic and pathogenic isolates. Virulent group of the isolates was determined according to DSI; non-pathogenic (DSI =1), low (DSI $\leq$ 3.50), moderate (DSI > 3.50 - 4.50), and high (DSI > 4.50). The most aggressive isolate was selected to use for further experiment.

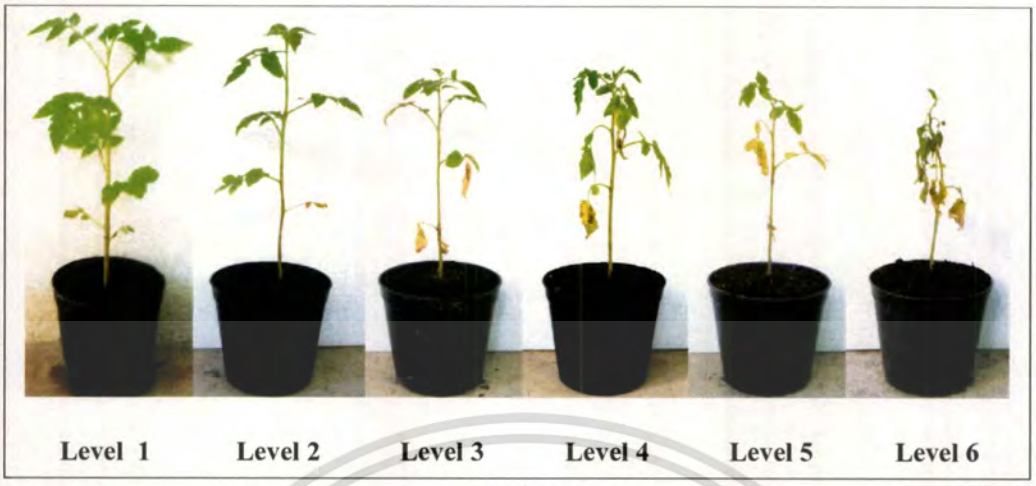


Figure 3.1 Disease severity index of tomato Fusarium wilt.

3.2 Study on molecular phylogeny

The molecular phylogeny of those isolates was done to distinguish the species and forma speciales among them since there have been variations in their morphological characters. Study on molecular phylogeny was conducted as follows:

3.2.1 DNA extraction

DNA extraction was prepared as the protocol of GF-1 Plant DNA Extraction User's Guide (Vivantis Co., Ltd., Selangor DE, Malaysia). Stationary cultures were grown in potato dextrose broth (PDB) for 3 days at room temperature (28-32°C). About 0.2 g of fresh weight mycelium was ground to fine powder with mortar and pestle using liquid nitrogen and mixed with 280 µl of lysis buffer (Buffer PL) to obtain a homogeneous solution. Protinase K (20 mg/ml) was added and mixed thoroughly. After incubation at 60°C for 1 hour in waterbath, the mixture was kept on ice for 3 minutes and then centrifuged for 5 minutes at 10,000 *g* to precipitate any insoluble or undigested materials. The supernatant was added with 20 µl of RNase A (20 mg/ml) and was incubated at 37°C for 5 minutes. The samples were homogenized by adding equal volumes of a mixture of buffer PB (600 µl) and were incubated for further 10 minutes at 60°C. DNA was precipitated by adding 200 µl of absolute ethanol and then the sample was transferred into a column and centrifuged at 10,000 *g* for 1 minute. DNA pellets were washed with 750 µl wash buffer and

repeated again. DNA was eluted by adding 50 µl of 10 mM Tris-HCL, pH 8.5 (Elution buffer) and centrifuged for 1 minute at 10,000 *g*. DNA was stored at -20°C until use.

3.2.2 Polymerase chain reaction (PCR) and DNA sequencing

To amplify the fragments of DNA, polymerase chain reaction (PCR) was conducted using modified method of Yasushi and Tsutomu (2006). The region of the ribosomal, including a small portion of 18S rDNA, ITS 1, 5.8S rDNA, ITS2, and a small portion of the 28S rDNA, was amplified by PCR. The primers used, PN3: 5′-CGTTGGTGAACCAGCGGAGGGATC-3′ and PN16: 5′-TCCCTTTCAACAATTTCACG-3′, were described by Neuvéglise *et al.* (1994). PCR reactions were carried out in 25 µl volume containing 200 ng of DNA template, 4 µl (1.25 mM) dNTPs, 1 µl of each primer (20 *p*mol/µl), 1.5 µl of $MgCl_2$, 0.2 µl of *Taq* DNA polymerase (Vivantis Co., Ltd., Malaysia) and 2.5 µl of 10X PCR buffer. Amplification was performed using a Thermal Cycler. A cycle was composed of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 2 minutes, annealing at 55°C for 2 minutes and extension at 72°C for 3 minutes. Five microlitres of PCR products were electrophoresed in a 1.5% agarose gel in 1xTAE buffer, which was stained with ethidium bromide for observation of the amplicons.

PCR products were sent to purify and sequence at Tech Dragon Limited, Hong Kong. The sequences were aligned and adjusted using Clustal X program for phylogenetic analysis.

3.2.3 Phylogenetic analysis

DNA sequences of tested *Fusarium* spp. were edited and aligned with BioEdit, version 7.0.5 program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequences were multiple aligned with Clustal X version 1.83 before the analysis using maximum parsimony parameter with (PAUP) 4.0b (Swofford. 1998) was performed. For maximum parsimony analyses, bootstraps of 1,000 replicates were performed to examine the relative of each isolate. Maximum parsimony trees were calculated via fast stepwise addition with the representative isolates of *Fusarium* spp. from GenBank (http://www.ncbi.nlm.nih.gov) as shown in Table 3.1 and *Verticillium dahliae* was used to analyze as outgroup. Genetic relative among *Fusarium* spp. isolates in ITS regions of rDNA sequences was determined as cluster in phylogenetic tree.

Table 3.1 *Fusarium* sequences from GenBank used in this study.

Fusarium species	Locality	ITS GenBank accession No.
F. oxysporum f.sp. *lycopersici*	India	EU214564.1
F. incarnatum	Japan	AY633745.1
F. chlamydosporum	P.R. China	AB369435.1
F. redolens	P.R. China	FJ441013.1
F. solani	USA	AF161222.1
F. sporotrichioides	P.R. China	FJ238107.1

3.3 Testing antagonistic fungi and their abilities to control Fusarium wilt pathogen

3.3.1 Dual-culture test

To test the abilities of antagonistic fungi to inhibit the growth of Fusarium wilt pathogen, dual-culture test was performed as the following method. The antagonistic fungi e.g. *C. globosum* KMITL-N0802, *C. lucknowense* CLT and *T. harzianum* PC01 were provided by Assoc.Prof. Dr. Kasem Soytong; KMITL. Dual-culture test was conducted using the method of Soytong (1992). The experiment was designed in Completely Randomized Design (CRD) with four replications. The most aggressive isolates of *F. oxysporum* f.sp. *lycopersici* from pathogenicity test was used in the experiment. The edge of radial growth of pathogenic fungus and antagonist was cut with 3 mm diameter sterilized cork borer and one agar plug of each fungus was transferred to PDA plate at one side (about 2.0 cm from center). For control treatment, either agar plug of pathogenic fungus or antagonist was placed on PDA plate at 2.5 cm from center on one side of the medium. The tested plates were incubated at room temperature (28-30 °C) for 5-7 days or more. The data were collected as colony diameter, number of conidia of pathogenic fungus. Percentage of growth and conidia inhibition of pathogen was calculated as the following formula:

$$\text{Inhibition (\%)} = \frac{A - B}{A} \times 100$$

A = colony diameter or conidial number of pathogen in control

B = colony diameter or conidial number of pathogen in dual culture plate

All data were statistically computed for analysis of variance (ANOVA). Treatment means were compared by using Duncan's New Multiple Range Test (DMRT) at P=0.05 and P=0.01.

3.3.2 Testing antagonistic substances

The antagonistic substances were extracted from *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT, and *Trichoderma harzianum* PC01 as crude extracts. These antagonistic substances were tested for their abilities to inhibit the growth of Fusarium wilt pathogen.

3.3.2.1 Testing crude extract to inhibit *Fusarium oxysporum* f. sp. *lycopersici*

The crude extraction from antagonistic fungi was performed using the method of Kanokmedhakul *et al.* (2006) as seen in Figure 3.2. Antagonistic fungi e.g. *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT, and *Trichoderma harzianum* PC01 were cultured in PDB at room temperature (28-30 °C) for 30 days. Mycelial mats were removed from PDB, filtered through cheesecloth and air-dried overnight. Fresh weight and dry weight of mycelial mats were weighed. Dried mycelial mats were ground with electrical blender, extracted with 200 ml hexane (H) and shaken for 24 hour at room temperature. The ground mycelia were separated by filtration through Whatman No. 4 filter paper. The marc was extracted again with hexane using method described above. The filtrates were evaporated in *vacuo* to yield crude extract. The marc was further extracted with ethyl acetate (EtOAc) and methanol (MeOH), respectively using the same procedure as hexane. Each crude extract was weighed, then kept in refrigerator at 5°C until use.

The crude extracts of antagonistic fungi were tested for inhibition of the most aggressive isolate of *F. oxysporum* f.sp. *lycopersici* obtained from previous experiment. The experiment was conducted by using 3x6 factorials in Completely Randomized Design (CRD) with four replications. Factor A represented crude extracts which consisted of hexane crude, ethyl acetate crude and methanol crude and factor B represented concentrations of 0, 10, 50, 100, 500, and 1,000 µg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxide (DMSO), then mixed into PDA before being autoclaved at 121°C, 15 lbs/inch^2 for 30 minutes. The tested pathogen was cultured on PDA and incubated at room temperature for 5 days, then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA plate (5.0 cm diameter) in each concentration and incubated at room temperature (28-30°C) for four days. Data were collected as colony diameter and number of conidia. Percentage of inhibition was computed as

described above. Data were statistically computed analysis of variance. Treatment means were computed with DMRT at P= 0.05 and P=0.01. The effective dose (ED_{50}) was computed by using probit analysis. The comparisons between normal and abnormal propagules in control and dual-culture were observed under compound microscope.

3.3.2.2 Testing on bioactive compounds to inhibit *Fusarium oxysporum* f. sp. *lycopersici*

The bioactive compounds from antagonistic fungi were provided by Assoc. Prof. Dr. Somdej Kanokmedhakul, Khon Kaen University, Khon Kaen, Thailand. The experiment was conducted using 3x4 Factorials in Completely Randomized Design with four replications. Factor A represented chaetoglobosin C, chaetomanone A and trichotoxin A 50, while factor B represented the concentration of 0, 10, 25, 50, and 100 µg/ml. The tested method and data collection were followed as described above.

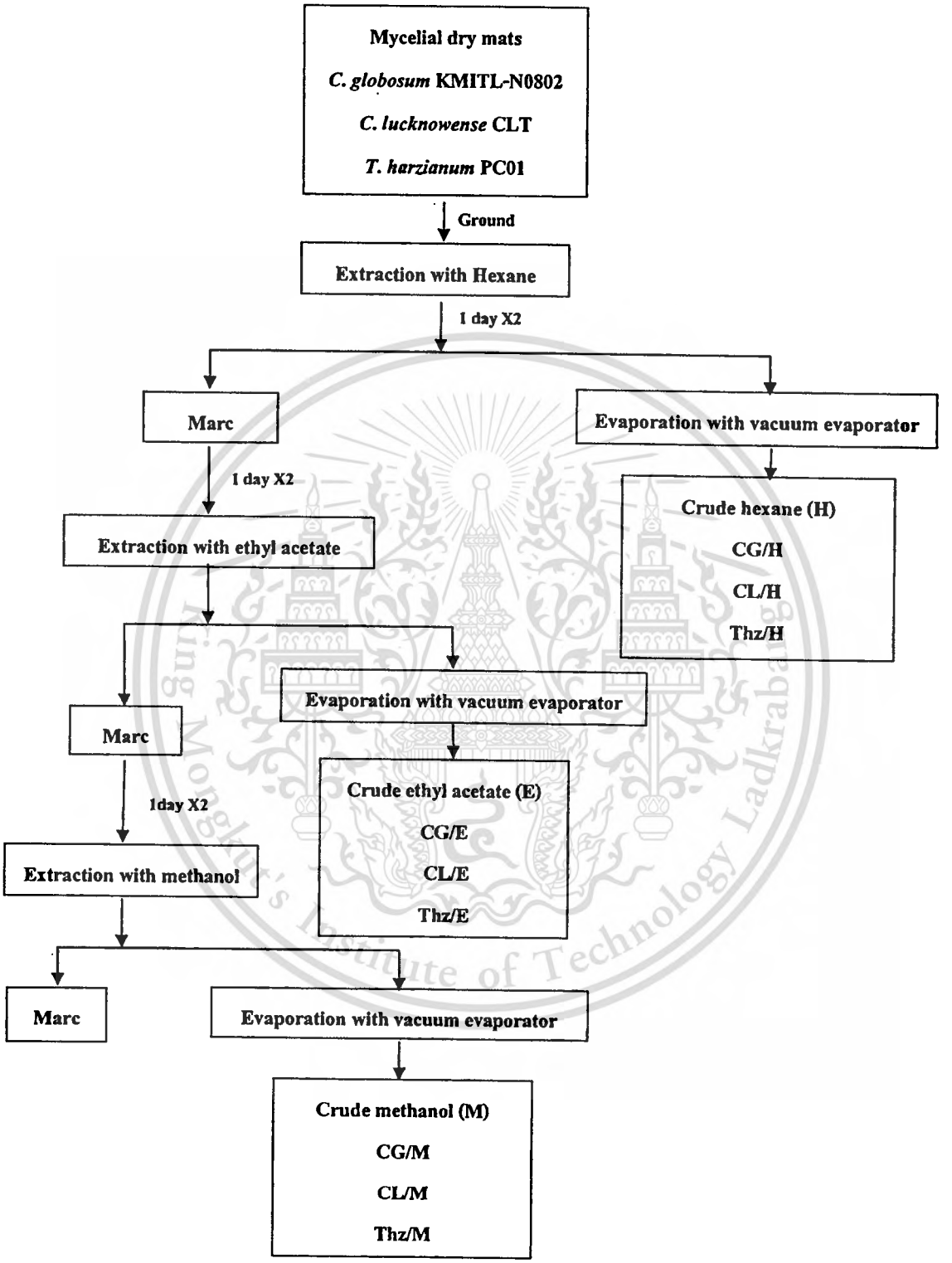


Figure 3.2 Flow chart of crude extraction from antagonistic fungi.

3.4 Microbial elicitors inducing plant resistance

The experiment was performed to detect α-tomatine in laboratory. The trial was conducted with modified methodology of Melton *et al.* (1998) and designed in Completely Randomized Design (CRD) with six treatments and three replications. Twenty days seedlings var. Sida were inoculated with conidial suspension of *F. oxysporum* f. sp. *lycopersici* at concentration of 2×10^6 conidia/ml and planted into 4 inch diameters plastic pot containing sterilized soil mixture as previous experiment and followed by spraying each bioactive compound or chemical fungicide on seedling leaves. Treatments were as follows:

Treatment 1 = inoculated with pathogen and sprayed with chaetoglobosin C at 50 μg/ml

Treatment 2 = inoculated with pathogen and sprayed with chaetomanone A at 50 μg/ml

Treatment 3 = inoculated with pathogen and sprayed with trichotoxin A50 at 50 μg/ml

Treatment 4 = inoculated with pathogen and sprayed with prochloraz at 20 g/20 L of water.

Treatment 5 = inoculated with pathogen

Treatment 6 = non-inoculated control

Disease severity index (DSI) was determined before seedlings were harvested for detection of α-tomatine quantification as previous experiment. Percentage of plant disease resistance (PDR) on treated tomato seedlings were analyzed using formula: DSI of inoculated control – DSI of each treatment / DSI of inoculated control x 100. The α-tomatine was extracted from 6 g of stems and leaves of tomato which harvested at 5, 10 and 15 days after treatment. Then plant tissues were ground in 95% methanol with a pestle and mortar. The extract was evaporated in vacuum evaporator and adjusted with methanol and then they were applied to thin layer chromatography (TLC) with standard comparison of α-tomatine. The spot of extracts from tested tomato which showed Rf = 0.23 as α-tomatine was expected to be α-tomatine of each treatment.

The α-tomatine quantification of the extracts was performed by using HPLC (high performance liquid chromatography) with modified method of Friedman *et al.* (1994). The crude extracts from tested plants were weighed for 1 mg and were dissolved with 1 ml of mixed solvent comprised of 50% methanol - 0.1% acetic acid and filtered through 0.45 μm nylon membrane. The filtrate at 50 μl was subjected into HPLC (Agilent Co. ltd.). The HPLC eluent for α -tomatine

analysis was prepared by combining solvent system with HPLC grade of 80% water: 15% acetonitride (CH_3CN): 5% methanol. Flow rate was set to 1.0 ml/min. The C_8 chromatography column was eluted with HPLC solvent system before injected the filtrates. The α-tomatine quantification was done using linear regression curve which was analyzed from peak area of standard α-tomatine at concentration of 500, 1,000, 1,500 and 2,000 ppm. Then the equation from linear regression curve was used to analyze for the quantity of α-tomatine. Data of the α-tomatine quantification were statistically computed analysis of variance. Treatment means were compared with DMRT at P= 0.05 and P= 0.01.

3.5 Evaluation of microbial elicitors to induce plant resistance for tomato wilt *in vivo*

The bioactive compounds, chaetoglobosin C, chaetomanone A and trichotoxin A50 were determined for the efficacies as microbial elicitors to induce plant resistance for tomato wilt *in vivo*. Randomized Completely Block Design (RCBD) was done with four replications and treatments were designed using extended concentrations of bioactive compounds from previous experiment as follows:-

T1 = chaetoglobosin C at 10 μg/ml

T2 = chaetoglobosin C at 50 μg/ml

T3 = chaetoglobosin C at 100 μg/ml

T4 = chaetomanone A at 10 μg/ml

T5 = chaetomanone A at 50 μg/ml

T6 = chaetomanone A at 100 μg/ml

T7 = trichotoxin A50 at 10 μg/ml

T8 = trichotoxin A50 at 50 μg/ml

T9 = trichotoxin A50 at 100 μg/ml

T10 = chemical fungicide (prochloraz 50% WP) at 20 g/20 L of water

T11 = inoculated with pathogen

T12 = non-inoculated control

Tomato seedlings var. Sida at 30 days old were inoculated with the most aggressive isolate of *F. oxysporum* f. sp. *lycopersici* at concentration of $2x10^6$ conidia/ml using dipped root method as previous experiment. Inoculated seedlings were transferred into 11 inch plastic pot which contained with sterilized mix soil (soil:sand:compost, 4:1:1). Each experimental unit was planted to 8 seedlings of tomato. The bioactive compounds in each concentration and prochloraz was sprayed over tomato leaves immediately and every two weeks after transplanting. Data were collected as growth parameters every 15 days such as plant height (cm) and diameter of plant canopy (cm). Disease severity index (DSI) was scaled as previous experiment and percentage of plant disease resistance (PDR) was analyzed. Data were subjected to analysis of variance (ANOVA) and treatment mean was compared with Duncan's New Multiple Range Test (DMRT) at P=0.05 and P=0.01. Disease reduction and increase in yield were computed using formula as previous experiment.

3.6 Evaluation of Bio-agent formulations to control Fusarium wilt of tomato *in vivo*

3.6.1 Preparation of Bio-agent formulations

Bio-agent formulations were separately formulated as oil formulation according to the method of Soytong (2001) by using antagonistic fungi namely N0802 (*C. globosum*), CLT (*C. lucknowense*), and PC01 (*T. harzianum*). Each antagonistic fungus was cultured by transferring one agar plug which cut with 3 mm diameter sterilized cork borer into PDB and incubated at room temperature (28-32 °C) for 30 days. After that, the culture was filtered through two layer cheesecloth to get mycelial mat and spore mass, then put in the electrical mixer and each Bio-agent formulation was adjusted to 2.5×10^6 spores/ml before being added in sterilized palm's oil.

3.6.2 *In vivo* experiment

The Bio-agent formulations were tested for the abilities as biofungicides to control tomato wilt *in vivo*. The experiment was conducted in 11 inch diameter pot. Tomato seedlings var. Sida at 30 days old were inoculated with conidial suspension of *F. oxysporum* f. sp. *lycopersici* which adjusted to $2x10^6$ conidia/ml by dipping root (Marlatt *et al.* 1996) for 15 min and transplanted into plastic pot contained with sterilized mix soil (soil:sand:compost, 4:1:1). Randomized Completely Block Design (RCBD) was performed with four replications. Treatment was designed as follows:- inoculated control (T_1), treated with Bio-agent formulations N0802 (T_2), treated with Bio-agent

formulations CLT (T_3), treated with Bio-agent formulations PC01 (T_4), treated with chemical fungicide (prochloraz 50% WP) at 20 g/20 L of water (recommendation rate) (T_5) and non treated control (T_6). Each experimental treatment used 20 seedlings of tomato (5 plants / replication). Bio-agent formulations were separately treated at 10 ml/20 L of water and interval sprayed of 20 ml/plant at every 15 days as chemical fungicide. Data were collected at every 30 days as growth parameters such as plant height (cm) and diameter of plant canopy (cm). Plant fresh weight (g), plant dry weight (g) and yield (g) were recorded at harvest. Yield was recorded as fruit size (cm) and fruit weight (g). Disease severity index (DSI) was scaled every 30 days as previous experiment. Data were subjected to analysis of variance (ANOVA) and treatment mean was compared with Duncan's New Multiple Range Test (DMRT) at P=0.05. Disease reduction was computed by using formula: % disease reduction = (Disease severity index of control – Disease severity index of treatment)/ Disease severity index of control X 100. Data of plant fresh weight (g), plant dry weight, and fruit weight (g) were recorded at harvested day. % increase in yield was analyzed using formula: (Yield per plant of treatment – Yield per plant of control)/ Yield per plant of treatment x 100.

CHAPTER 4

RESULTS

4.1. Sample collection, isolation, identification and pathogenicity test

4.1.1 Sample collection and isolation of *Fusarium* spp.

Disease samples were collected from infested tomatoes in the fields in Bangkok, Pathumthani, Nakhon Ratchasima, Burirum, Nong Khai, Sakon Nakhon, Khon Kaen, and Mukdahan provinces. Samples were taken from rhizosphere soil and infected tomato plant which showing wilt symptom. Seven isolates of *Fusarium* species were isolated from infected root, stem and rhizosphere soil from Nakhon Ratchasima province and named in NRC04, NSC01, NSC02, NSC07, NSC09, NSoC01 and NSoC04. The isolates BKFC01, BKFC03, BKFC04, BKFC06, BKFC07, BKFC12, BKRC02 and BKSC02 were isolated from fruits, roots and rhizosphere soil in Bangkok. Five isolates namely PSC01, PSC02, PSC03, PSC04 and PSC05 were isolated from tomato stems from Pathumthani province. Three isolates from Burirum province were isolated from tomato roots and named in BRC01, BRC02 and BRC03. Four isolates namely SRC02, SSoC02, SSoC03 and SSoC04 were isolated from roots and rhizosphere soil from Sakon Nakhon province. The isolates from Khon Kaen province, KSoC01, KSoC02, KSoC03, KSoC04, were isolated from rhizosphere soil and the isolate No. KK2 was provided by Assoc.Prof. Dr. Weerasak Saksirirat and Ms. Manasawee Chaiphad from Khon Kaen University. The isolates from Nong Khai province were isolated from infected roots, stems and rhizosphere soil and named as NKRC02, NKRC04, NKRC09, NKRC11, NKSC01, NKSC02 and NKSoC01. Six isolates namely MRC02, MSC02, MSC04, MSoC01, MSoC02 and MSoC03 were isolated from roots, stems, and rhizosphere soil from Mukdahan province (Table 4.1).

All isolates were studied for morphological characters such as colony, conidiophore, macroconidia and microconidia on potato sucrose agar (PSA) which incubated at room temperature (28-32 °C). Chlamydospores were cultured on corn meal agar (CMA). Morphological characters of all isolates were identified under microscope and measured for their sizes as seen in Table 4.2 and Figure 4.1-4.45.

Table 4.1 Isolates of *Fusarium* spp. from collection sites.

Locating	Isolates No.*	Samples	Fungal species
Bangkok	BKFC01	fruit	*F. incarnatum*
	BKFC03	fruit	*F. solani*
	BKFC04	fruit	*F. incarnatum*
	BKFC06	fruit	*F. incarnatum*
	BKFC07	fruit	*F. chlamydosporum*
	BKFC12	fruit	*F. incarnatum*
	BKRC02	root	*F. solani*
	BKSC02	stem	*F. solani*
Pathumthani	PSC01	stem	*F. incarnatum*
	PSC02	stem	*F. incarnatum*
	PSC03	stem	*F. incarnatum*
	PSC04	stem	*F. incarnatum*
	PSC05	stem	*F. incarnatum*
Nakhon Ratchasima	NRC04	root	*F. incarnatum*
	NSC01	stem	*F. oxysporum*
	NSC02	stem	*F. incarnatum*
	NSC07	stem	*F. incarnatum*
	NSC09	stem	*F. solani*
	NSoC01	rhizosphere soil	*F. solani*
	NSoC04	rhizosphere soil	*F. chlamydosporum*
Burirum	BRC01	root	*F. solani*
	BRC02	root	*F. solani*
	BRC03	root	*F. oxysporum*
Sakon Nakhon	SRC02	root	*F. oxysporum*
	SSoC02	rhizosphere soil	*F. solani*
	SSoC03	rhizosphere soil	*F. oxysporum*
	SSoC04	rhizosphere soil	*F. oxysporum*

Table 4.1 (continued)

Locating	Isolates No.*	Samples	Fungal species
Khon Kaen	KK2**	stem	*F. oxysporum*
	KSoC01	rhizosphere soil	*F. solani*
	KSoC02	rhizosphere soil	*F. oxysporum*
	KSoC03	rhizosphere soil	*F. incarnatum*
	KSoC04	rhizosphere soil	*F. solani*
Nong Khai	NKSC01	stem	*F. oxysporum*
	NKSC02	stem	*F. oxysporum*
	NKRC02	root	*F. oxysporum*
	NKRC04	root	*F. oxysporum*
	NKRC09	root	*F. oxysporum*
	NKRC11	root	*F. redolens*
	NKSoC01	rhizosphere soil	*F. redolens*
Mukdahan	MRC02	root	*F. solani*
	MSC02	stem	*F. solani*
	MSC04	stem	*F. solani*
	MSoC01	rhizosphere soil	*F. incarnatum*
	MSoC02	rhizosphere soil	*F. solani*
	MSoC03	rhizosphere soil	*F. solani*

* 1st alphabet means provinces:- BK = Bangkok, P = Pathumthani, N = Nakhon Ratchasima, B = Burirum, S = Sakon Nakhon, K = Khon Kaen, NK = Nong Khai and M = Mukdahan

2nd alphabet means samples:- S = stem, R = root, F = fruit, So = rhizosphere soil

3rd alphabet means name of collector:- C = Chamaiporn

4th alphabet means number of isolates

** Isolate KK2 was provided by Ms.Manasawee Chayphad and Assoc.Prof. Dr. Weerasak Saksirirat, Faculty of Agriculture, Khon Kaen University

Table 4.2 Morphological characters of *Fusarium* spp. isolated from collecting samples.

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum BKFC01	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores scattered in the aerial mycelium, loosely branched, philides slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 14.1-25.5 x 3.2-4.3 µm.	Microconidia have fusiform shape, straight with slightly curved, 6.8-17.5 x 2.8-4.5 µm.	Chlamydospores are globose, intercalary, pale, formed singly, in pairs or in chains, smooth wall, 3.8-4.9 x 3.4-4.2 µm.
Fusarium solani BKFC03	Colony white, rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32°C	Conidiophores produced in the aerial mycelium, singly, slender, cylindrical shape.	Macroconidia produced on shorter, branched conidiophores, usually moderately curved, with short, blunt apical and indistinctly pedicellate basal cells, 3 -5 septate, 27.5-41.2 x 4.2-6.5 µm.	Microconidia usually abundant, produced on elongate, sometimes verticillate, 7.2-15.8 x 3.7-15.3 µm.	Chlamydospores produced singly, in terminal, lateral, or intercalary, smooth wall, 2.4-3.7 x 2.5-3.5 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum BKFC04	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming pinkish or peach.	Conidiophores produced in aerial mycelium, singly, slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 18.3-31.1 x 2.6-4.8 µm.	Microconidia have fusiform shape, straight with slightly curved, 0-1 septate, 9.5-17.9 x 1.9-3.4 µm.	Chlamydospores are globose, intercalary, formed singly or in chains, smooth or roughen wall, 2.9-4.5 x 2.3-4.3 µm.
Fusarium incarnatum BKFC06	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores produced in aerial mycelium, singly, slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 18.0-25.4 x 3.0-4.8 µm.	Microconidia shaped in elliptical or cylindrical, straight with slightly curved, 0-1 septate, 8.7-16.6 x 2.2-4.3 µm.	Chlamydospores are globose, intercalary, formed singly, in pairs or in chains, smooth or roughen wall, 2.4-4.1 x 2.4-4.6 µm.
Fusarium chlamydosporum BKFC07	Colony fast growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium abundantly developed, pink.	Conidiophores scattered over aerial mycelium, richly branched.	Macroconidia have fusiform shape with 3-5 septate, slightly curved, 11.3-19.4 x 1.7-2.8 µm.	Microconidia fusiform or elliptical, 3.5-6.4 x 1.5-2.5 µm.	Chlamydospores formed singly, in pairs or in chains, intercalary, smooth or roughen wall, 3.1-5.3-2.8-5.1 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum BKFC12	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming brown yellow.	Conidiophores scattered in the aerial mycelium, loosely branched, philides slender, cylindrical.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 19.8-32.5 x 2.8-5.2 µm .	Microconidia have fusiform shape, straight with slightly curved, 0-1 septate, 5.2-10.9 x 1.2-2.3 µm.	Chlamydospores are often sparse, globose, intercalary, pale, formed singly or in chains, smooth or roughen wall, 2.4-5.5-1.9-4.2 µm.
Fusarium solani BKRC02	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C	Conidiophores formed singly or branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 23.3-42.1 x 3.7-5.7 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 10.9-18.9 x 2.9-4.5 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth walled, 2.9-4.1 x 2.4-3.8 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium solani BKSC02	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 26.4-37.4 x 3.5-4.8 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 9.2-16.4 x 2.6-4.3 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth walled, 2.3-4.2 x 2.2-4.6 µm.
Fusarium solani BRC01	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 36.7-54.5 x 5.5-7.2 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 7.5-15.3 x 2.1-3.8 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, smooth walled, 3.0-4.0 x 2.8-4.4 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium solani BRC02	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or short branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 16.0-36.3 x 2.7-5.1 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 7.5-14.6 x 2.6-4.2 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, smooth walled, 3.0-4.1 x 3.0-3.9 µm.
Fusarium oxysporum BRC03	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 13.2-34.4 x 2.5-3.8 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 4.1-9.3 x 1.5-3.0 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.2-3.9-2.0-3.5 µm.
Fusarium oxysporum KK2	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with violet tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 14.1-27.4 x 2.1-3.7 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 4.2-9.6x 1.3-3.7 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.2-4.9-1.9-3.1 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium solani KSoC01	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 15.4-39.6 x 3.0-6.0 µm.	Microconidia usually abundant, shaped in elliptical or cylindrical with 0-1 septate, 8.2-14.9 x2.0-4.7 µm.	Chlamydospores produced singly, in terminal, lateral, or intercalary, smooth wall, 2.9-4.2 x 2.5-3.9 µm.
Fusarium oxysporum KSoC02	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 18.4-28.4 x 2.7-3.9 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 3.1-10.1 x 0.7-2.9 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.4-4.7 x 2.0-3.2 µm.
Fusarium incarnatum KSoC03	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores produced in the aerial mycelium, singly or branched, slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 15.0-23.4 x 2.5-4.4 µm.	Microconidia shaped in elliptical or cylindrical, straight with slightly curved, 0-1 eptate, 6.5-11.4 x 1.6-3.9 µm.	Chlamydospores are globose, intercalary, pale, formed singly, in pairs or in chains, 2.3-4.1 x 2.1-3.5 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium solani KSoC04	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 24.2-41.9 x 3.5-5.4 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 10.0-16.3 x 2.9-4.8 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth wall, 2.3-3.2 x 2.3-3.1 µm.
Fusarium solani MRC02	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or short branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 19.2-40.5 x 3.8-5.2 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 9.0-16.9 x 2.3-4.7 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth or roughen wall, 2.5-4.7 x 2.3-4.5 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium solani MSC02	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or short branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 20.8-35.7 x 3.0-5.3 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 8.6-17.6 x 2.9-5.1 µm.	Chlamydospores produced singly or in paired, in terminal or intercalary, or formed from macro and microconidia, smooth wall, 2.8-4.2 x 2.7-4.2 µm.
Fusarium solani MSC04	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or short branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 20.38-29.8 x 2.5-3.6 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 8.7-11.9 x 2.4-3.6 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth or roughen wall, 2.8-4.6 x 2.5-3.7 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum MSoC01	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, whitish.	Conidiophores produced in the aerial mycelium, singly or branched, slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 15.6-24.6 x 2.1-4.0 µm.	Microconidia have elliptical or cylindrical shape, straight with slightly curved, 0-1 septate, 7.4-16.2 x 2.0-4.4 µm.	Chlamydospores are globose, intercalary, formed singly, in pairs or in chains, smooth or roughen wall, 2.6-4.9 x 2.6-4.4 µm.
Fusarium solani MSoC02	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores produced in the aerial mycelium, singly, slender, cylindrical shape.	Macroconidia shaped in slightly or moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 20.3-31.3 x 2.3-4.3 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 8.4-12.8 x 3.0-4.5 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth or roughen wall, 2.2-3.6 x 2.0-3.4 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium solani MSoC03	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores produced in the aerial mycelium, singly, slender, cylindrical shape.	Macroconidia shaped in slightly or moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 16.2-33.9 x 2.3-3.6 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 8.2-15.9 x 1.4-3.4 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth or roughen wall, 2.6-3.6 x 2.0-3.7 µm.
Fusarium oxysporum NKRC02	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 26.5-42.1 x 3.5-5.0 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 10.1-18.1 x 3.1-4.8 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.4-3.9 x 1.9-3.2 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium oxysporum NKRC04	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with violet tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 15.3-26.1 x 2.6-3.9 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 3.4-10.3 x 1.7-3.2 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.6-3.6 x 1.7-3.1 µm.
Fusarium oxysporum NKRC09	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 22.7-33.1 x 2.2-3.9 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 7.0-9.9 x 2.0-3.0 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.3-3.8 x 2.0-3.6 µm.
Fusarium redolens NKRC11	Colonies rather fast growing, reaching 9 cm diameter in 7-9 days at 28-32° C, Aerial mycelium sparse to abundant and becoming felted, white with purple or violet tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia are fusiform, often formed in pale sporodochia, distingly 3-5 septate, 15.5-29.7 x 2.5-4.0 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, 5.0-10.8 x 2.0-3.3 µm.	Chlamydospores arising in the mycelium, terminal and intercalary, smooth wall, 2.6-4.3 x 2.2-4.1 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium oxysporum NKSC01	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 16.0-32.8 x 2.2-3.5 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 3.5-6.0 x 1.5-2.9 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.6-3.7 x 2.2-3.4 µm.
Fusarium oxysporum NKSC02	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 21.3-31.2 x 2.2-3.6 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 5.6-11.7 x 1.4-2.4 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.4-3.5 x 2.1-3.3 µm.
Fusarium redolens NKSoC01	Colonies rather fast growing, reaching 9 cm diameter in 7-9 days at 28-32° C, Aerial mycelium sparse to abundant and becoming felted, with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia are fusiform, often formed in pale sporodochia, distingly 3-5 septate, 15.4-35.4 x 2.2-3.6 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, 4.3-8.9 x 1.9-2.7 µm.	Chlamydospores arising in the mycelium, terminal and intercalary, smooth wall,2.4-3.8 x 2.3-3.5 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum NRC04	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, whitish.	Conidiophores produced in the aerial mycelium, singly or branched, slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 12.4-21.3 x 1.8-2.7 µm.	Microconidia have elliptical or cylindrical shape, straight with slightly curved, 0-1 septate, 5.3-12.7 x 1.4-2.3 µm.	Chlamydospores are globose, intercalary, formed singly, in pairs or in chains, smooth wall, 2.5-4.0 x 2.2-3.4 µm.
Fusarium oxysporum NSC01	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 15.5-25.6 x 2.4-3.8 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, straight, 4.1-10.7 x 1.3-2.7 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.3-4.5 x 2.0-4.1 µm.
Fusarium incarnatum NSC02	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores scattered in the aerial mycelium, loosely branched, philides slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 11.1-21.7 x 1.9-2.7 µm.	Microconidia have fusiform shape, straight with slightly curved, 6.4-15.6 x 1.4-2.1 µm.	Chlamydospores are globose, intercalary, pale, formed singly or in chains, smooth wall, 2.4-4.8 x 2.4-3.9 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum NSC07	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores scattered in the aerial mycelium, loosely branched, phillides slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 14.0-22.5 x 1.7-2.7 µm.	Microconidia have fusiform shape, straight with slightly curved, 7.0-14.5 x 1.3-2.0 µm.	Chlamydospores are globose, intercalary, pale, formed singly or in chains, smooth wall, 2.0-3.9 x 1.6-3.3 µm.
Fusarium solani NSC09	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or short branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 23.0-40.5 x 4.5-7.0 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 11.5-20.5 x 3.3-5.5 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth or roughen wall, 2.7-4.5 x 2.4-3.9 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium solani NSoC01	Colony white, rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or short branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 21.1-25.8 x 3.2-4.6 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 9.1-13.2 x 2.1-4.1 µm.	Chlamydospores produced singly or in paired, in terminal, intercalary, or formed from macro and microconidia, smooth or roughen wall, 2.8-4.0 x 2.2-3.8 µm.
Fusarium chlamydosporum NSoC04	Colonies rather fast growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium abundantly developed, pale yellow.	Conidiophores scattered over the aerial mycelium, branched.	Macroconidia produced on normal phialides, fusiform shape with 3-5 septate, slightly curved, 15.1-21.5 x 2.8-4.1 µm.	Microconidia fusiform or elliptical, 10.0-16.1 x 2.4-3.7 µm.	Chlamydospores formed singly, in pairs or in chains, intercalary, smooth or roughen wall, 2.9-4.3-2.2-4.3 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum PSC01	Colony fast growing, reaching 9 cm diameter in 5-7 days at 28-32° C, aerial mycelium, cottony, at first whitish but later becoming yellow brown.	Conidiophores produced richly tree branch.	Macroconidia scattered in the aerial mycelium, fusiform with slightly curved, 3-5 septate, 15.7-34.2 x 2.9-4.9 µm.	Microconidia shaped in pyriform, 0-1 septate, 6.5-13.4 x 2.9-4.5 µm.	Chlamydospores numerous, intercalary or rarely terminal, often in chains or clusters, smooth wall, 2.5-4.4 x 2.1-3.4 µm.
Fusarium incarnatum PSC02	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores scattered in aerial mycelium, loosely branched, philides slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 18.5-29.6 x 2.9-3.9 µm.	Microconidia have fusiform shape, straight with slightly curved, 11.1-16.8 x 2.4-3.3 µm.	Chlamydospores are globose, intercalary, pale, formed singly or in chains, smooth wall, 2.6-3.9 x 1.9-3.3 µm.
Fusarium incarnatum PSC03	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C. Aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores scattered in aerial mycelium, loosely branched, philides slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 18.4-30.2 x 2.6-4.7 µm.	Microconidia have fusiform shape, straight with slightly curved, 9.7-16.0 x 2.2-3.3 µm.	Chlamydospores are globose, intercalary, pale, formed singly or in chains, smooth wall, 2.6-4.9 x 2.2-4.5 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium incarnatum PSC04	Colony fast growing, reaching 9 cm diameter in 5-7 days at 28-32° C, aerial mycelium, cottony, at first whitish but later becoming yellow brown.	Conidiophores produced richly tree branch.	Macroconidia scattered in the aerial mycelium, fusiform with slightly curved, 3-5 septate, 20.1-34.2 x 2.6-4.7 µm.	Microconidia shaped in pyriform, 0-1 septate, 10.2-18.9 x 1.6-3.6 µm.	Chlamydospores numerous, intercalary or rarely terminal, often in chains or clusters, , smooth wall, 2.4-4.0 x 2.2-3.7 µm.
Fusarium incarnatum PSC05	Colony fast-growing, reaching 9 cm diameter in 5-7 days at 28-32° C, aerial mycelium floccose, at first whitish but later becoming peach.	Conidiophores scattered in the aerial mycelium, loosely branched, philides slender, cylindrical shape.	Macroconidia shaped in fusiform, almost straight, with slightly bent, beaked apical, 3-5 septate, 13.6-25.6 x 1.5-2.8 µm.	Microconidia have fusiform shape, straight with slightly curved, 5.7-13.3 x 1.2-2.2 µm.	Chlamydospores are globose, intercalary, pale, formed singly or in chains, smooth or roughen wall, 2.0-4.1 x 1.6-3.4 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium oxysporum SRC02	Colony fast-growing, reaching 9 cm diameter. in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 17.9-28.9 x 3.0-4.4 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, 5.1-10.3 x 1.9-3.4 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.6-4.1 x 2.3-4.0 µm.
Fusarium solani SSoC02	Colony rather fast growing with little aerial mycelium, white or cream, reaching 9 cm diameter in 5-7 days at 28-32° C.	Conidiophores formed singly or short branched, slender, cylindrical shape.	Macroconidia shaped in moderately curved, with short, blunt apical, and pedicellate basal cells 3 -5 septate, 34.7-52.8 x 4.0-5.9 µm.	Microconidia usually abundant, shaped in elliptical with 0-1 septate, 10.1-15.6 x 3.0-4.7 µm.	Chlamydospores produced singly or in paired, in terminal or intercalary, or formed from macro and microconidia, smooth wall, 2.6-4.2 x 2.1-3.6 µm.

Table 4.2 (continued).

Fungal isolates	Colony on PSA	Conidiophores	Macroconidia	Microconidia	Chlamydospores
Fusarium oxysporum SSoC03	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 15.7-32.4 x 2.4-4.3 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, 4.0-7.5 x 1.6-3.2 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.4-3.7 x 2.4-3.5 µm.
Fusarium oxysporum SSoC04	Colony fast-growing, reaching 9 cm diameter in 7-9 days at 28-32° C. Aerial mycelium sparse to abundant and becoming felted, white with purple tinge.	Conidiophores are shorted, formed singly and branched.	Macroconidia shaped in fusiform, slightly curved, 3-5 septate, 15.8-32.8 x 2.5-4.1 µm.	Microconidia abundant, 0-1 septate, elliptical or cylindrical, 6.3-10.7 x 1.9-3.5 µm.	Chlamydospores terminal or intercalary in hyphae, smooth or roughen wall, 2.3-4.3 x 2.2-4.3 µm.

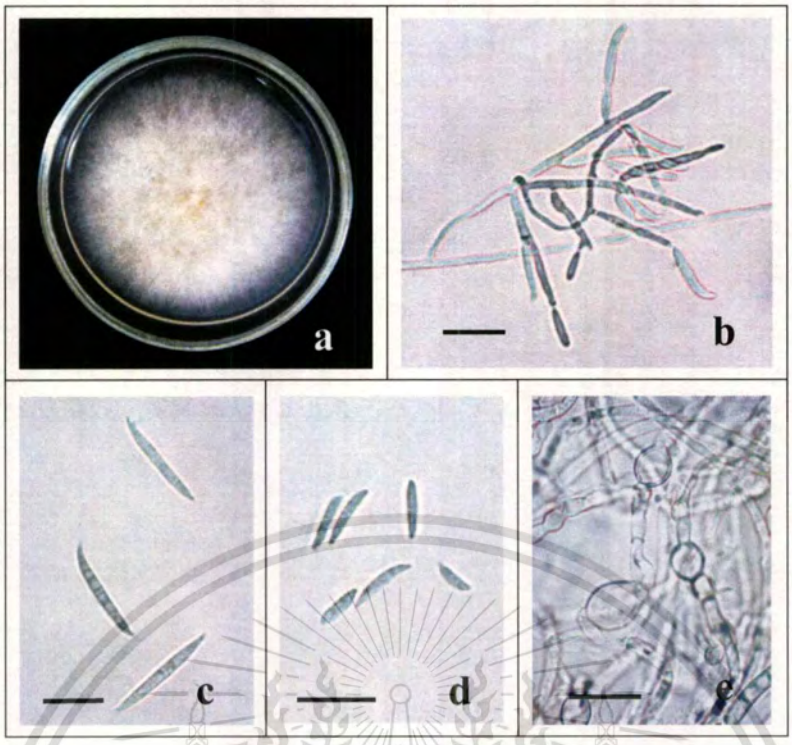


Figure 4.1 Morphological character of *Fusarium incarnatum* BKFC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

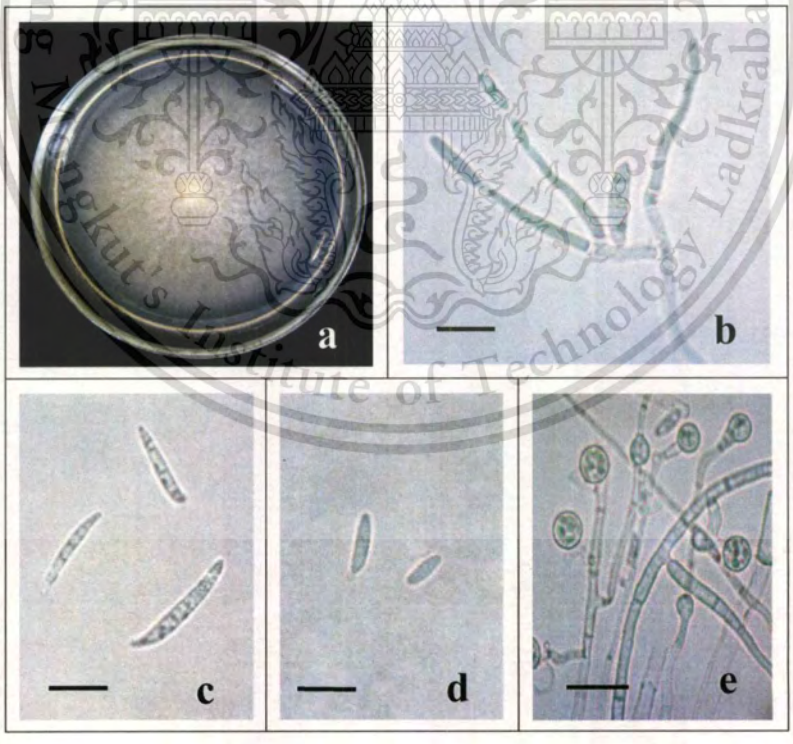


Figure 4.2 Morphological character of *Fusarium solani* BKFC03.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

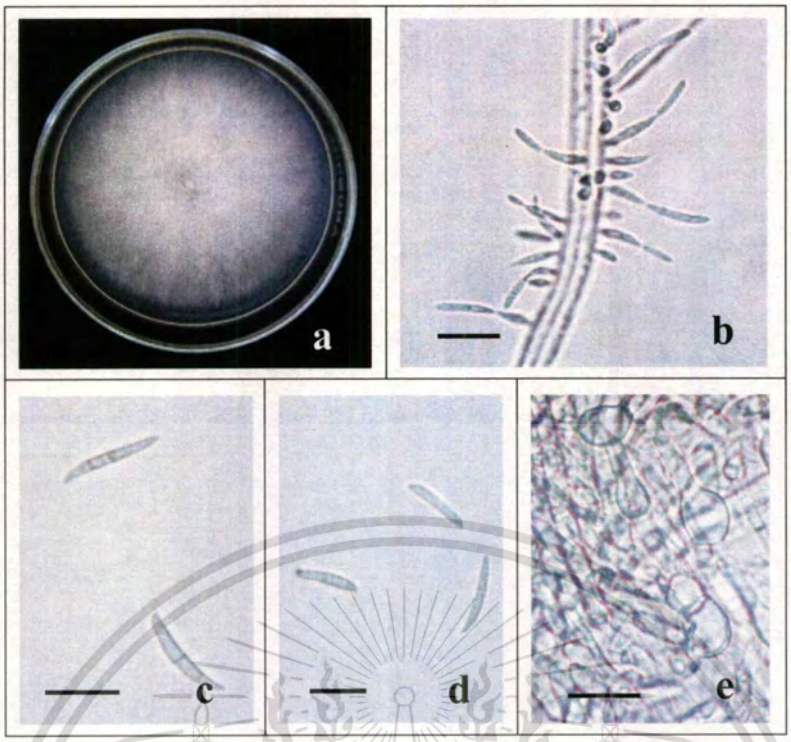


Figure 4.3 Morphological character of *Fusarium incarnatum* BKFC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

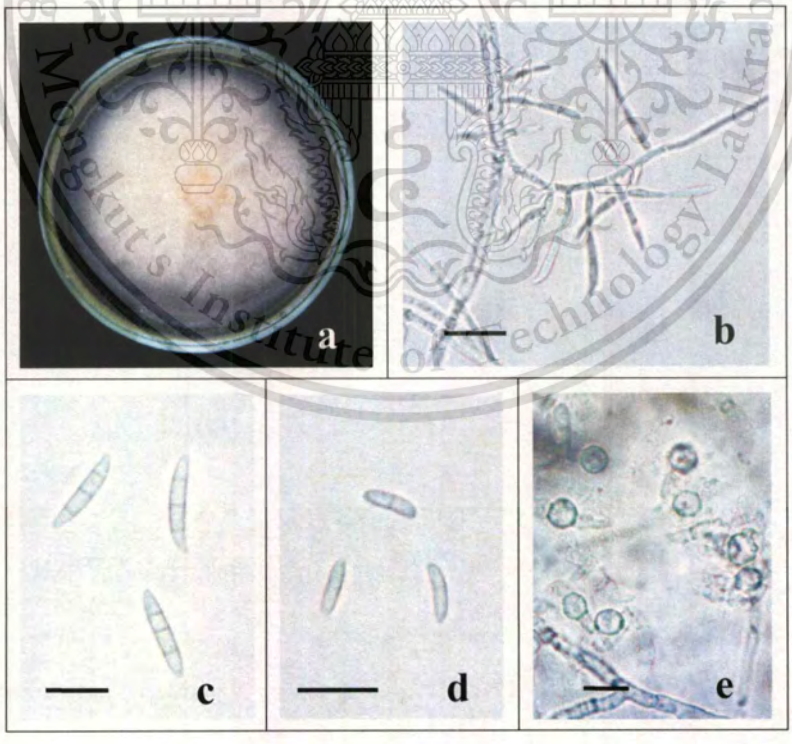


Figure 4.4 Morphological character of *Fusarium incarnatum* BKFC06.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

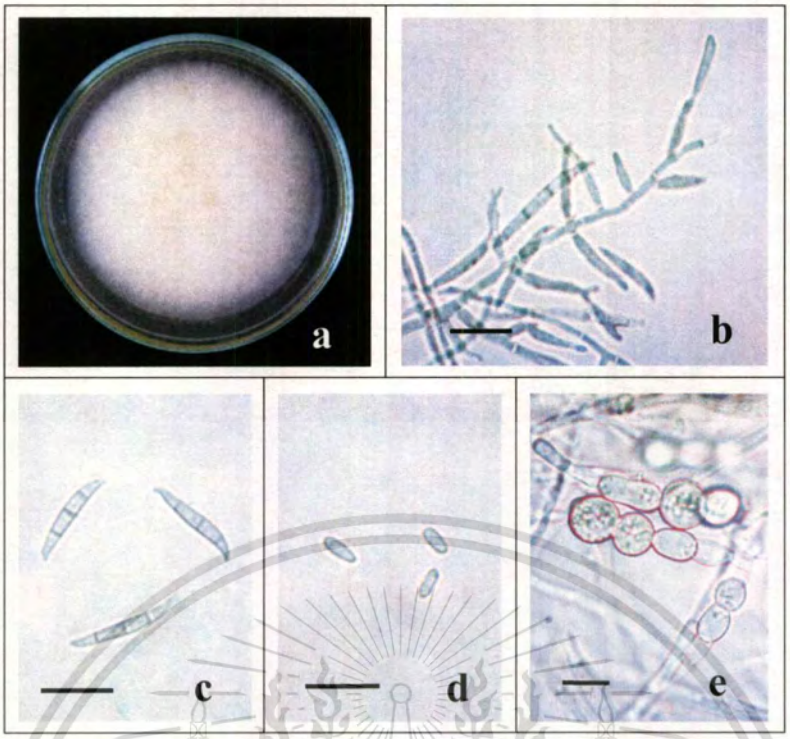


Figure 4.5 Morphological character of *Fusarium chlamydosporum* BKFC07.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

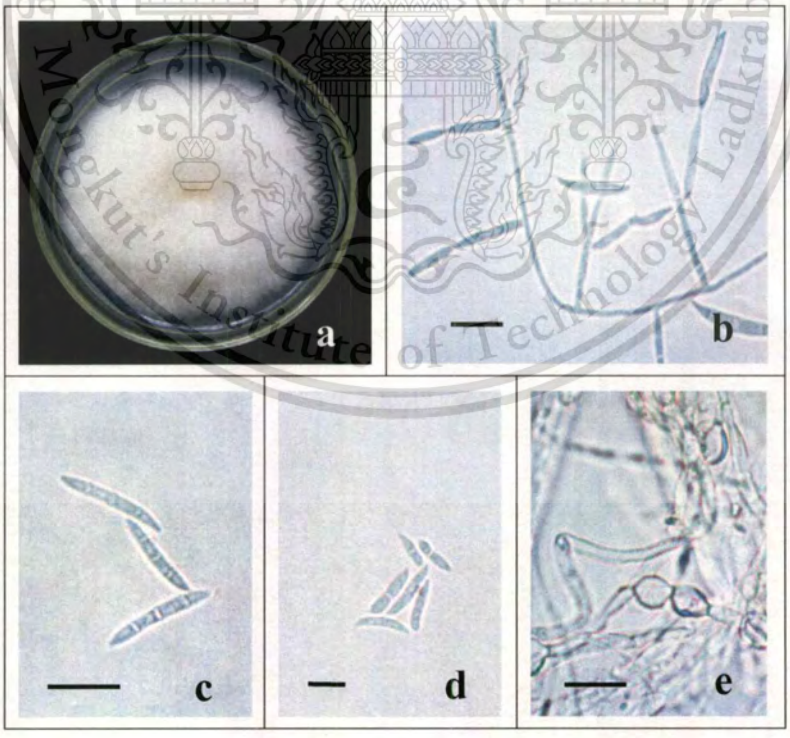


Figure 4.6 Morphological character of *Fusarium incarnatum* BKFC12.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

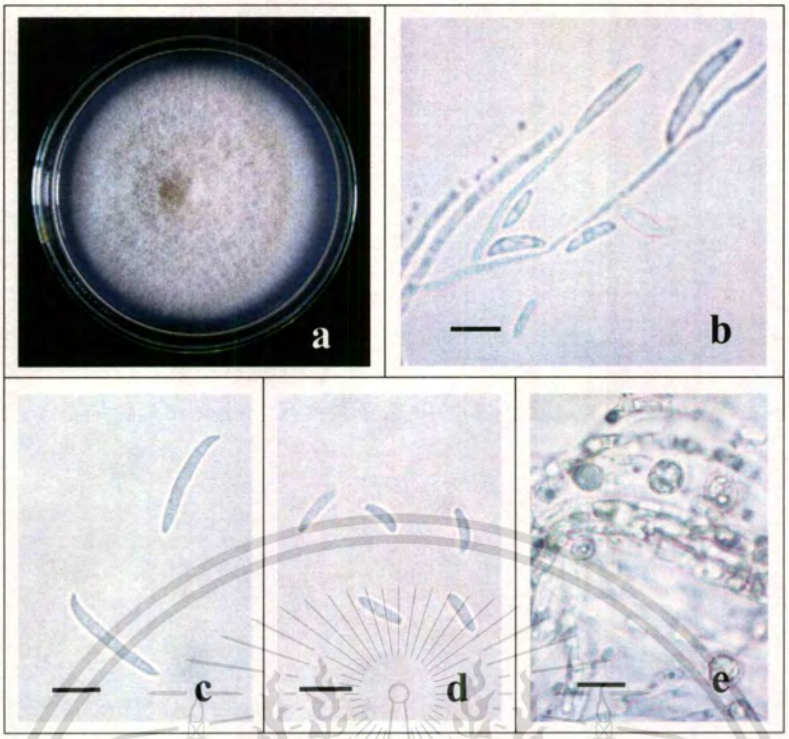


Figure 4.7 Morphological character of *Fusarium solani* BKRC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

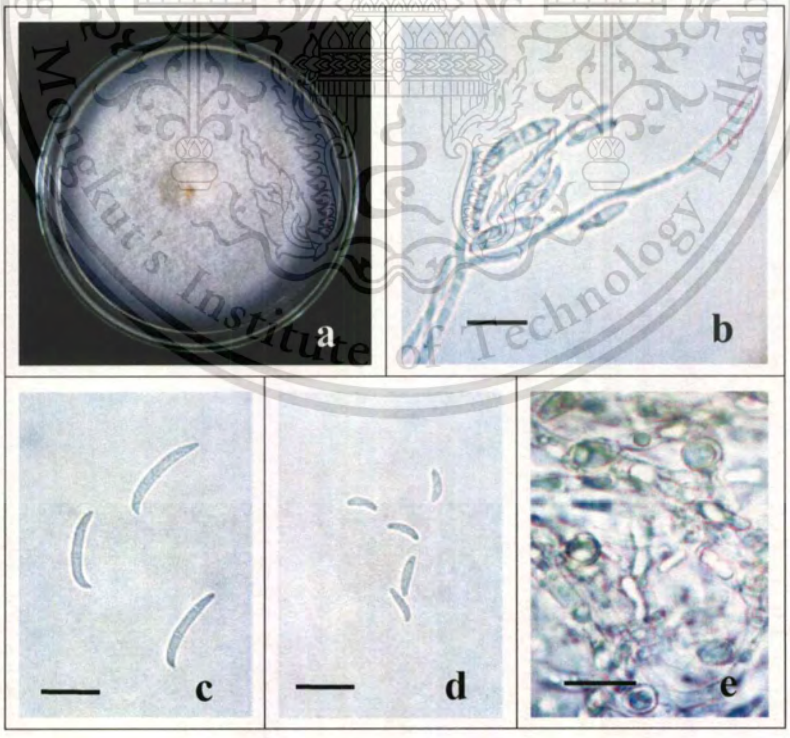


Figure 4.8 Morphological character of *Fusarium solani* BKSC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

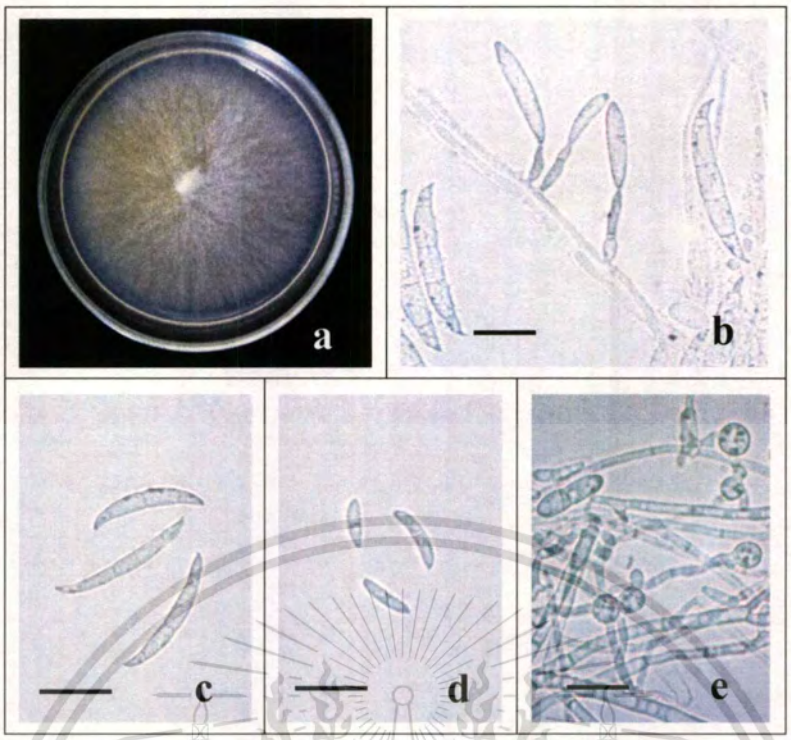


Figure 4.9 Morphological character of *Fusarium solani* BRC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

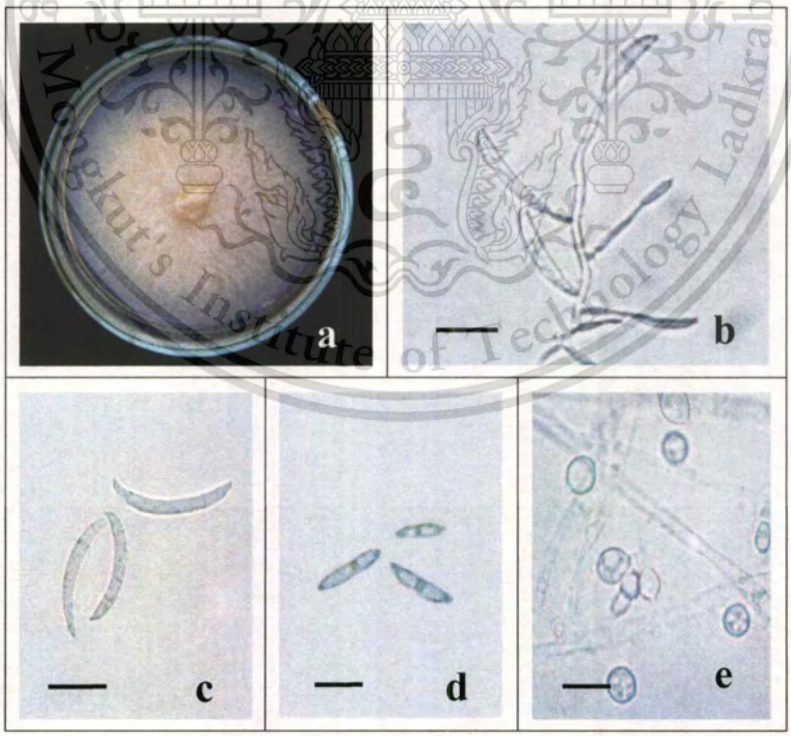


Figure 4.10 Morphological character of *Fusarium solani* BRC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

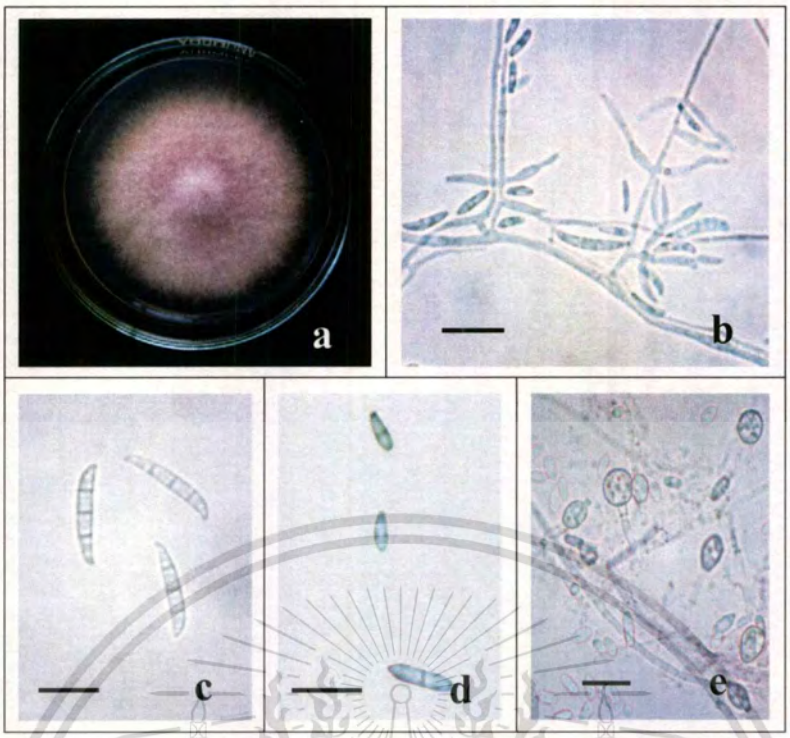


Figure 4.11 Morphological character of *Fusarium oxysporum* BRC03.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

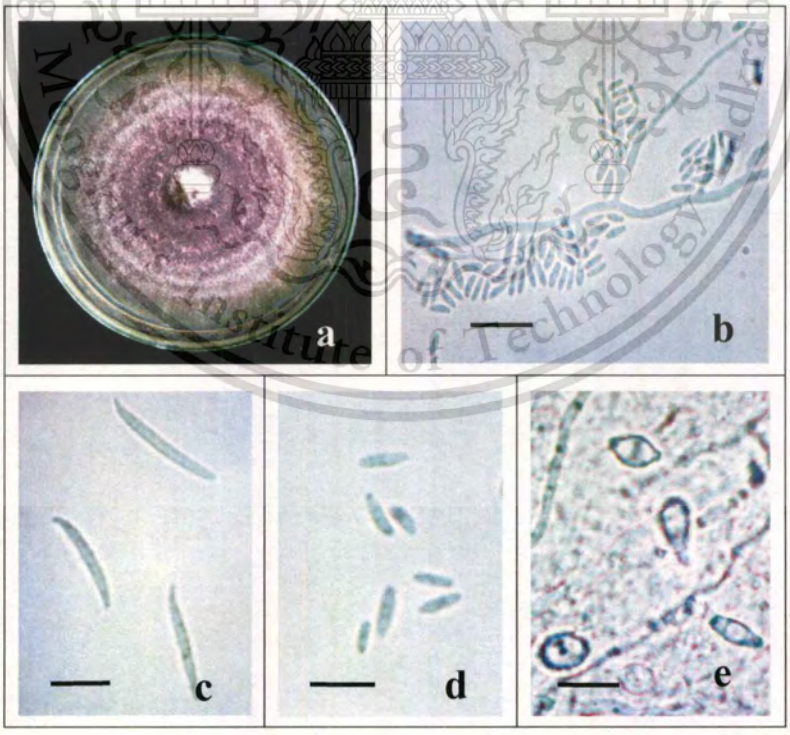


Figure 4.12 Morphological character of *Fusarium oxysporum* KK2.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

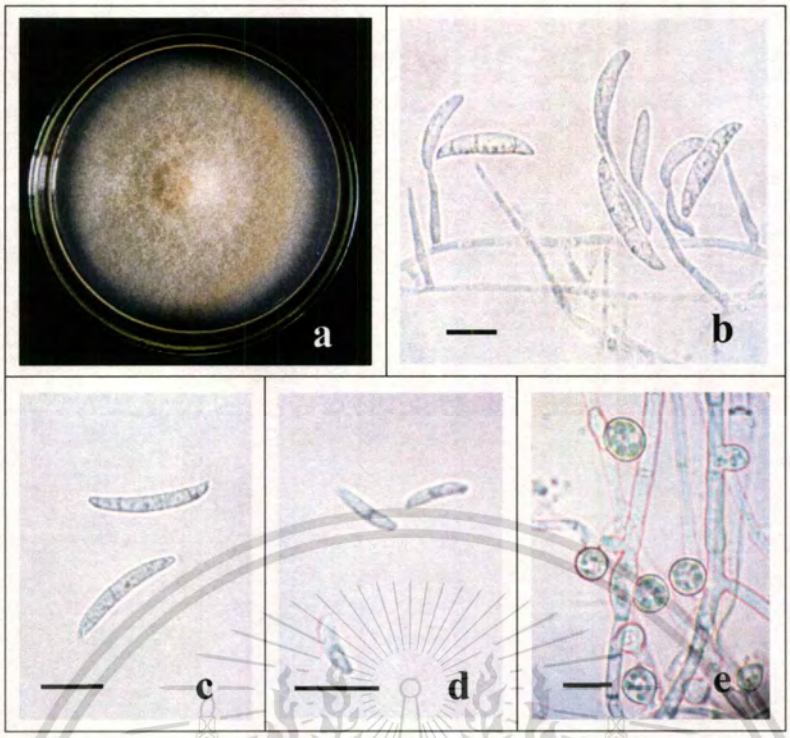


Figure 4.13 Morphological character of *Fusarium solani* KSoC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

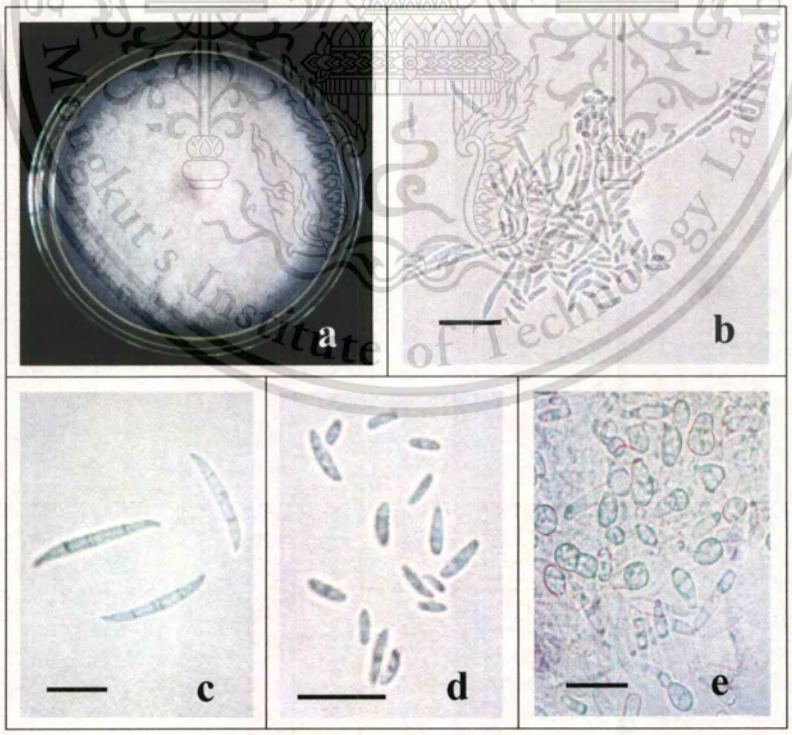


Figure 4.14 Morphological character of *Fusarium oxysporum* KSoC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

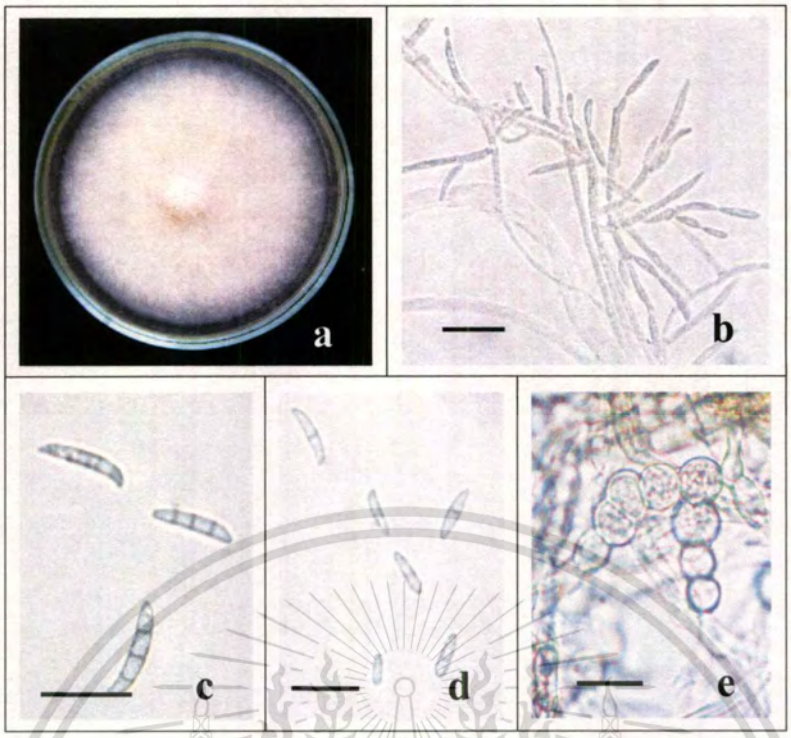


Figure 4.15 Morphological character of *Fusarium incarnatum* KSoC03.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

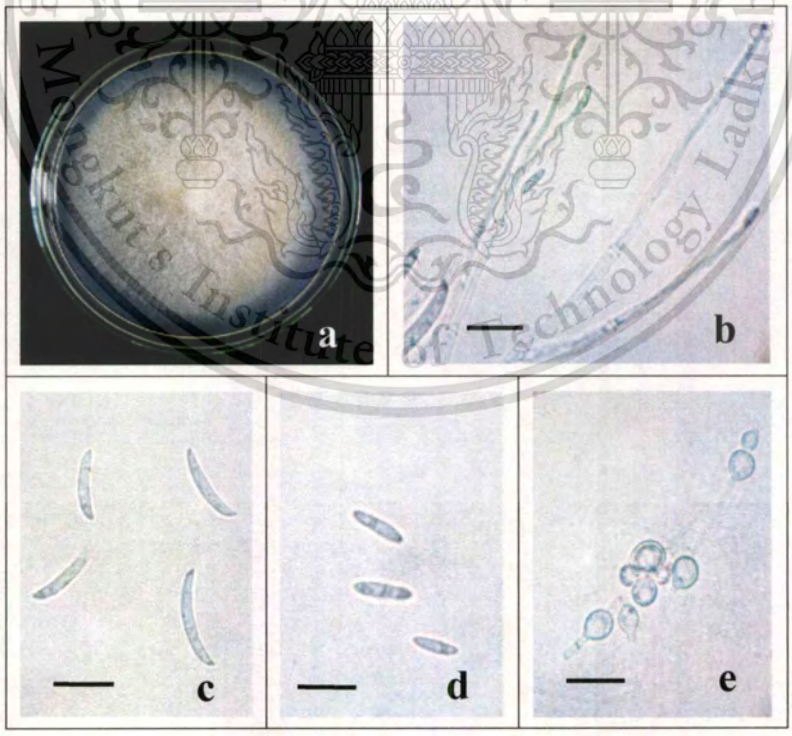


Figure 4.16 Morphological character of *Fusarium solani* KSoC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

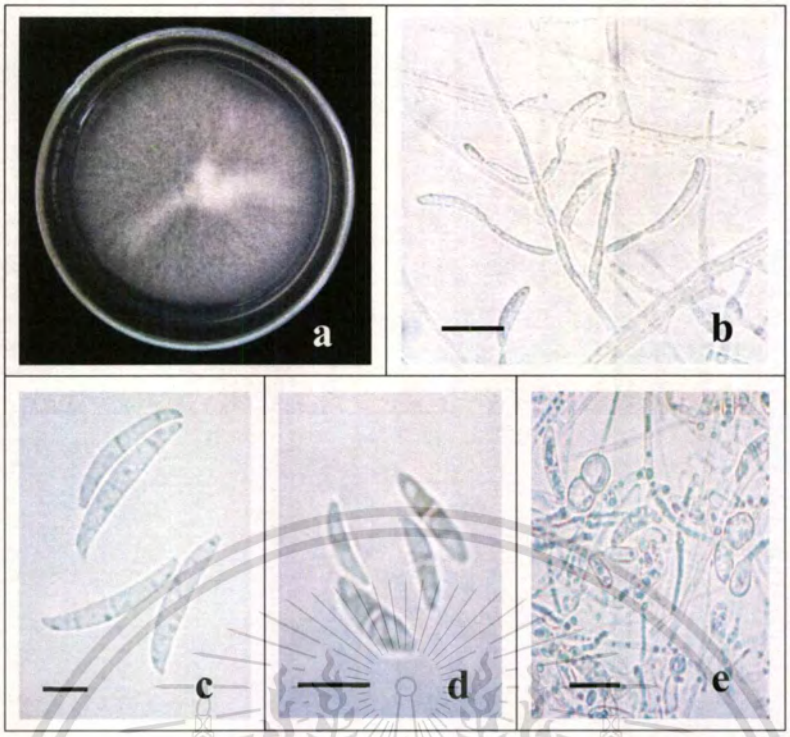


Figure 4.17 Morphological character of *Fusarium solani* MRC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

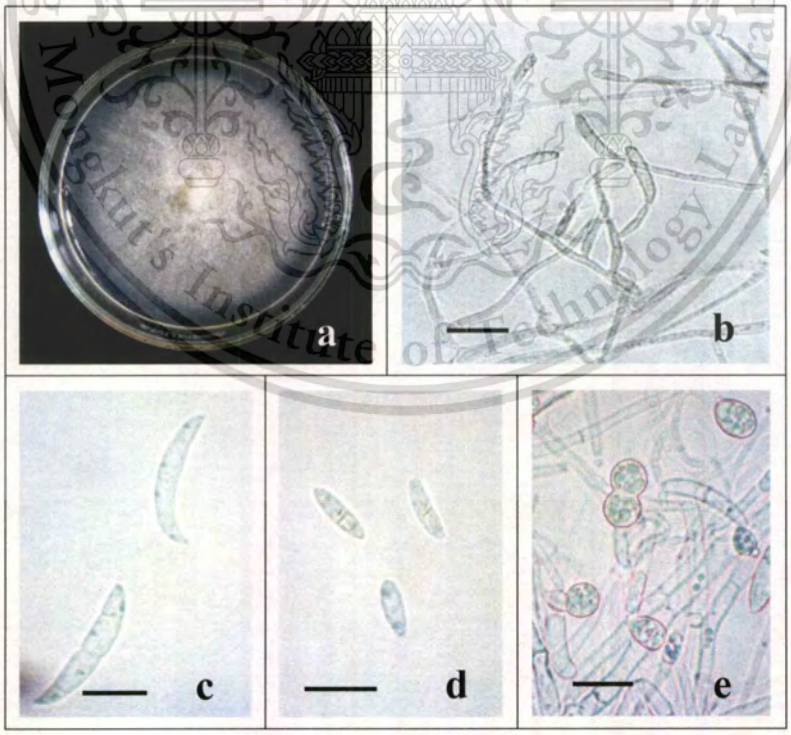


Figure 4.18 Morphological character of *Fusarium solani* MSC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

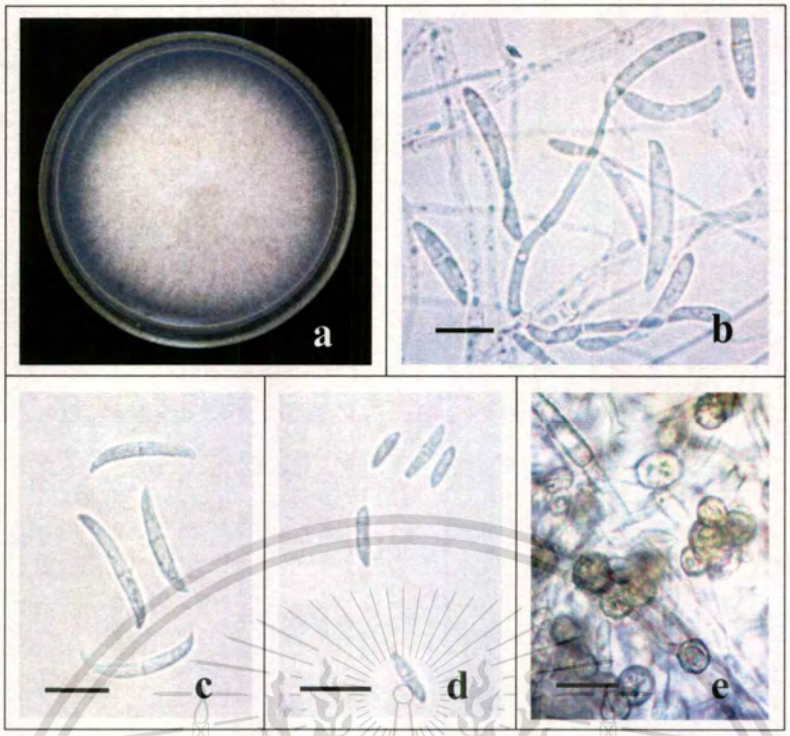


Figure 4.19 Morphological character of *Fusarium solani* MSC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

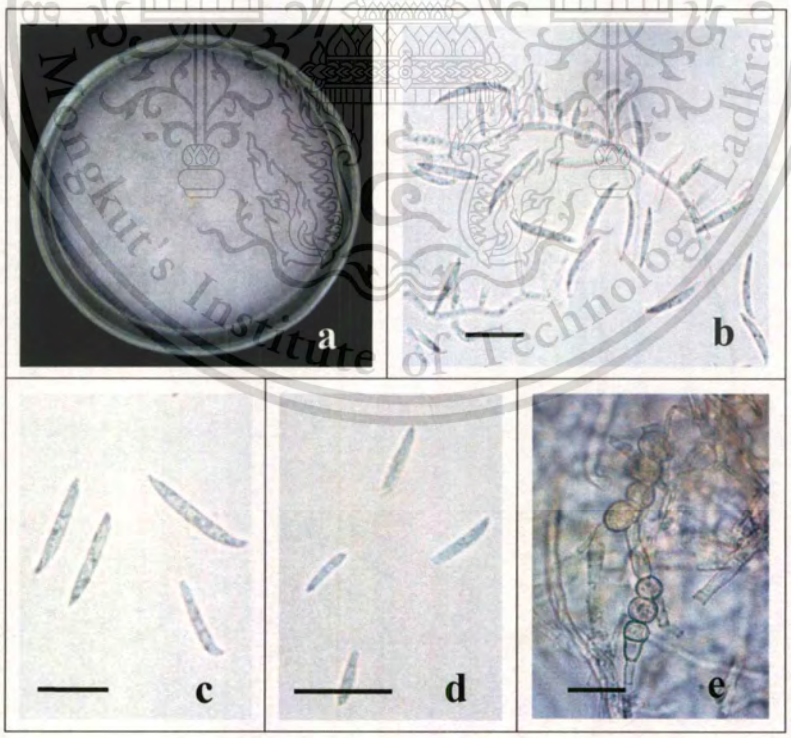


Figure 4.20 Morphological character of *Fusarium incarnatum* MSoC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

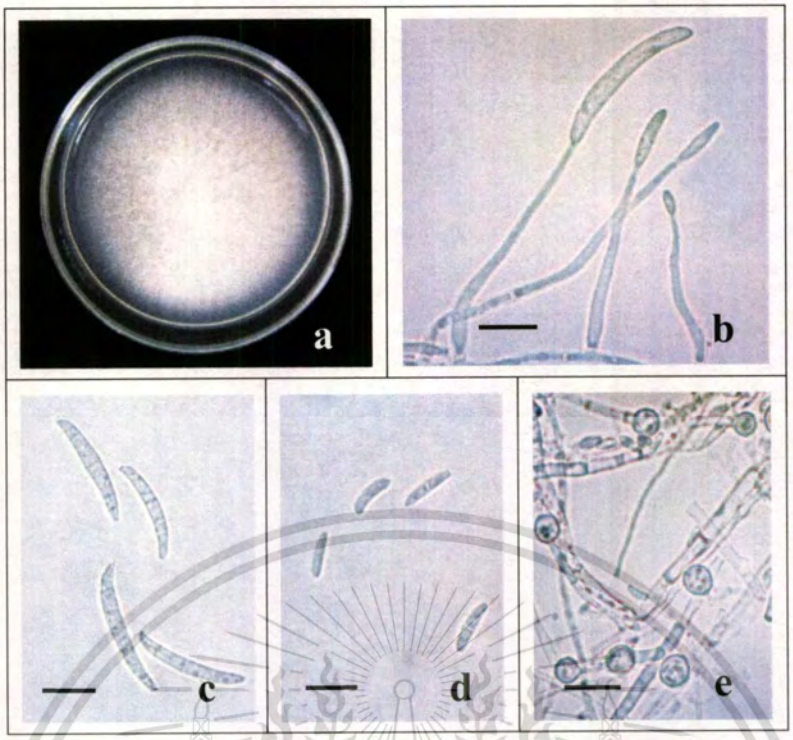


Figure 4.21 Morphological character of *Fusarium solani* MSoC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

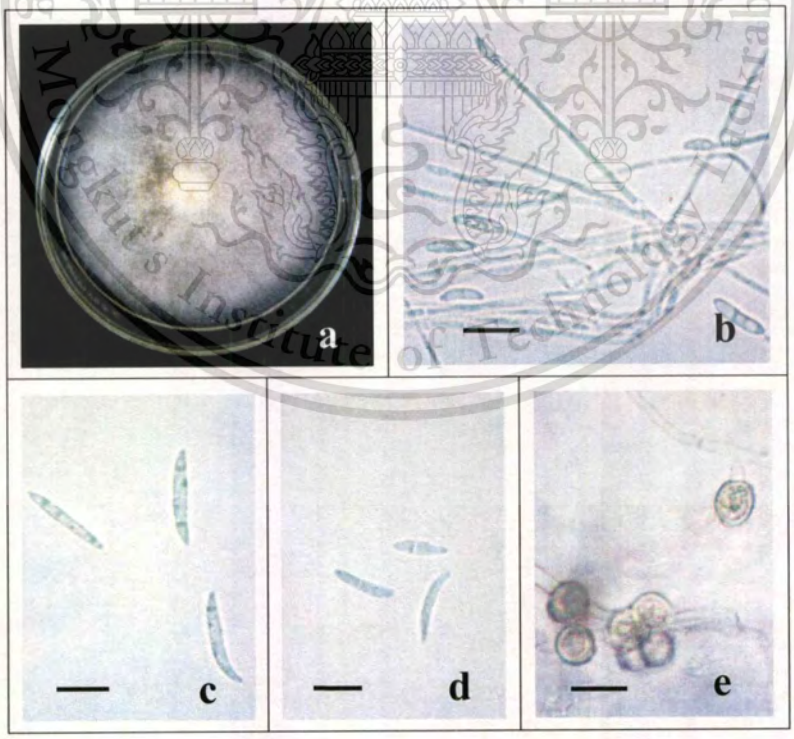


Figure 4.22 Morphological character of *Fusarium solani* MSoC03.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

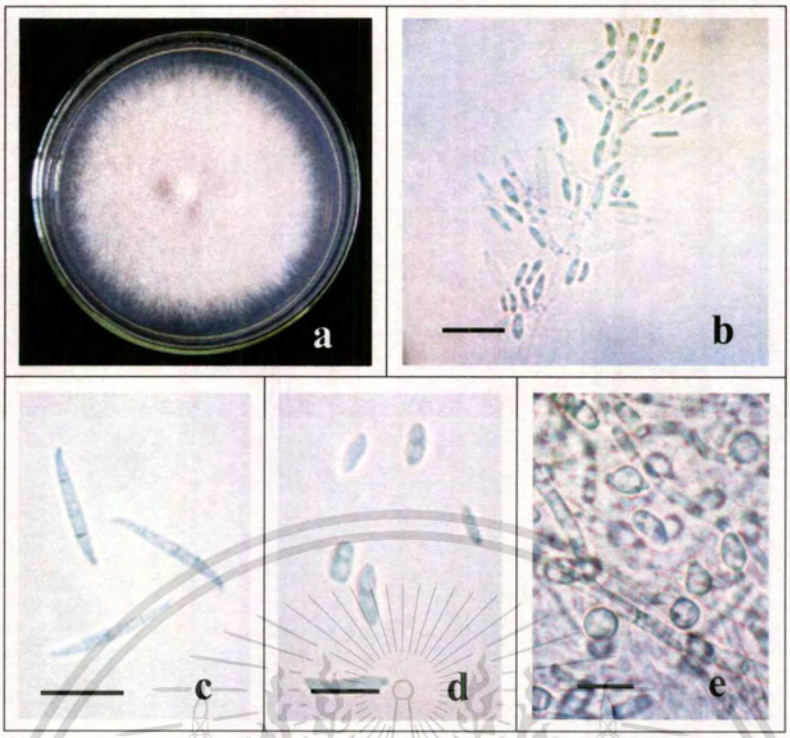


Figure 4.23 Morphological character of *Fusarium oxysporum* NKRC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

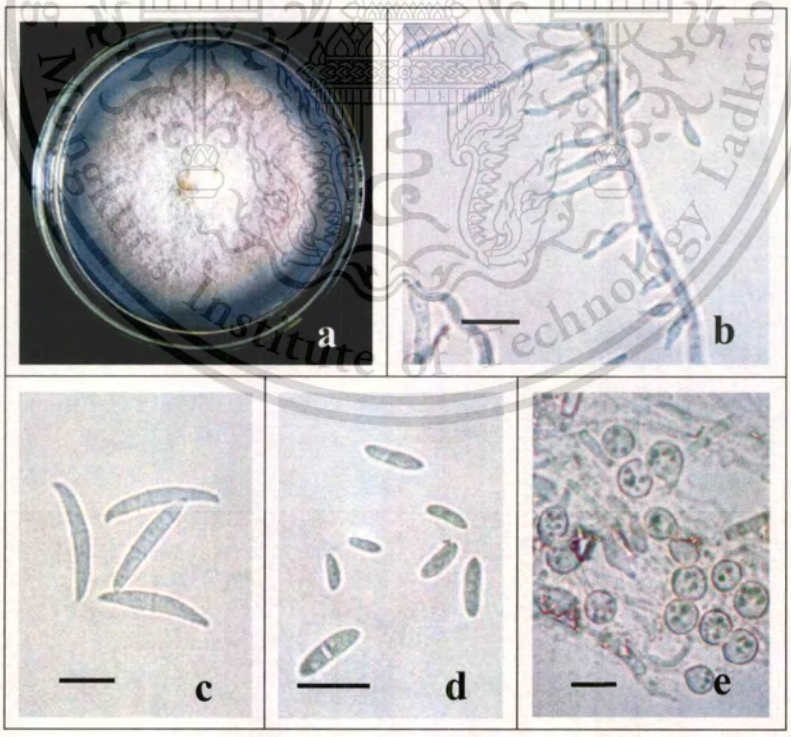


Figure 4.24 Morphological character of *Fusarium oxysporum* NKRC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

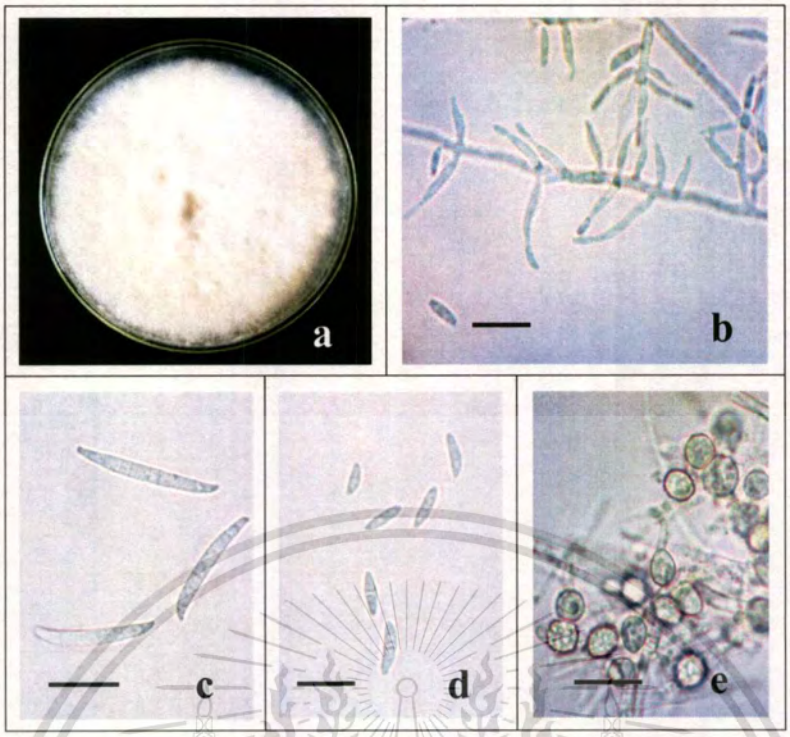


Figure 4.25 Morphological character of *Fusarium oxysporum* NKRC09.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

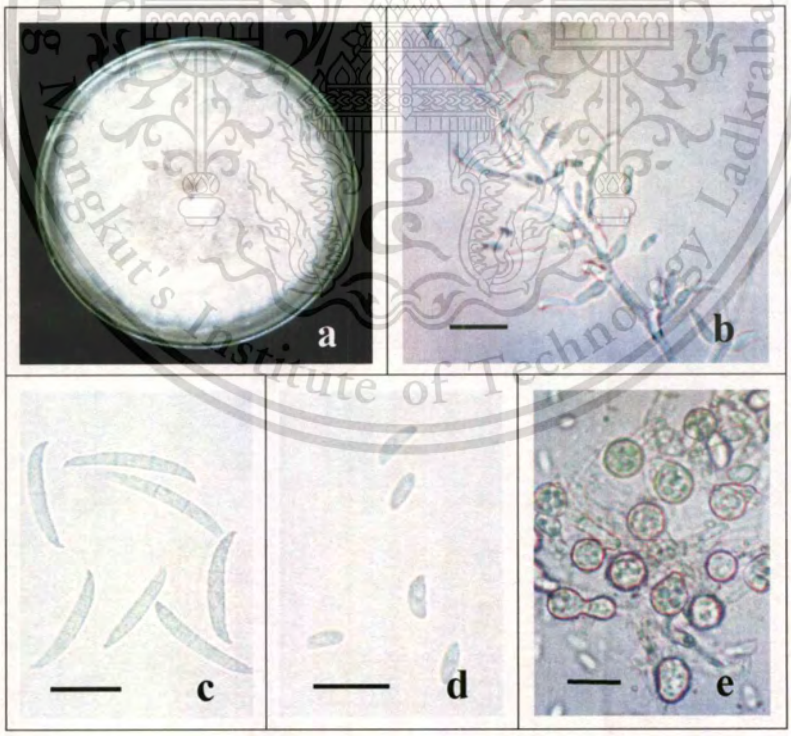


Figure 4.26 Morphological character of *Fusarium redolens* NKRC11.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

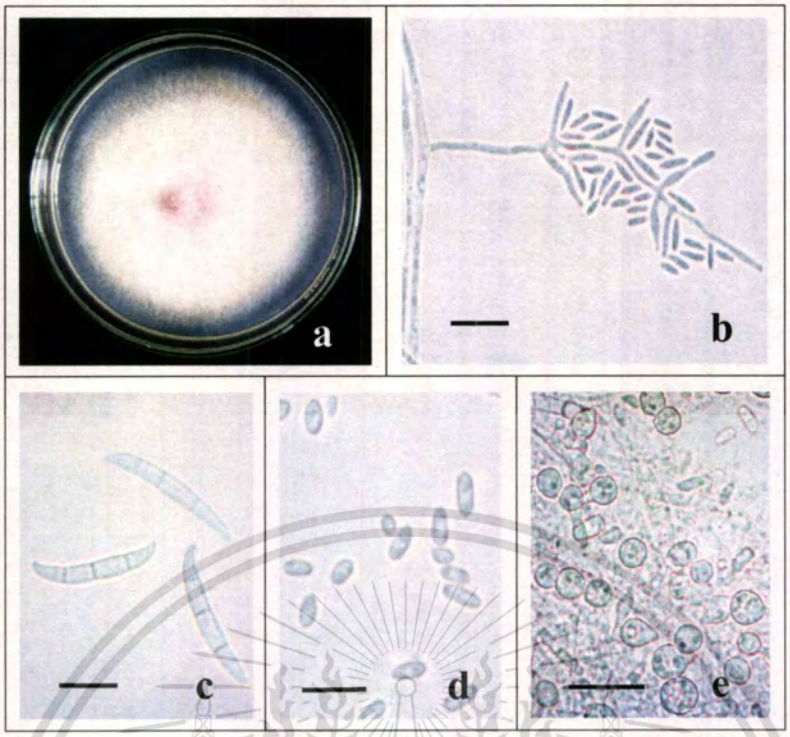


Figure 4.27 Morphological character of *Fusarium oxysporum* NKSC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

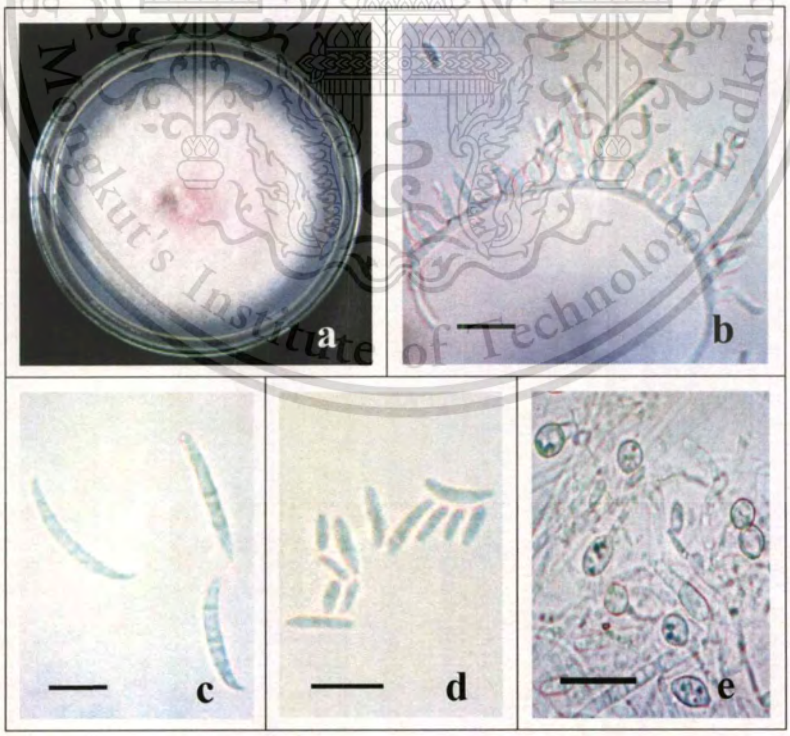


Figure 4.28 Morphological character of *Fusarium oxysporum* NKSC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

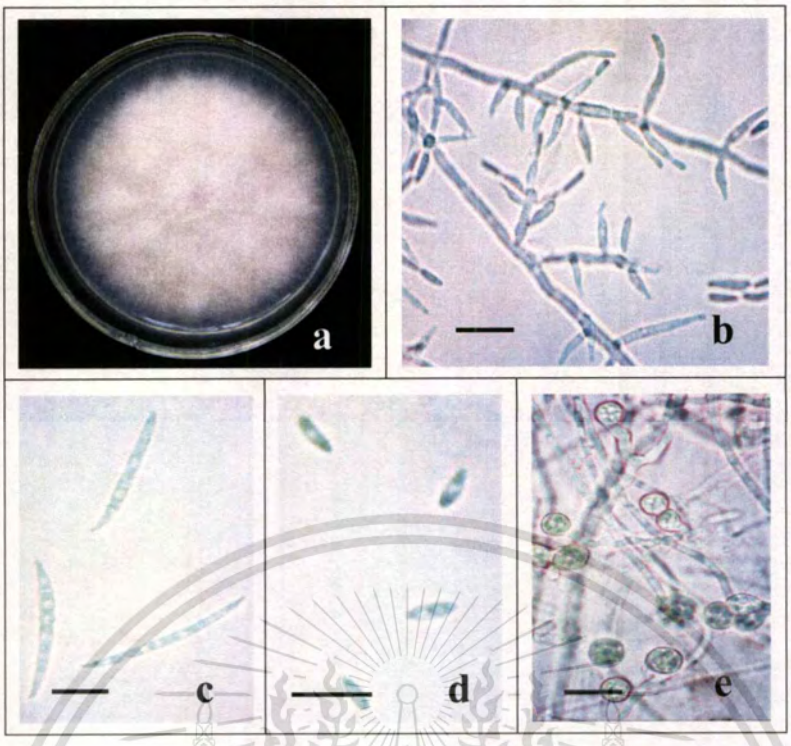


Figure 4.29 Morphological character of *Fusarium redolens* NKSoC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

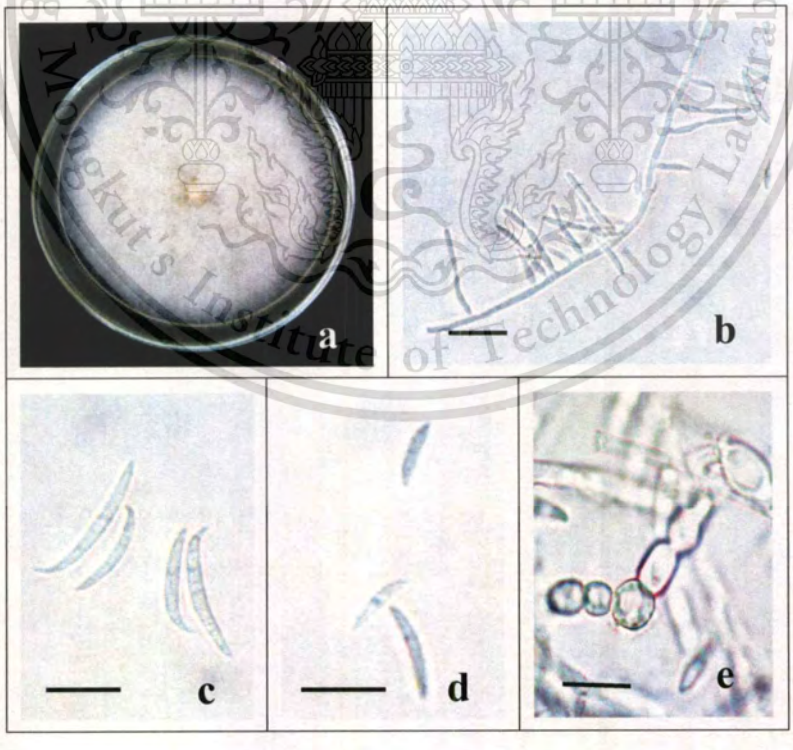


Figure 4.30 Morphological character of *Fusarium incarnatum* NRC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

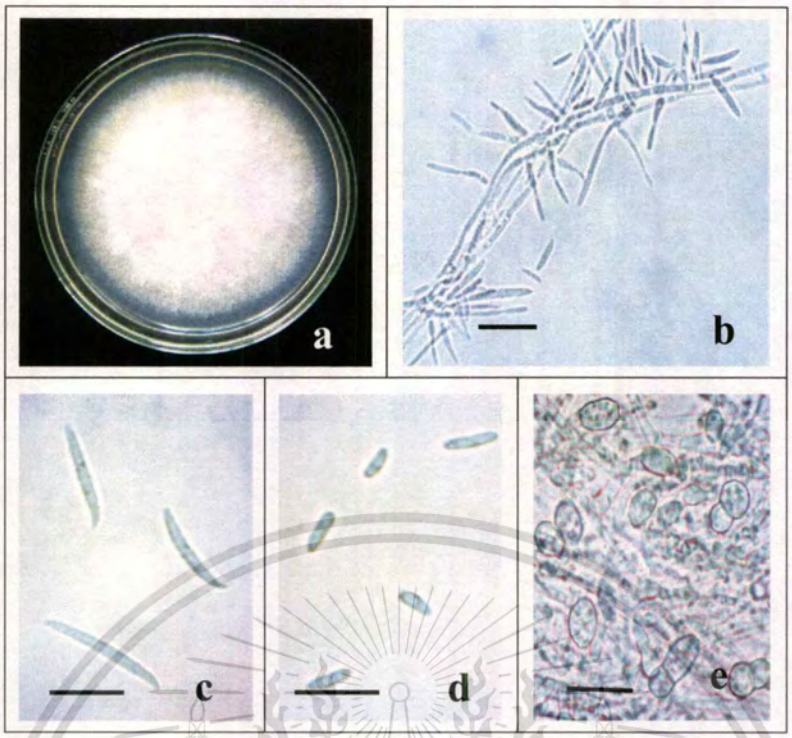


Figure 4.31 Morphological character of *Fusarium oxysporum* NSC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

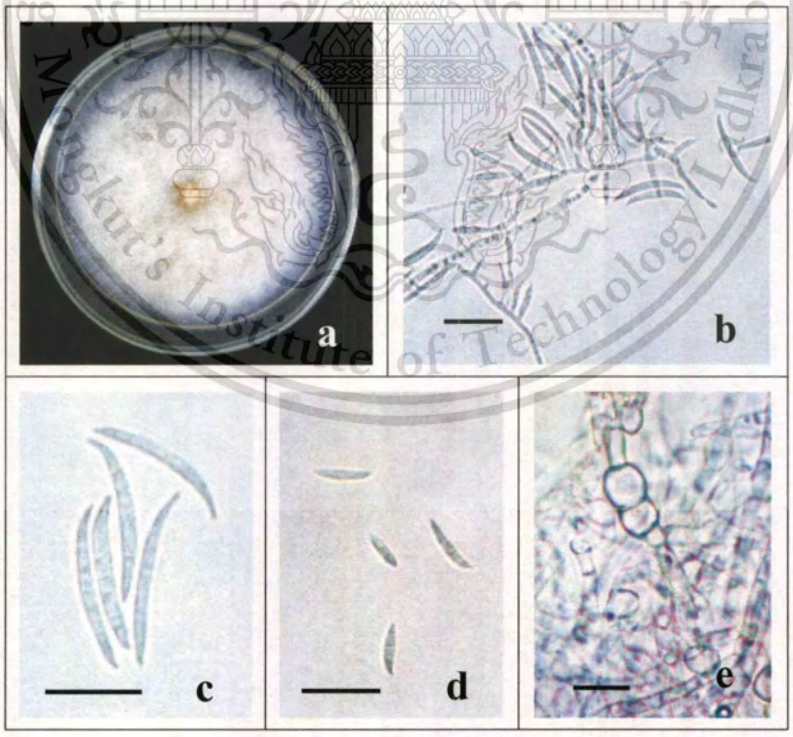


Figure 4.32 Morphological character of *Fusarium incarnatum* NSC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

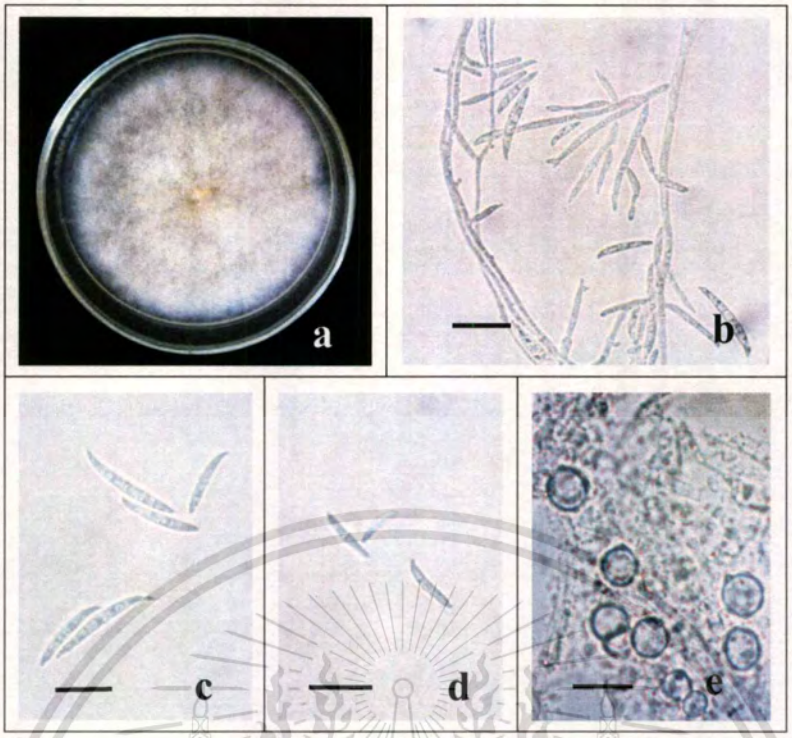


Figure 4.33 Morphological character of *Fusarium incarnatum* NSC07.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

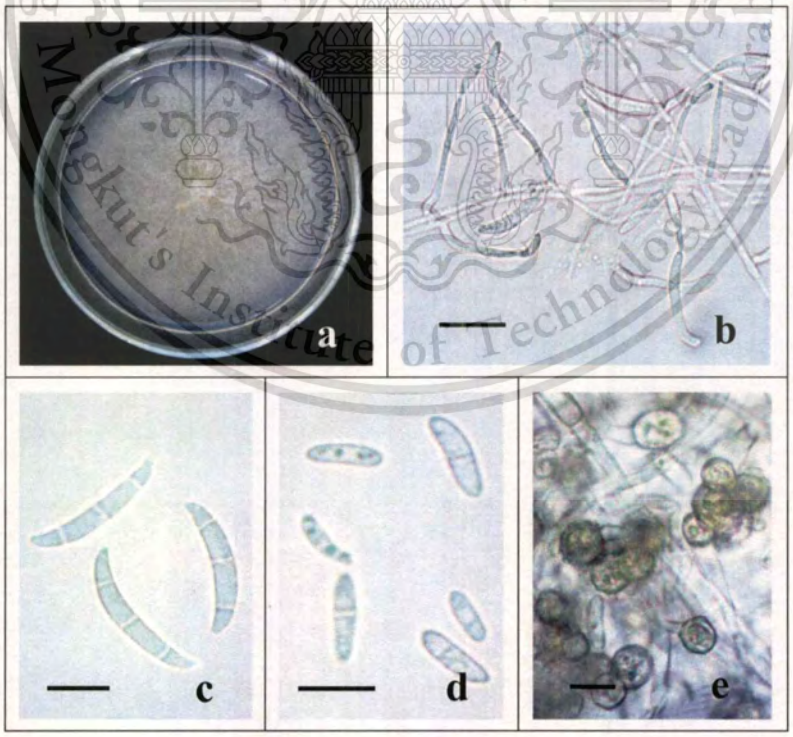


Figure 4.34 Morphological character of *Fusarium solani* NSC09.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

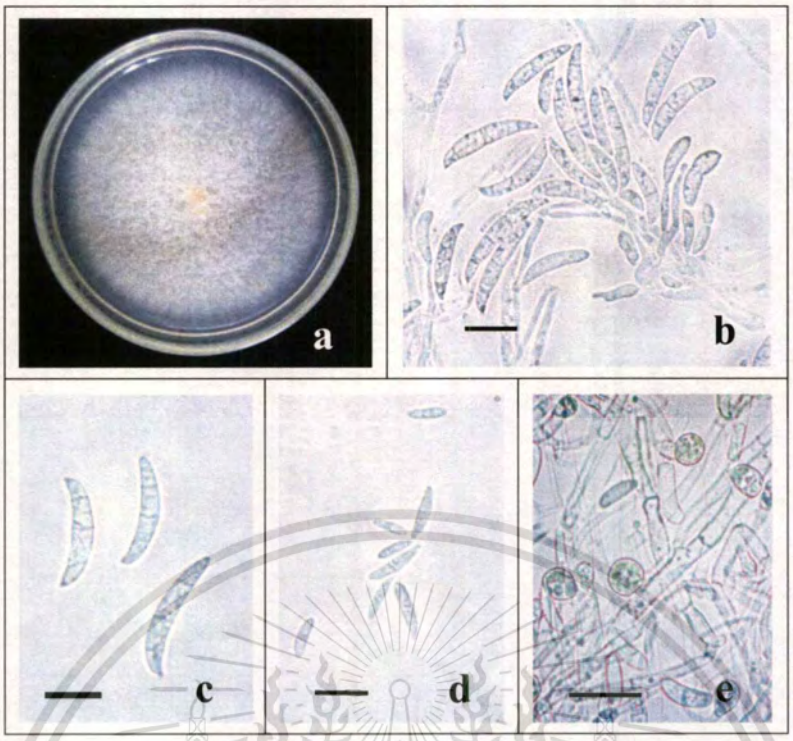


Figure 4.35 Morphological character of *Fusarium solani* NSoC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

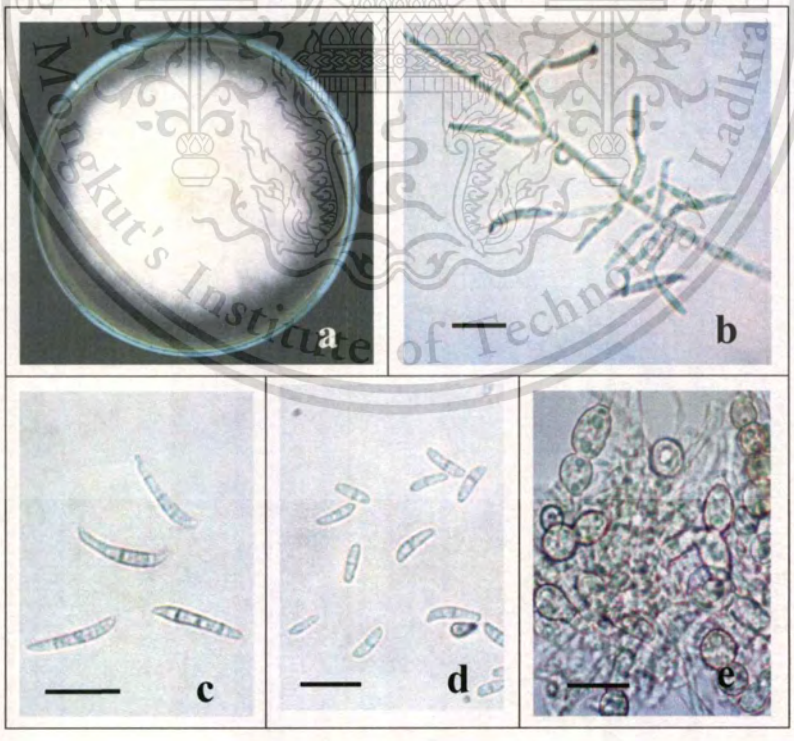


Figure 4.36 Morphological character of *Fusarium chlamydosporum* NSoC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

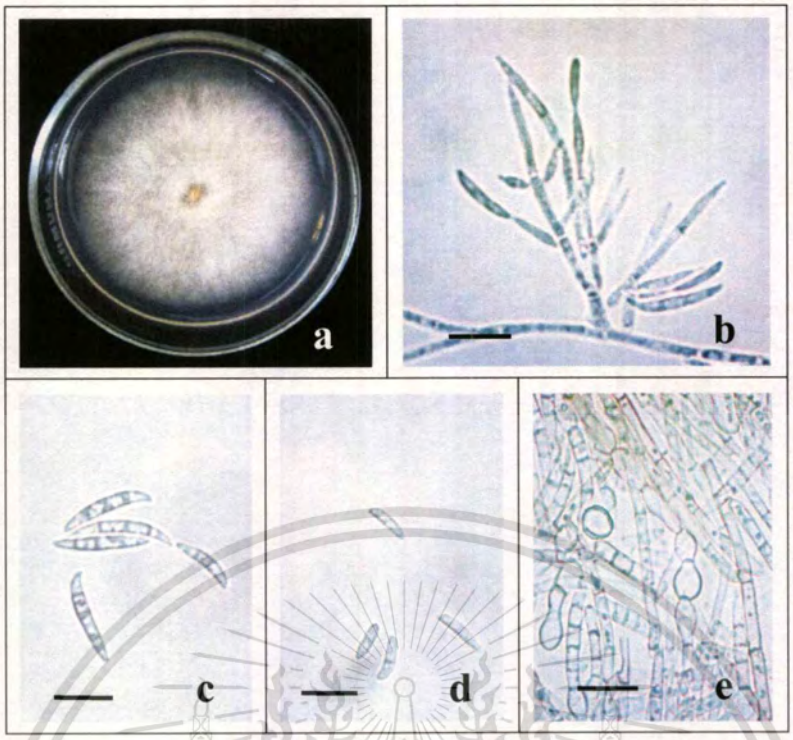


Figure 4.37 Morphological character of *Fusarium incarnatum* PSC01.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

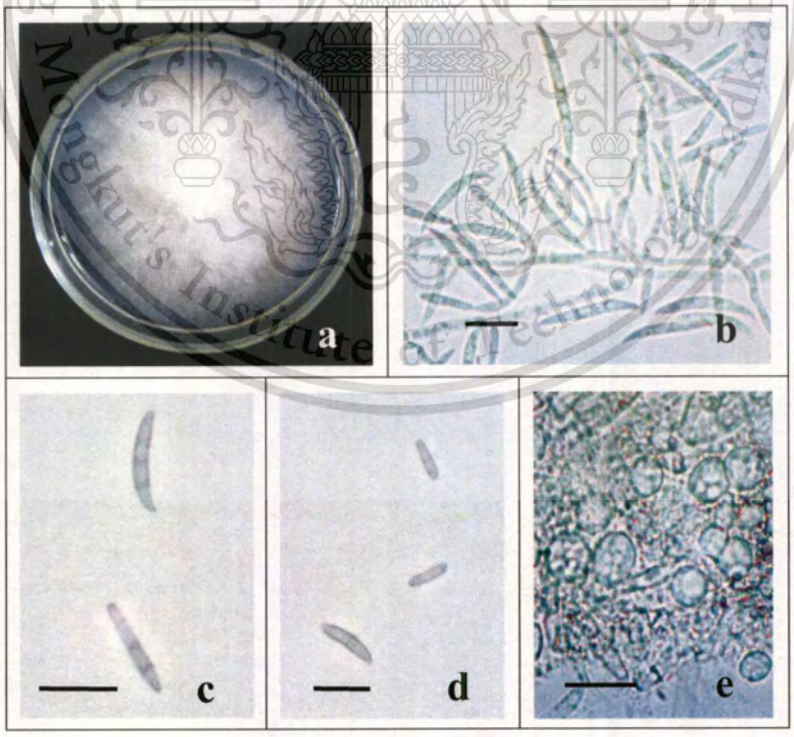


Figure 4.38 Morphological character of *Fusarium incarnatum* PSC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

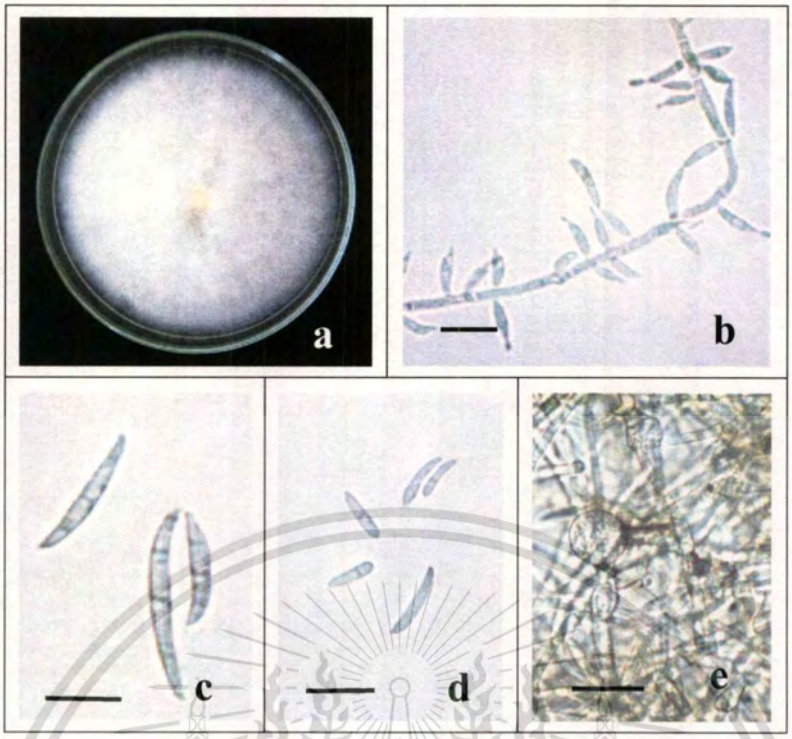


Figure 4.39 Morphological character of *Fusarium incarnatum* PSC03.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

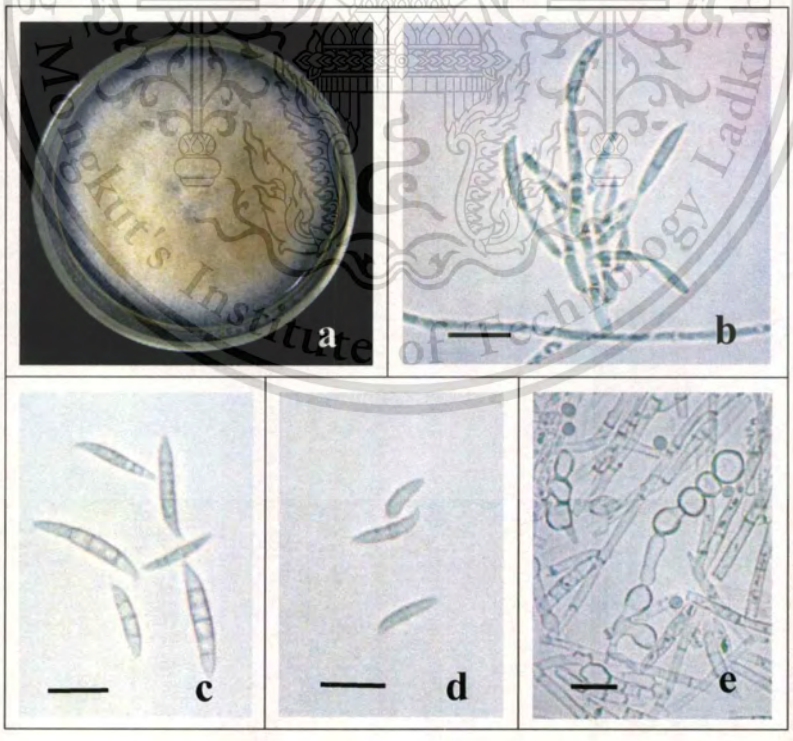


Figure 4.40 Morphological character of *Fusarium incarnatum* PSC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

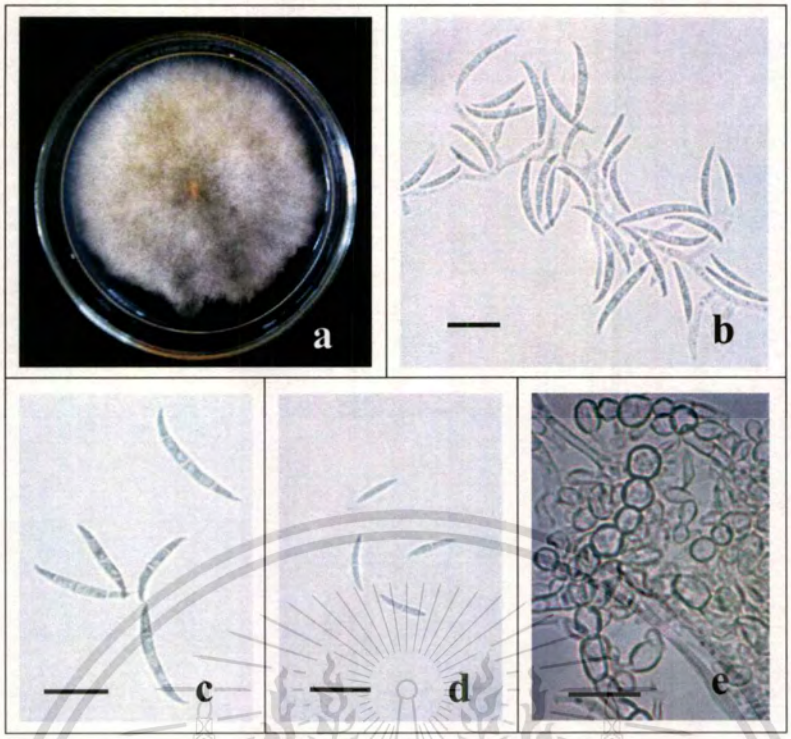


Figure 4.41 Morphological character of *Fusarium incarnatum* PSC05.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

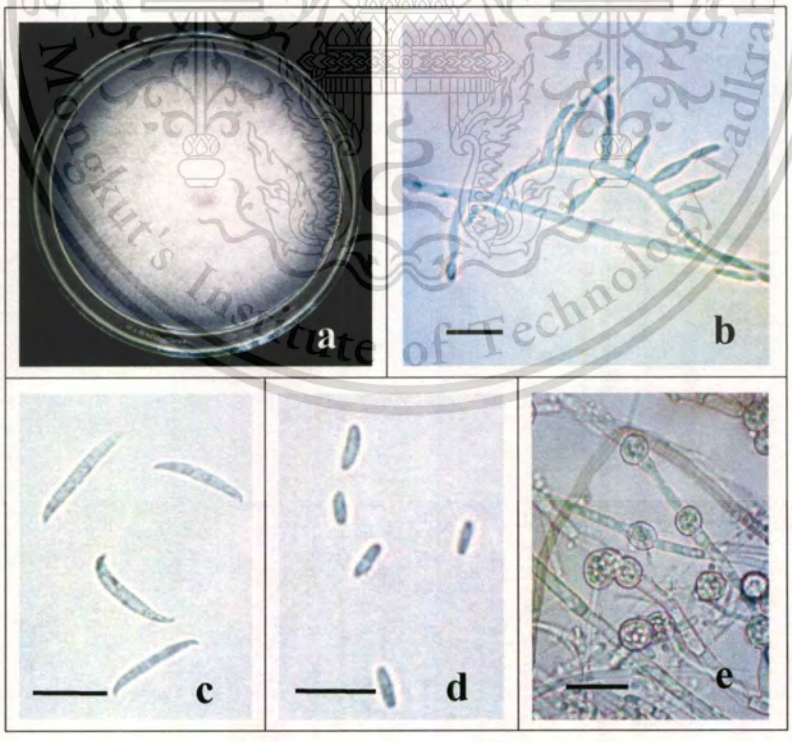


Figure 4.42 Morphological character of *Fusarium oxysporum* SRC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

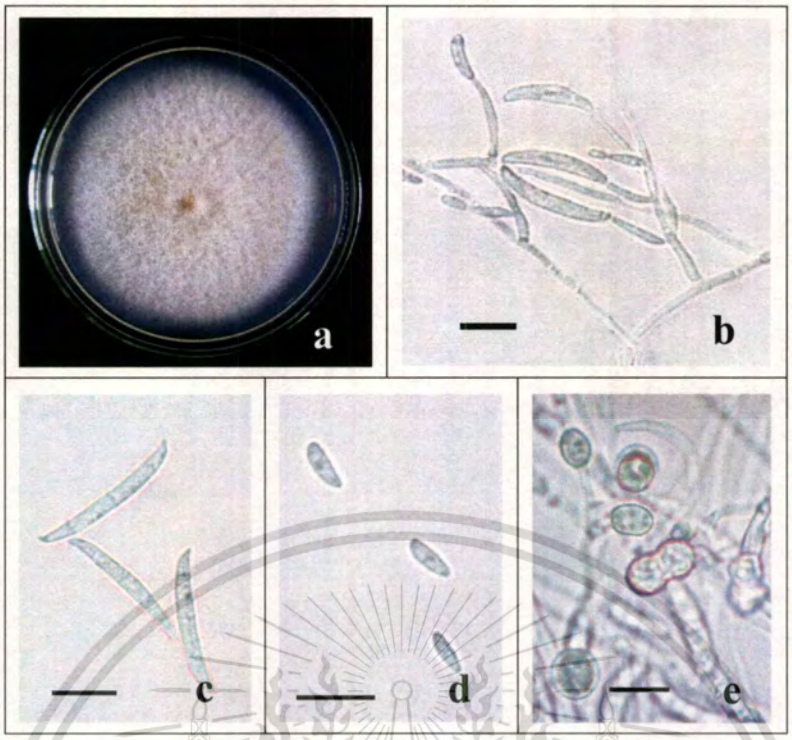


Figure 4.43 Morphological character of *Fusarium solani* SSoC02.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

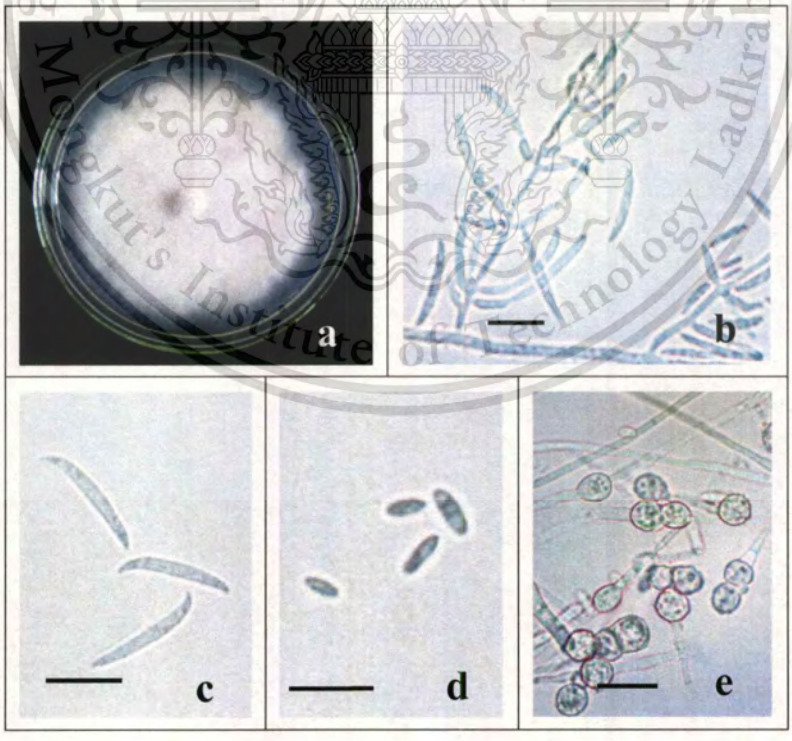


Figure 4.44 Morphological character of *Fusarium oxysporum* SSoC03.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

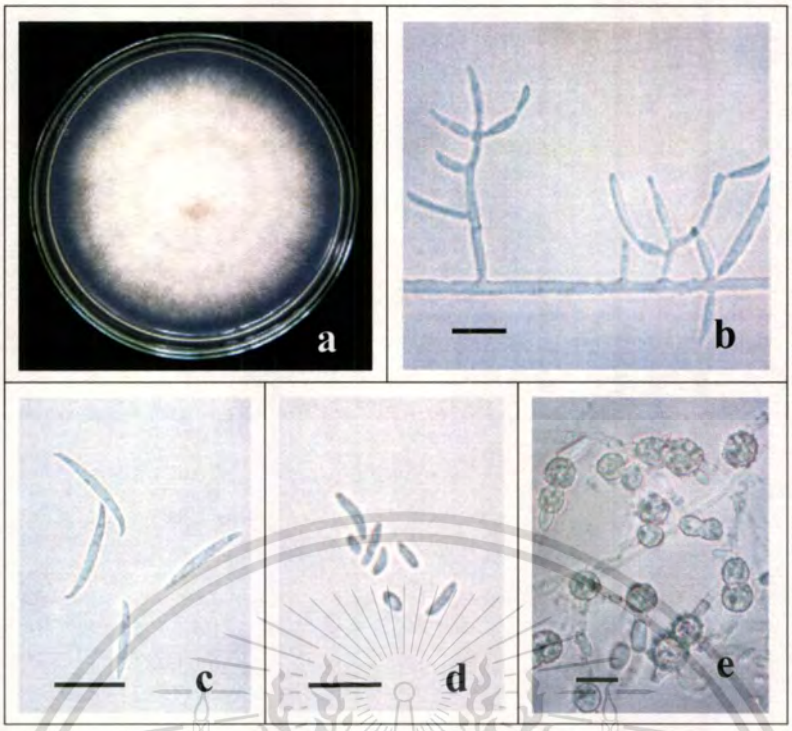


Figure 4.45 Morphological character of *Fusarium oxysporum* SSoC04.

a = colony on PSA, b = conidiophore, c = macroconidia, d = microconidia, e = chlamydospores, scale bar = 10 µm.

4.1.2 Pathogenicity tests

Disease severity index (DSI) was scored at 21 days after inoculation based on the modified disease severity scale of Silva and Bettiol (2005) as follows:- level 1= no symptom; level 2= plant showed yellowing leaves and wilting 1-20%, level 3= plant showed yellowing leaves and wilting 21-40%, level 4= plant showed yellowing leaves and wilting 41-60%, level 5= plant showed yellowing leaves and wilting 61-80%, and level 6= plant showed yellowing leaves and wilting or die 81-100%. Data in Table 4.3 and Figure 4.47 resulted in disease severity index and virulent group of pathogenicity test on tomato seedlings var. Sida. The results revealed that tomato seedlings did not show any wilt symptom after they were inoculated with isolates of *F. incarnatum*, *F. chlamydosporum*, *F. solani*, *F. redolens* and *F. sporotrichiodes*. While twelve isolates were identified as *F. oxysporum* showed to be pathogenic isolates on tomato seedling with three virulent groups. The isolates NKSC01, NKSC02, and KK2 presented to be high virulence isolates which DSI was 6.00, 5.88, and 5.75, respectively. While isolates SRC02, NKRC04, NSC01, and NKRC02 presented moderate virulence which DSI was 4.38, 4.38, 4.31, and 4.13, respectively. The isolates KSoC02, NKRC09, BRC03, SSoC03, and SSoC04 showed significantly DSI less than the isolates mentioned above which DSI was 3.38, 3.38, 3.19, 3.13, and 3.06, respectively and were grouped in low virulence.

Table 4.3 Disease severity index of tomato wilt at 21 days after inoculation.

Provinces	Isolates	DSI [1]	Virulent group[3]	Provinces	Isolates	DSI	Virulent group
Bangkok	BKFC01	1.00d [2]	NP	Khon Kaen	KK2	5.75a	H
	BKFC03	1.00d	NP		KSoC01	1.00d	NP
	BKFC04	1.00d	NP		KSoC02	3.38c	L
	BKFC06	1.00d	NP		KSoC03	1.00d	NP
	BKFC07	1.00d	NP		KSoC04	1.00d	NP
	BKFC12	1.00d	NP	Nong Khai	NKSC01	6.00a	H
	BKRC02	1.00d	NP		NKSC02	5.88a	H
	BKSC02	1.00d	NP		NKRC02	4.13b	M
Pathumthani	PSC01	1.00d	NP		NKRC04	4.38b	M
	PSC02	1.00d	NP		NKRC09	3.38c	L
	PSC03	1.00d	NP		NKRC11	1.00d	NP
	PSC04	1.00d	NP		NKSoC01	1.00d	NP
	PSC05	1.00d	NP	Sakon Nakhon	SRC02	4.38b	M
Nakhon	NRC04	1.00d	NP		SSoC02	1.00d	NP
Ratchasima	NSC01	4.31b	M		SSoC03	3.13c	L
	NSC02	1.00d	NP		SSoC04	3.06c	L
	NSC07	1.00d	NP	Mukdahan	MRC02	1.00d	NP
	NSC09	1.00d	NP		MSC02	1.00d	NP
	NSoC01	1.00d	NP		MSC04	1.00d	NP
	NSoC04	1.00d	NP		MSoC01	1.00d	NP
Burirum	BRC01	1.00d	NP		MSoC02	1.00d	NP
	BRC02	1.00d	NP		MSoC03	1.00d	NP
	BRC03	3.19c	L		Control	1.00d	

[1]Disease severity index (DSI) was scored at 21 days after inoculation. 1= no symptom; 2= plant showed yellowing leaves and wilting 1-20%, 3= plant showed yellowing leaves and wilting 21-40%, 4= plant showed yellowing leaves and wilting 41-60%, 5= plant showed yellowing leaves and wilting 61-80%, and 6= plant showed yellowing leaves and wilting or die 81-100%.

[2]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.

[3]Virulent group of the isolates was determined according to DSI; NP = Non-pathogenic, L = low, M = moderate, H = high.

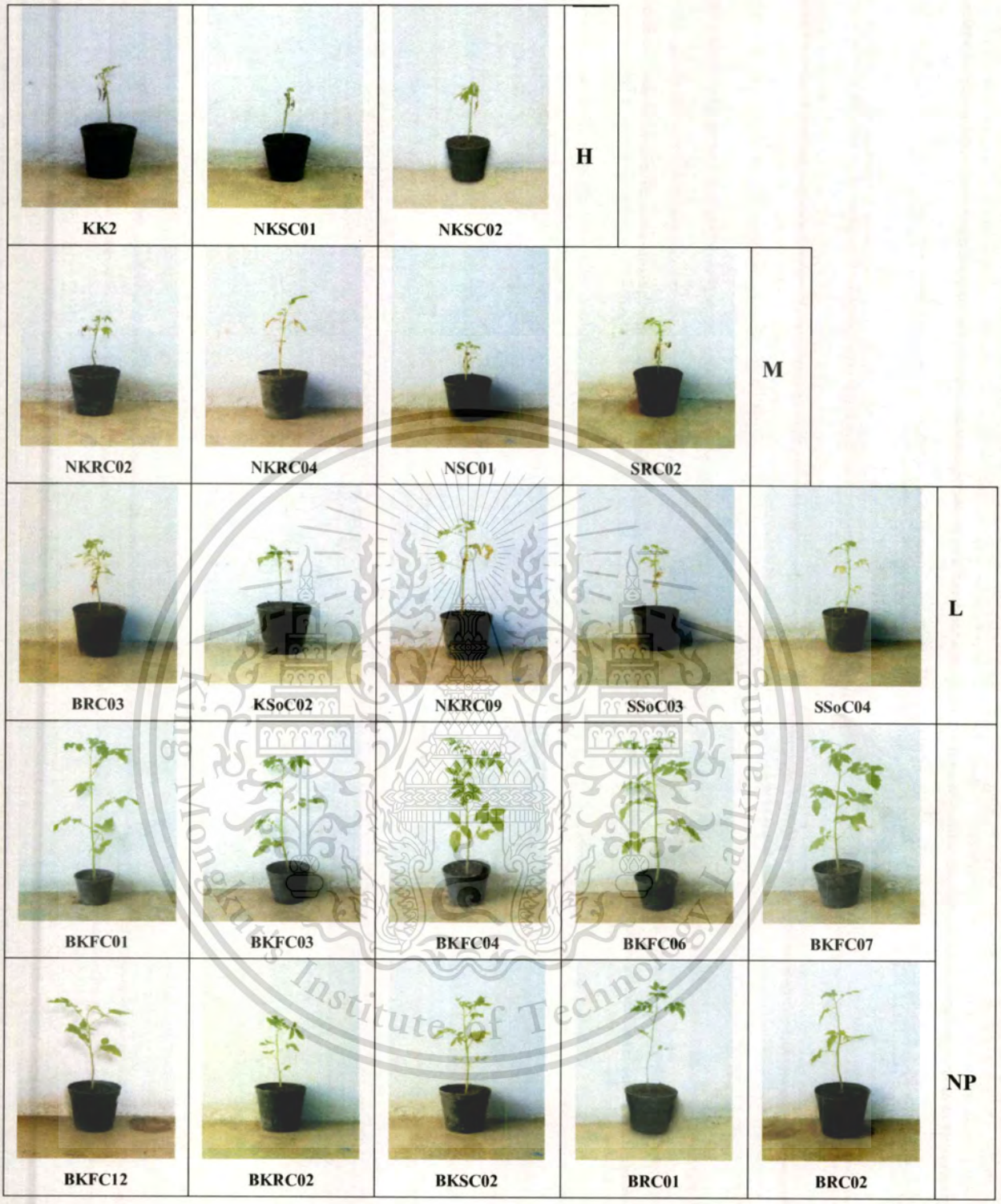


Figure 4.46 Disease severity index of tomato seedlings var. Sida after inoculated with *Fusarium* spp. 21 days.

H = high virulent, M = moderate virulent, L = low virulent, NP = non- pathogenic.

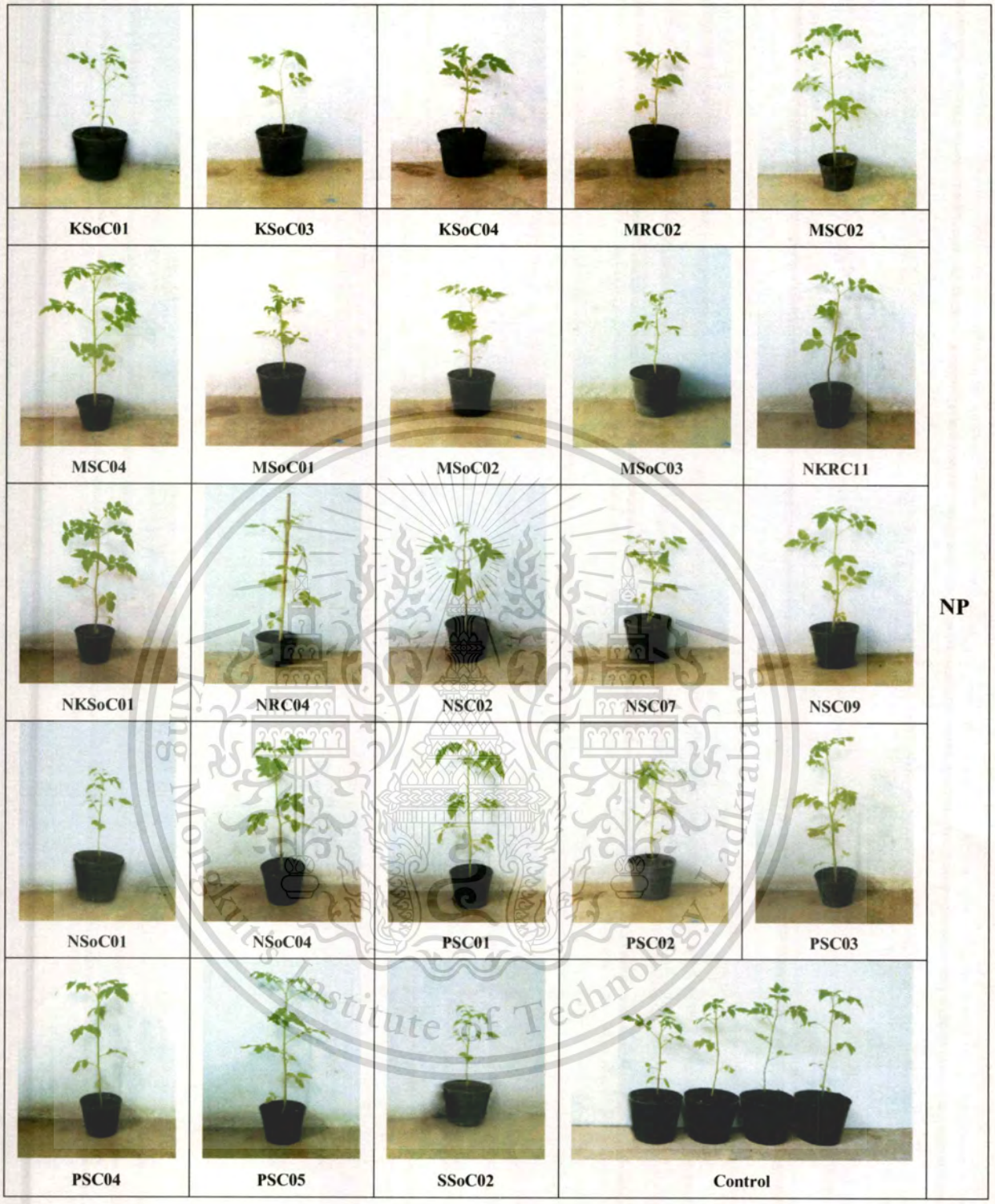


Figure 4.46 (continued).

4. 2 Study on molecular phylogeny

Amplification of the ITS1 and ITS2 regions from all isolates of *Fusarium* spp. by using primer PN3 and PN16 resulted in approximately 900 bp products.

4.2.1 Phylogenetic analysis

The phylogenetic tree from PAUP analysis presented cluster of the *Fusarium* spp. into five major groups (Fig. 4.47). Twelve isolates belonged to *F. oxysporum* which were NKSC01, NKSC02, KK2, NKRC02, NKRC04, SRC02, NSC01, BRC03, KSoC02, SSoC03, SSoC04, and NKRC09. These *F. oxysporum* isolates were divided into three subgroups and classified as *F. oxysporum* f.sp. *lycopersici*. Group of *F. solani* was separated into two subgroups. Isolates BKFC03, BKRC02, BKSC02, KSoC01, KSoC04, MRC02, MSC04, MSoC02, MSoC03, NSC09, NSoC01 were in the first subgroup and BRC01, BRC02, MSC02, SSoC02 were in the second group. The isolates NKRC11 and NKSoC01 were identified as *F. redolens*. The isolates BKFC01, BKFC04, BKFC06, BKFC12, KSoC03, MSoC01, NRC04, NSC02, NSC07, PSC01, PSC02, PSC03, PSC04, and PSC05 were grouped in *F. incarnatum*. BKFC07 and NSoC04 were classified as *F. chlamydosporum*.

4.2.2 Relationship among isolates of *Fusarium* species

Phylogenetic analysis was studied for the relationship with collection sites, morphological characters, and pathogenicity test. The dendrogram revealed no relationship between groups of *Fusarium* spp. and collection sites. In these forty five isolates, only twelve isolates from five provinces, Nong Khai, Khon Kaen, Sakon Nakhon, Nakhon Ratchasima, and Burirum, presented to be pathogenic isolates to tomato var. Sida. The results showed that morphological characters of *F. solani* were clearly different from other species. Colonies of isolates in group of *F. solani* had little and thin aerial mycelium. Macroconidia and microconidia were larger and conidiophores were longer and slender than the others. The isolates which in groups of *F. oxysporum* and *F. redolens* showed almost similar in colonies with purple tinge colour, short singly or branched conidiophores, fusiform macroconidia and elliptical or cylindrical microconidia. While the groups of *F. incarnatum*, *F. chlamydosporum*, and *F. sporotrichioides* presented closely relation to morphological characters.

The relationship among phylogenetic analysis and pathogenicity test were studied. Twelve isolates which in group of *F. oxysporum* were proved to be pathogenic strains causing wilt on tomato seedlings while another thirty-three isolates did not show any wilt symptoms on tomato seedlings. With this, the isolates which were divided in three subgroups of *F. oxysporum* exhibited different levels of disease incidence as mentioned in the results of pathogenicity test.

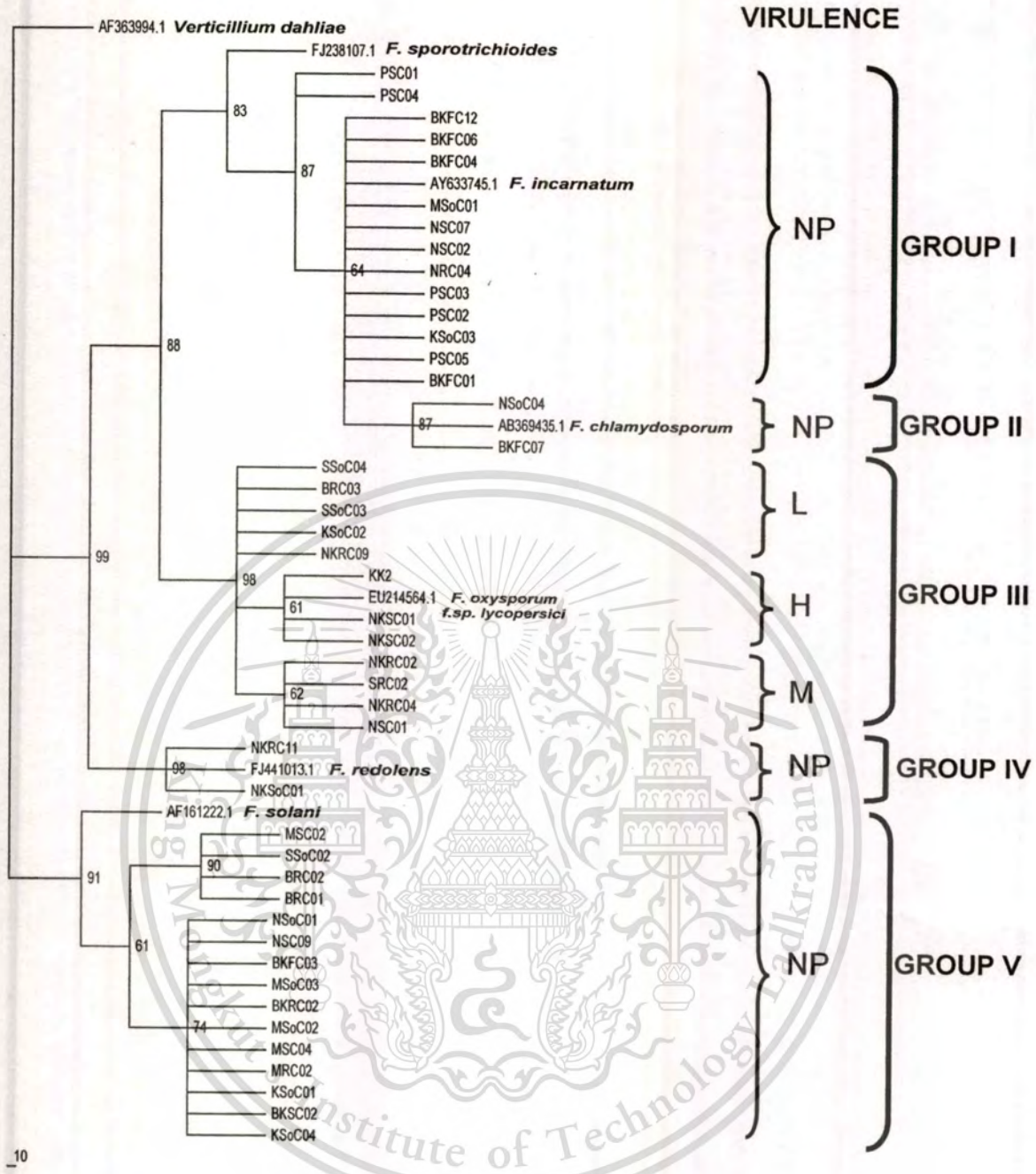


Figure 4.47 Phylogenetic relationships of *Fusarium* spp. inferred by the parsimony analysis of rDNA sequences. Phylogenetic tree was obtained from analysis by the parsimony method using the PAUP*4.0b program. *Verticillium dahliae* was used as an outgroup. The numbers above the lines represent the 1000 replicates parsimony bootstrap values. Virulence group; NP = non- pathogenic, L = low virulent, M = moderate virulent, H = high virulent.

4.3 Testing antagonistic fungi and their abilities to control Fusarium wilt pathogen

4.3.1 Antagonistic fungi

The antagonistic fungi; *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT and *Trichoderma harzianum* PC01 were provided by Assoc.Prof. Dr. Kasem Soytong; KMITL. Morphological characters of these antagonistic fungi were studied after cultured on PDA.

Chaetomium globosum KMITL-N0802: Colonies grew full 9.0 cm diameter Petri dish in 10 days at room temperature (28-32°C) with little aerial mycelium. Colonies were green brown or olivaceous or grey. Ascomata were dark brown, globose or subglobose with ascomatal size 245.0-322.5 x 212.0-328.0 µm. Lateral hairs were dark brown with paler tips 3.8 µm wide, terminal hairs were dark olive-brown with paler tips, loosely coiled, 3.2 µm wide. Asci shaped in clavate with 8 ascospores, stalked, 25.8-34.5 x 13.8-16.3 µm. Ascospores were dark olive brown, lemon shaped with bi-apiculate, thick walled, 6.5-8.5 x 5.5-6.8 µm with apical germ pore (as seen in Figure 4.48).

Chaetomium lucknowense CLT: Colonies grew full plate (9 cm diameter) in 10-12 days at room temperature (28-32°C), olive green to brown or grey when mature. Ascomata were grey green, superficial, spherical or ovate shaped, with size 178.4-205.2 µm. Ascomatal hairs numerous, flexous. Asci shaped in clavate with short stalks, contained 8 ascospores, 24.3-27.8 x 12.0-13.5 µm. Ascospores shaped in ovate, olivaceous brown, 6.3-8.3 x 5.3-6.8 µm, with a distinct germ pore (as seen in Figure 4.49).

Trichoderma harzianum PC01: Colonies reaching 9 cm diameter in 3 days at room temperature (28-32°C), dull to dark green, floccose or with compact conidiophore tufts. Conidia shaped in subglobose to short oval, smooth walled, 2.8-4.3 x 2.5-3.5 µm. Conidiophores had a complicated branching system. Phialides disposed in numbers of 3 (as seen in Figure 4.50).

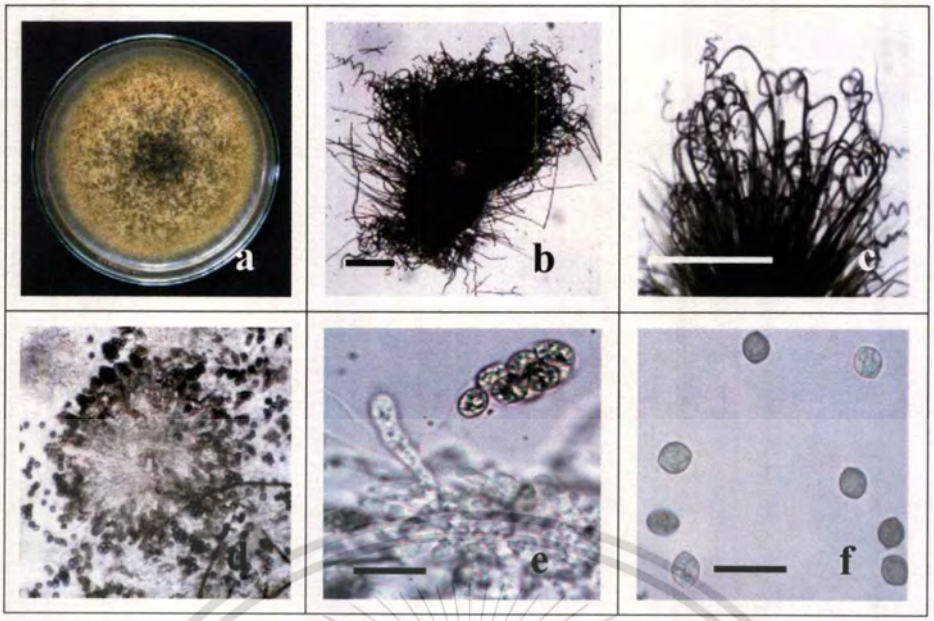


Figure 4.48 Morphological characters of *Chaetomium globosum* KMITL-N0802.

a = colony on PDA, b = ascomata, bar = 100 µm, c= ascomatal hairs; bar = 100 µm, d, e = asci, bar = 10 µm, f = ascospores; bar = 10 µm.

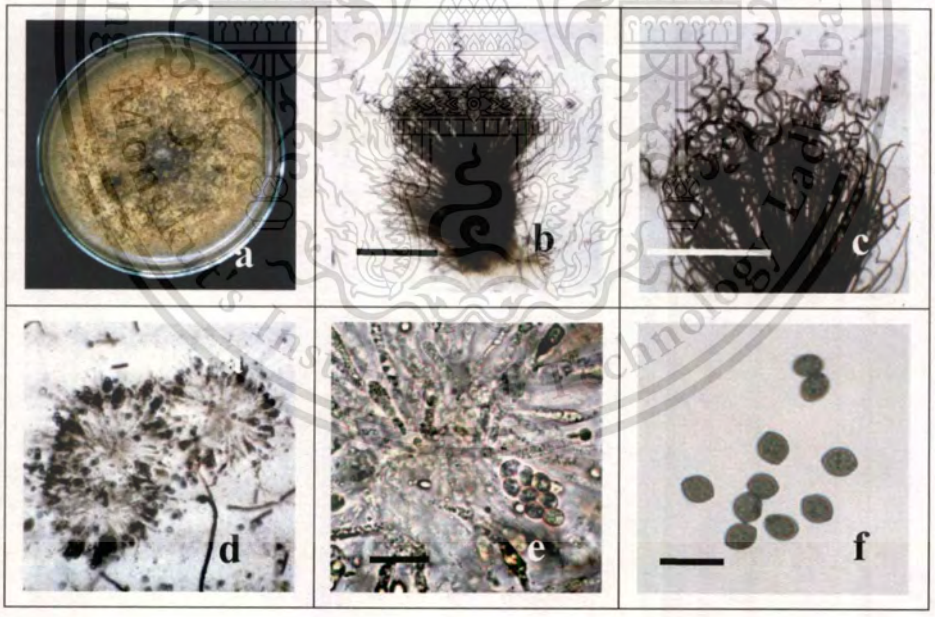


Figure 4.49 Morphological characters of *Chaetomium lucknowense* CLT.

a = colony on PDA, b = ascomata, ; bar = 100 µm, c= ascomatal hairs; bar = 100 µm, d, e = asci bar = 10 µm, f = ascospores; bar = 10 µm.

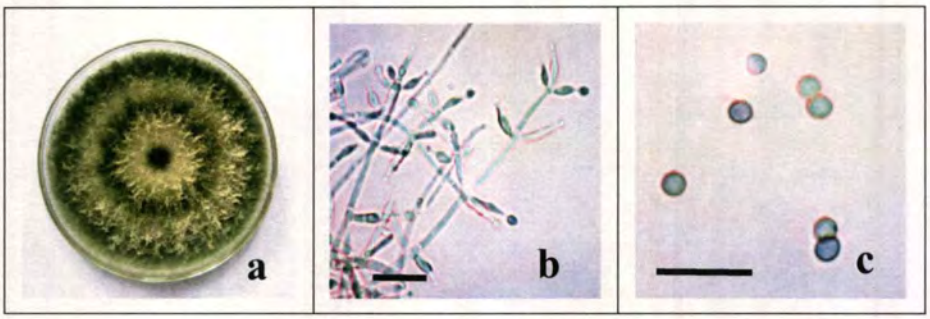


Figure 4.50 Morphological characters of *Trichoderma harzianum* PC01.

a = colony on PDA, b = conidiophore, c = conidia; bar = 10 µm.

4.3.2 Dual-culture test

The antagonistic fungi e.g. *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT and *Trichoderma harzianum* PC01 were tested for the abilities to inhibit the growth of *F.* oxysporum f.sp. lycopersici NKSC01. The results revealed that *T. harzianum* PC01 and *C. lucknowense* CLT gave higher significantly inhibited the mycelial growth of the pathogen than *C. globosum* N0802 which percentage of inhibition at 90.56%, 88.89% and 71.11%, respectively. But, all tested antagonists were not significantly inhibited the conidia production which were 99.99%, 92.54% and 92.14%, respectively (Table 4.4 and Figure 4.51).

Table 4.4 Inhibition of mycelium growth and sporulation of *Fusarium oxysporum* f.sp. *lycopersici* in dual culture

Antagonistic fungi	Mycelial inhibition [1/] (%)	Conidial inhibition (%)
Chaetomium globosum KMITL-N0802	71.11 [2/] b	92.14a
Chaetomium lucknowense CLT	88.89a	92.54a
Trichoderma harzianum PC01	90.56a	99.99a

[1/] Inhibition % = colony diameter or number of conidia of pathogen in control – colony diameter or number of conidia of pathogen in dual culture plate / colony diameter or number of conidia of pathogen in control x 100.

[2/] Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.

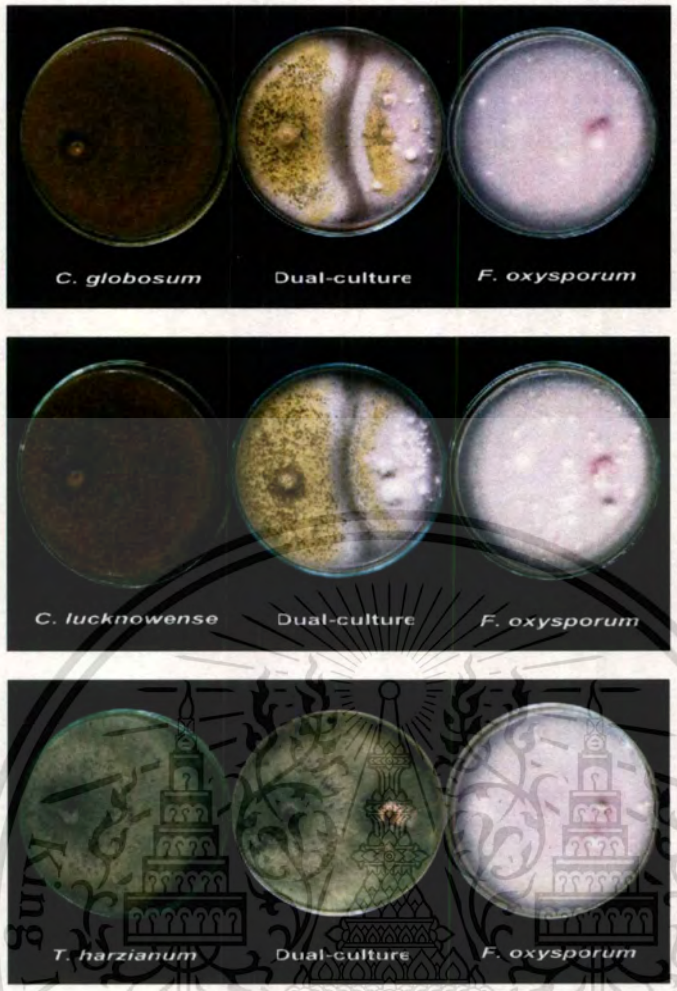


Figure 4.51 Dual-culture tests between antagonistic fungi and *F. oxysporum* f.sp. *lycopersici* at 30 days.

a = *Chaetomium globosum* KMITL-N0802, b = *Chaetomium lucknowense* CLT, c = *Trichoderma harzianum* PC01.

4.3.3 Testing crude extract to inhibit *F. oxysporum* f.sp. *lycopersici*

The antagonistic fungi; *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT, and *Trichoderma harzianum* PC01 were cultured in PDB (2.5 litre each). After 30 days, fungal macelial mats were removed from PDB and dried. Dried mycelial mats of *C. globosum* KMITL-N0802, *C. lucknowense* CLT and *T. harzianum* PC01 were weighted as 35, 30, and 25 g, respectively before extraction.

Crude extracts from *C. globosum* KMITL-N0802 which extracted with hexane was orange oil, crude ethyl acetate and crude methanol were brown solid. Weight of hexane crude, ethyl acetate crude and methanol crude were 0.46, 0.80, and 3.60 g, respectively. Crude extracts

from *C. lucknowense* CLT which extracted with hexane was yellow or orange oil, ethyl acetate crude and methanol crude were brown solid. Weight of hexane crude, ethyl acetate crude and methanol crude were 0.29, 0.76, and 1.64 g, respectively. Crude extracts from *T. harzianum* PC01 which extracted with hexane was white oil, ethyl acetate crude was yellow oil and methanol crude was yellow brown solid. Weight of hexane crude, ethyl acetate crude and methanol crude were 1.38, 0.68, and 2.12 g, respectively as seen in Figure 4.52 and Table 4.5.

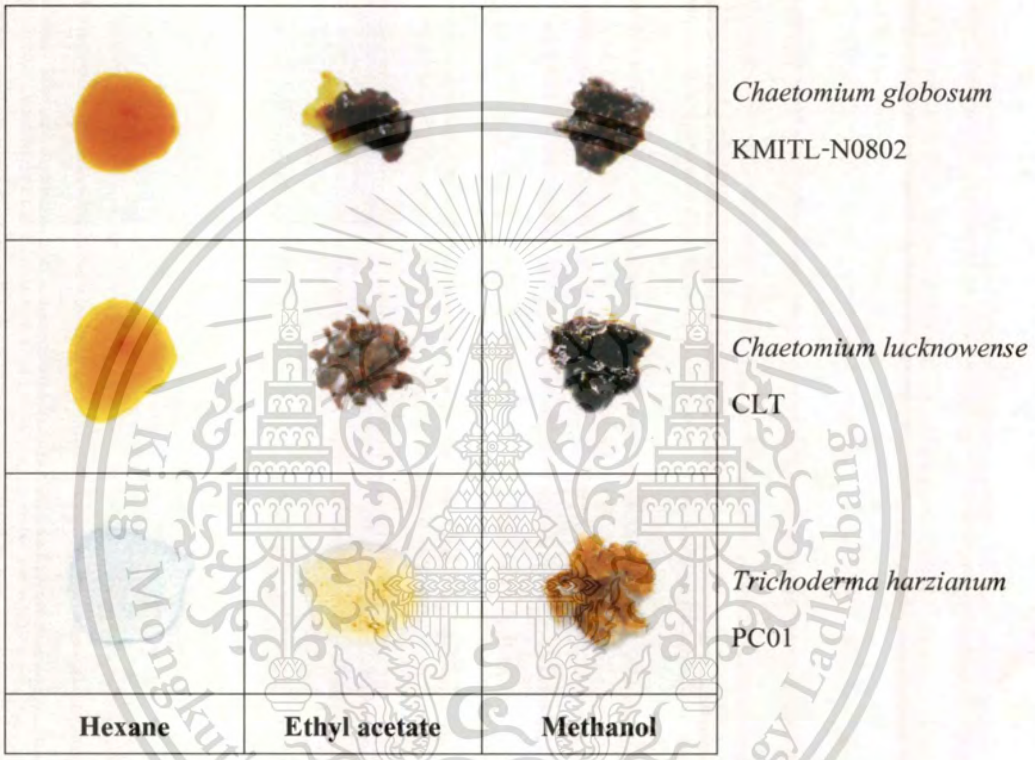


Figure 4.52 Characteristics of crude extracts from antagonistic fungi.

Table 4.5 Yields of mycelial mats and crude extracts of antagonistic fungi.

Antagonistic fungi	Dried weight (g)	Hexane (g)	% yield	EtOAC (g)	% yield	MeOH (g)	% yield
***C. globosum* KMITL-N0802**	35	0.46	1.31	0.80	2.29	3.60	10.29
***C. lucknowense* CLT**	30	0.29	0.97	0.76	2.53	1.64	5.47
***T. harzianum* PC01**	25	1.38	5.52	0.68	2.72	2.12	8.48

Crude extracts of antagonistic fungi at concentrations of 0, 10, 50, 100, 500, and 1,000 g/ml were tested for inhibition of *F. oxysporum* f. sp. *lycopersici* NKSC01 which obtained from previous experiment. The results showed in Table 4.6 and Figure 4.53. Crude extracts of tested antagonists could inhibit conidial production of the pathogen at the concentrations ranged from 10-1,000 µg/ml and showed the best properties to inhibit conidial production of *F. oxysporum* f.sp. *lycopersici* NKSC01 at 1,000 µg/ml. The highest conidial inhibition was presented when used crude hexane and crude ethyl acetate of *C. globosum* KMITL-N0802, crude hexane, crude ethyl acetate and crude methanol of *C. lucknowense* CLT, crude ethyl acetate and crude methanol of *T. harzianum* PC01 which were 63.13%, 62.58%, 74.85%, 69.55%, 72.62%, 96.93% and 97.50%, respectively. Crude hexane of *C. globosum* KMITL-N0802 showed the highest inhibition of conidial production of the pathogen which ED_{50} value was 157 µg/ml while crude ethyl acetate and crude methanol presented their abilities to inhibit conidial production at the ED_{50} values 339 and 302 µg/ml, respectively. Crude hexane of *C. lucknowense* CLT gave the highest inhibition which ED_{50} value was 188 µg/ml, followed by crude ethyl acetate and crude methanol which the ED_{50} values were 209 and 212 µg/ml, respectively. Crude extracts of *T. harzianum* PC01 which extracted from methanol showed the highest inhibition of conidial production which the ED_{50} value was 192 µg/ml while crude ethyl acetate and crude hexane were 232 and 861 µg/ml, respectively.

Table 4.6 Growth inhibition of crude extracts of *Chaetomium globosum* KMITL-N0802, *Chaetomium lucknowense* CLT and *Trichoderma harzianum* PC01 againsted *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Crude extracts	Concentrations (µg/ml)	Mycelial inhibition[1] (%)	Conidial inhibition (%)	ED_{50} (µg/ml)
Crude hexane of	10	0[2]f	32.63ef	157
C. globosum KMITL-	50	0f	43.83d	
N0802	100	2.0e	44.86d	
	500	6.0cd	56.49b	
	1000	6.5c	63.13a	
Crude ethyl acetate of	10	0f	27.05gh	339
C. globosum KMITL-	50	2.5e	31.13fg	
N0802	100	4.5d	35.02ef	
	500	9.0b	52.16bc	
	1000	16.0a	62.58a	
Crude methanol of	10	0f	24.71h	302
C. globosum KMITL-	50	0f	25.62h	
N0802	100	2.0e	37.11e	
	500	5.5cd	51.11c	
	1000	7.0c	56.57b	
CV (%)		27.30	7.98	
Crude hexane of	10	0d	32.37ef	188
C. lucknowense CLT	50	0d	40.83d	
	100	0d	40.83d	
	500	0d	49.17b	
	1000	0d	74.85a	
Crude ethyl acetate of	10	0d	20.31gh	209
C. lucknowense CLT	50	0d	26.09fg	
	100	1.5c	40.35ef	
	500	6b	51.77bc	
	1000	22.5a	69.55a	
Crude methanol of	10	0d	12.45h	212
C. lucknowense CLT	50	0d	28.99h	
	100	0d	46.85e	
	500	0d	57.92bc	
	1000	2.0c	72.62a	
CV (%)		41.79	10.44	

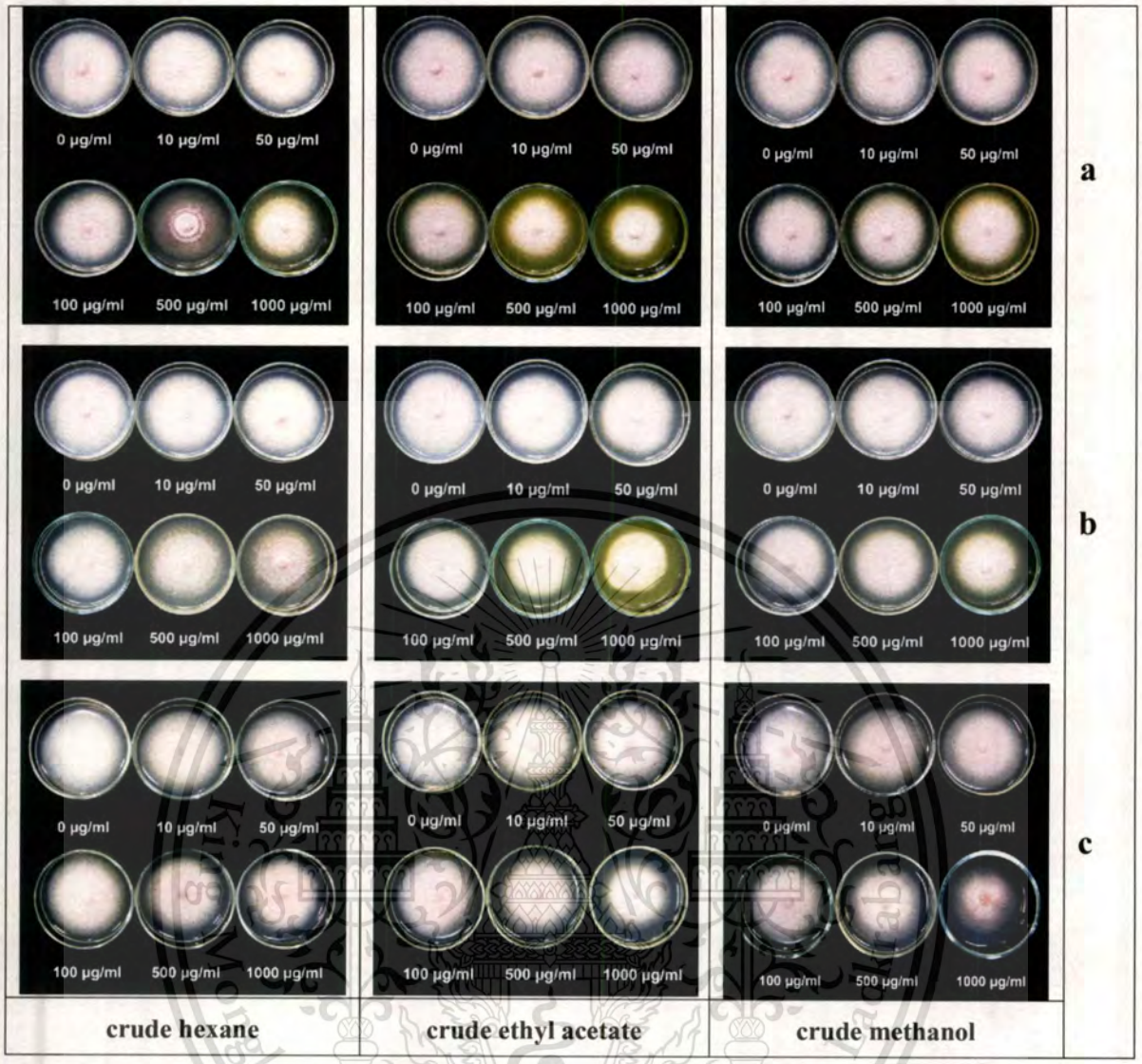
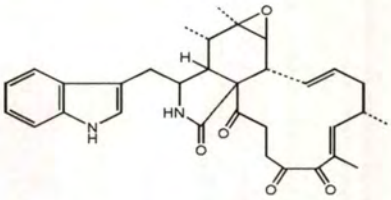


Figure 4.53 Testing crude extracts of antagonistic fungi against *F. oxysporum* f.sp. *lycopersici*. a = crude extracts from *Chaetomium globosum* KMITL-N0802, b = crude extracts from *Chaetomium lucknowense* CLT, c = crude extracts from *Trichoderma harzianum* PC01.

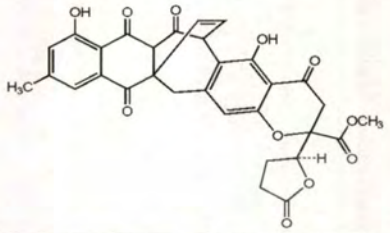
4.3.4 Testing on bioactive compounds to inhibit *F. oxysporum* f.sp. *lycopersici*

The bioactive compounds from antagonistic fungi; chaetoglobosin C from *C. globosum* KMITL-N0802 was white or cream solid, chaetomanone A from *C. lucknowense* CLT was yellow crystal and white solid trichotoxin A50 from *T. harzianum* PC01 were used to test for their abilities to control *F. oxysporum* f.sp. *lycopersici* NKSC01. Physical characters of bioactive compounds were shown in Figure 4.54. The bioactive compounds, chaetoglobosin C, chaetomanone A and trichotoxin A50 as concentration of 0, 10, 25, 50, and 100 µg/ml were tested

for their efficacies *in vitro* and the results were shown in Table 4.7 and Figure 4.55. All tested bioactive compounds exhibited strongly inhibition on mycelial growth and conidial production of pathogen at concentration 100 µg/ml over 80%. Chaetoglobosin C, chaetomanone A and trichotoxin A50 inhibited the mycelial growth of pathogen with ED_{50} values of 71, 68 and 71 µg/ml, respectively. Meanwhile, the sporulation of pathogen was inhibited by chaetoglobosin C, chaetomanone A and trichotoxin A50 with ED_{50} values of 17, 22 and 18 µg/ml, respectively. Moreover, bioactive compounds, chaetoglobosin C, chaetomanone A and trichotoxin A50 affected the bioactivity to the pathogen cells of *F. oxysporum* f sp *lycopersici.* It was observed that macroconidia of the tested pathogen showed abnormal cells which cell wall of pathogens collapsed and cytoplasm was clogged inside the cells and somewhat released from the pathogen cells (Fig.4.56).



Chaetoglobosin C



Chaetomanone A

Sequence

M	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18	Known name
1675A	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Ala-Ala-Ala-Aib-Pro-Leu-Aib-Aib-Gln-Vol	(T5D2)
1689A	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Aib-Gln-Vol	(T5E)
1689B	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Gly-Ala-Aib-Aib-Pro-Leu-Aib-Aib-Gln-Vol	(New)
1689C	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Ala-Ala-Ala-Aib-Pro-Leu-Aib-Iva-Gln-Vol	(T5F)
1689D	Ac-Aib-Gly-Aib-Leu-Ala-Gln-Aib-Aib-Ala-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Gln-Vol	New
1690E	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Aib-Glu-Vol	Known
1690F	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Gly-Ala-Aib-Aib-Pro-Leu-Aib-Aib-Glu-Vol	New
1703A	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Iva-Gln-Vol	(T5G)
1703B	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Gly-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Gln-Vol	New
1703C	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Aib-Gln-Vol	New
1703D	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Aib-Gln-Vol	New
1704E	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Iva-Glu-Vol	New
1704F	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Gly-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Glu-Vol	New
1704G	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Aib-Glu-Vol	Known
1704H	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Aib-Glu-Vol	New
1717A	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Gln-Vol	(T5I)
1717B	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Iva-Gln-Vol	(T5H)
1717C	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Iva-Gln-Vol	New
1718E	Ac-Aib-Gly-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Glu-Vol	Known
1718F	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Ala-Aib-Pro-Leu-Aib-Iva-Glu-Vol	New
1718G	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Aib-Glu-Vol	Known
1731A	Ac-Aib-Ala-Aib-Leu-Aib-Gln-Aib-Aib-Aib-Ala-Aib-Aib-Pro-Leu-Aib-Iva-Gln-Vol	(T5J)

Trichotoxin A50

Physical characters	**Chemical structures**

Figure 4.54 Physical characters and chemical structures of chaetoglobosin C, chaetomanone A and trichotoxin A50.

Table 4.7 Growth inhibition of chaetoglobosin C, chaetomanone A and trichotoxin A50 against *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Bioactive compounds	Concentrations (µg/ml)	Mycelial inhibition[1/] (%)	ED_{50} (µg/ml)	Conidial inhibition (%)	ED_{50} (µg/ml)
Chaetoglobosin C	10	1.50[2/]gh	71	36.27e	17
	25	5.00ef		57.40c	
	50	15.50d		73.06b	
	100	79.00b		95.76a	
Chaetomanone A	10	1.00gh	68	25.13f	22
	25	4.00f		51.34cd	
	50	24.00c		72.07b	
	100	83.00a		95.89a	
Trichotoxin A50	10	3.00fg	71	40.54e	18
	25	6.50e		49.73d	
	50	22.50c		68.55b	
	100	80.50b		98.45a	
CV (%)		6.1		7.38	

[1/]Inhibition % = colony diameter or number of propagules of pathogen in control – colony diameter or number of propagules of pathogen in dual culture plate / colony diameter or number of propagules of pathogen in control x 100.

[2/]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

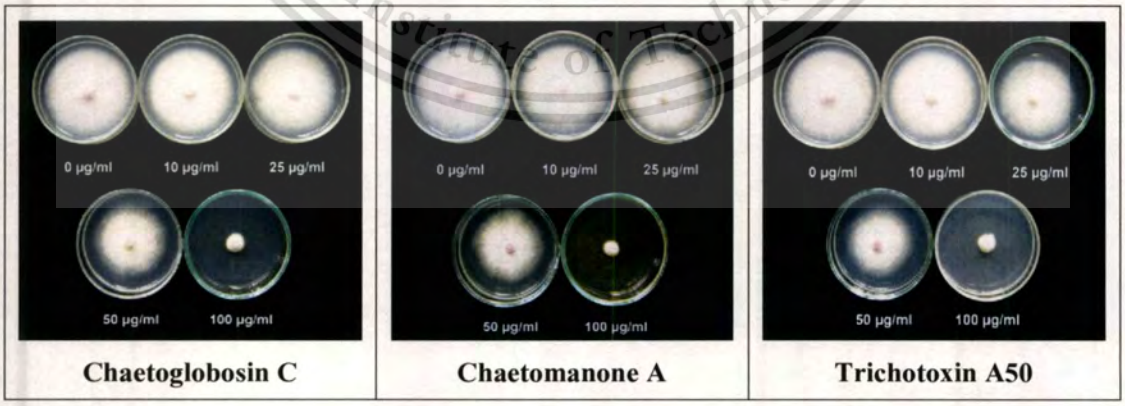


Figure 4.55 Testing bioactive compounds against *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

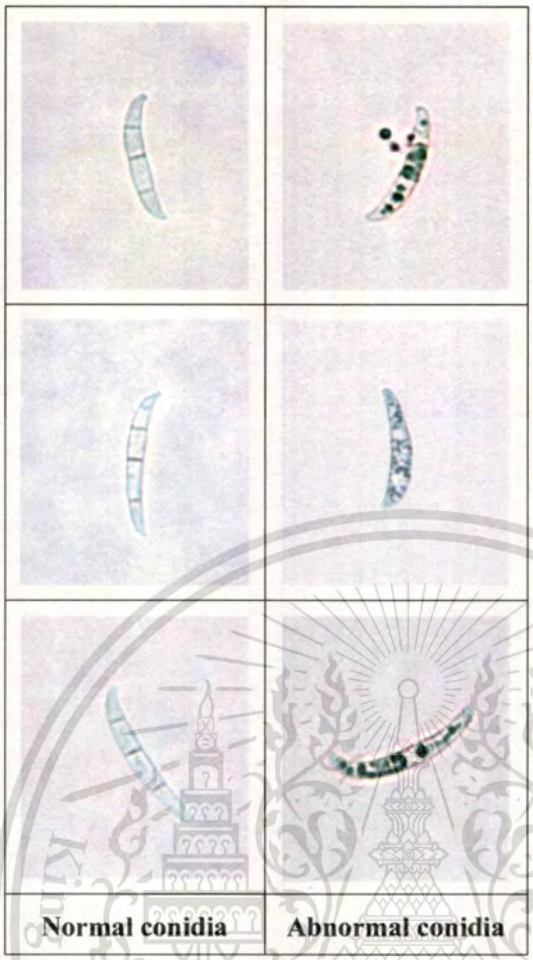


Figure 4.56 Abnormal conidia of *Fusarium oxysporum* f.sp. *lycopersici* NKSC01 affected by chaetoglobosin C, chaetomanone A and trichotoxin A50.

4.4 Microbial elicitors inducing plant resistance

The phytoanticipin, α-tomatine, was induced by bioactive compounds, chaetoglobosin C, chaetomanone A and trichotoxin A50. Disease severity index were determined before seedlings were harvested and analyzed for plant disease resistance (PDR) (Figure 4.57). Results showed that plant disease resistance (PDR) on treated tomato seedlings with chaetoglobosin c, chaetomanone A and trichotoxin A50 after 10 days were 44.97%, 35.18% and 39.43%, respectively when compared to prochloraz that showed PDR of 29.95%. The extracts from tested tomato were spotted to thin layer chromatography (TLC) paper with standard comparison α-tomatine. The spot of extracts on TLC paper presented green spot in all treatments with Rf = 0.23 as same as standard α –tomatine (Figure 4.57). The quantity of α-tomatine at five days were analyzed and the data revealed that inoculated seedlings which treated with chaetoglobosin C

presented the highest α-tomatine quantity as 746.67 ug/g of sample while inoculated seedlings which treated with chaetomanone A, and trichotoxin A50 showed significantly lower α-tomatine quantity as 535.01 and 599.79 ug/g of samples, respectively. Meanwhile, inoculated seedlings which treated with prochloraz, tomato seedlings which inoculated with pathogen only, and non-inoculated control showed non- significantly different in α-tomatine quantity as 368.68, 361.51, and 294.38 ug/g of samples, respectively. The treated with trichotoxin A50 showed α-tomatine quantity as 492.22 ug/g of sample which higher significantly different than treatments which treated with chaetoglobosin C, chaetomanone A, and non-inoculated control which showed α-tomatine quantity as 348.72, 322.98, and 321.19 ug/g of samples, respectively after ten days. In addition, inoculated seedlings which treated with prochloraz and tomato seedlings which inoculated with pathogen only showed lowest quantity of α-tomatine as 190.82 and 179.87 ug/g of samples. The quantity of α-tomatine showed less amount in all treatments at fifteen days except for non-inoculated control which revealed the content of α-tomatine as 365.91 ug/g of sample. Treatments which treated with chaetoglobosin c, chaetomanone A, and trichotoxin A50 showed not significantly different in quantity of α-tomatine as 207.87, 254.25, and 205.04 ug/g of samples, respectively while treated with α-tomatine were 131.56 and 77.46 ug/g of samples, respectively (Table 4.8).

Table 4.8 Disease severity index and α-tomatine quantification of tomato after inoculated with pathogen and sprayed with bioactive compounds.

Treatments	5 days		10 days		15 days		
	DSI	Quantity of α-tomatine (ug/g)	DSI	Quantity of α-tomatine (ug/g)	DSI	PDR[2] (%)	Quantity of α-tomatine (ug/g)
Chaetoglobosin C	1.0a[1]	746.67a	1.0a	348.72b	2.43b	44.97a	207.87b
Chaetomanone A	1.0a	535.01b	1.0a	322.98b	2.87b	35.18a	254.25b
Trichotoxin A50	1.0a	599.70b	1.0a	492.22a	2.67b	39.43a	205.04b
prochloraz	1.0a	368.68c	1.0a	190.82c	3.43ab	26.95a	131.56c
Inoculated control	1.0a	361.51c	1.0a	179.87c	4.37a	-	77.46c
Non-inoculated	1.0a	294.38c	1.0a	321.19b	1.0c	-	365.91a
CV (%)	ns	13.71	ns	13.74	20.8	25.85	14.62

[1]Average of three replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.

[2]Plant disease resistance (PDR) = (DSI of inoculated control - DSI of each treatment) / DSI of inoculated control x 100.

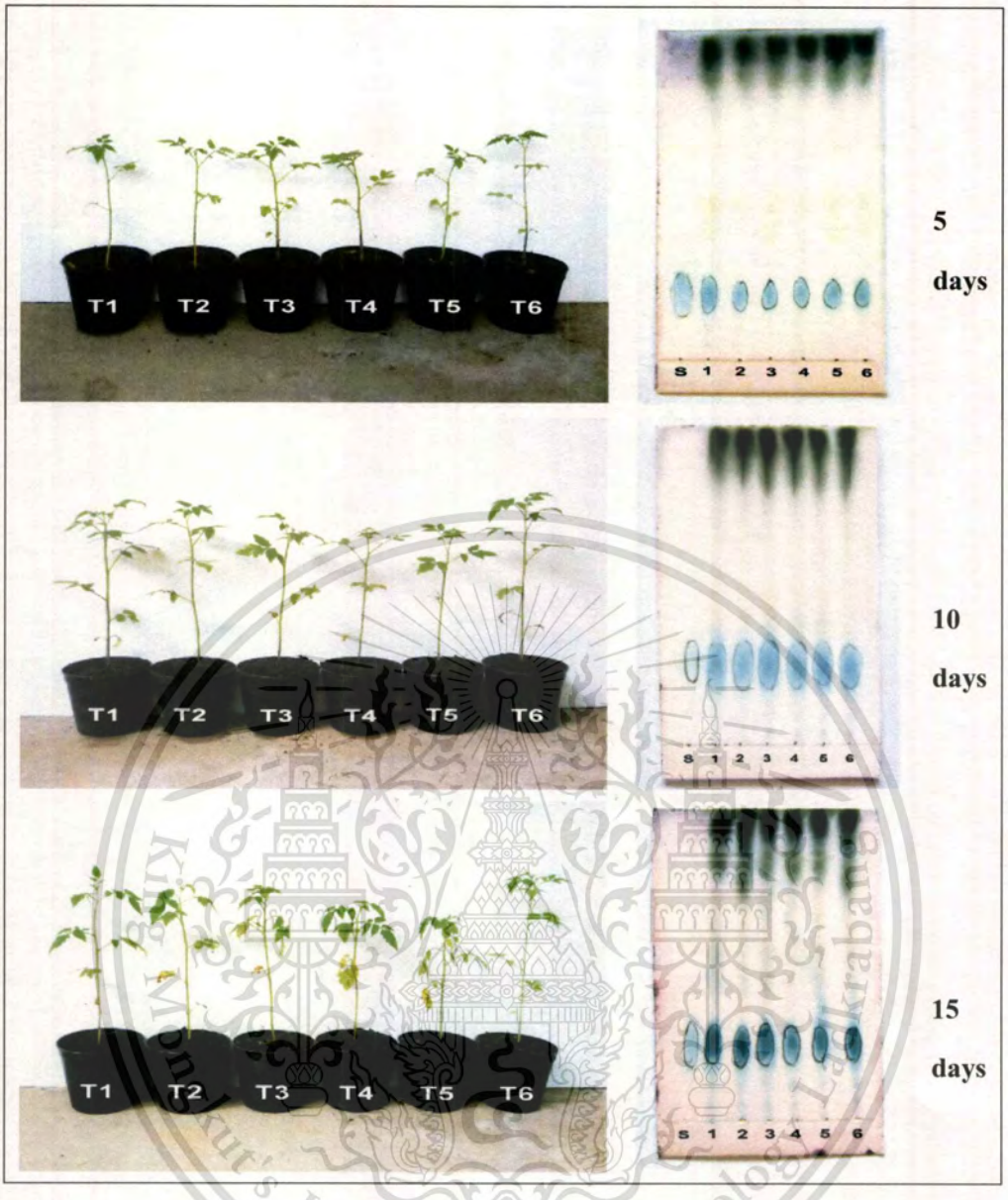


Figure 4.57 Detection of α-tomatine after inoculated with *Fusarium oxysporum* f.sp. *lycopersici* and treated with bioactive compounds for 5, 10 and 15 days.

T1 = treated with chaetoglobosin C, T2 = treated with chaetomanone A, T3 = treated with trichotoxin A50, T4 = treated with prochloraz, T5 = inoculated with pathogen only, T6 = non- inoculated control. Thin layer chromatography (TLC) S = standard α- tomatine, 1= treated with chaetoglobosin C, 2 = treated with chaetomanone A, 3 = treated with trichotoxin A50, 4 = treated with prochloraz, 5 = inoculated control, 6 = non- inoculated control.

4.5 Evaluation of microbial elicitors to induce plant resistance for tomato wilt *in vivo*

The bioactive compounds, chaetoglobosin C, chaetomanone A and trichotoxin A50 were determined for the efficacies as microbial elicitors to induce plant resistance for tomato wilt *in vivo*. At 30 days after treatments, disease severity index (DSI) of inoculated control showed the highest DSI of 3.88 while prochloraz treatment showed lower significantly DSI of 3.0. The treatment of all concentrations of bioactive compounds presented the same level of DSI which DSI between 1.50-2.25. Plant disease resistance (PDR) of treatment of trichotoxin A50 at 50 µg/ml revealed highest plant disease resistance of 63.96%. Treatments which treated with bioactive compounds of chaetoglobosin C at 10, 50 and 100 µg/ml, chaetomanone A at 10, 50 and 100 µg/ml and trichotoxin A50 at 10 and 100 µg/ml showed lower plant disease resistance which were 43.22%, 48.22%, 45.09%, 54.17%, 48.22%, 50.72%, 44.05%, 52.38%, respectively while prochloraz treatment showed the lowest plant disease immunity of 21.94%. Result showed that tomato which treated with chaetoglobosin C at 10, 50 and 100 µg/ml, chaetomanone A at 10 and 50 µg/ml, trichotoxin A50 at 10 µg/ml and non-inoculated control revealed plant height of 35.50, 36.0, 40.25, 37.50, 40.25, 38.75 and 40.0 cm, respectively. While the treatments of chaetomanone A at 100 µg/ml, trichotoxin A50 at 50 and 100 µg/ml, prochloraz and inoculated control showed lower significantly plant height of 31.63, 31.88, 31.0, 23.13 and 22.7 cm, respectively. Plant canopy of tomatoes which treated with chaetoglobosin C at 100 µg/ml and chaetomanone A at 50 µg/ml showed the highest result at 31.13 and 31.50 cm, respectively. Tomatoes which treated with chaetoglobosin C at 10 and 50 µg/ml, chaetomanone A at 10 and 100 µg/ml, trichotoxin A50 at 10 and 50 µg/ml and non-inoculated control showed non-significantly results of 24.63, 27.38, 27.63, 24.13, 27.50, 24.88 and 27.13 cm, respectively. While tomatoes which treated with prochloraz and inoculated control showed lower significantly plant canopy of 16.88 and 15.0 cm, respectively.

The results of the experiment at 45 days after treatments presented that inoculated control treatment showed the highest disease severity index (DSI) of 4.25 while prochloraz treatment showed lower significantly DSI of 3.13. The treatments of chaetoglobosin C at 10 µg/ml resulted in higher significantly DSI than another treatments of bioactive compounds which DSI of 2.0 while chaetoglobosin C at 50 and 100 µg/ml, chaetomanone A at 10, 50 and 100 µg/ml,

trichotoxin A50 at 10, 50 and 100 µg/ml showed DSI of 1.75, 1.75, 1.63, 1.88, 1.88, 1.88, 1.50 and 1.88, respectively. Plant disease resistance (PDR) of trichotoxin A50 at 50 µg/ml revealed highest plant disease resistance of 65.15%. While the treatment which treated with bioactive compounds, chaetoglobosin C at 10, 50 and 100 µg/ml, chaetomanone A at 10, 50 and 100 µg/ml and trichotoxin A50 at 10 and 100 µg/ml showed lower plant disease resistance which were 53.80%, 58.80%, 58.80%, 61.58%, 55.68%, 55.68%, 55.68% and 56.75%, respectively. The treatment of prochloraz showed lower significantly plant disease resistance than bioactive compounds which PDR of 31.13%. Result of plant height showed that tomato which treated with bioactive compounds in all concentrations and non-inoculated control presented non-significantly plant height which plant height of treatments of chaetoglobosin C at 10, 50 and 100 µg/ml, chaetomanone A at 10, 50 and 100 µg/ml, trichotoxin A50 at 10, 50, and 100 µg/ml and non-inoculated control were 47.88, 48.0, 50.75, 44.63, 51.63, 44.88, 45.50, 47.88, 42.50, and 50.38 cm, respectively. Plant canopy of treatments of chaetoglobosin C at 100 µg/ml, chaetomanone A at 50 µg/ml and non-inoculated control showed higher significantly plant canopy of 29.38, 29.0 and 28.63 cm, respectively when compared to treatments of trichotoxin A50 at 10 and 50 µg/ml which showed plant canopy of 27.88 and 27.27 cm, respectively. While treatments of chaetoglobosin C at 10 and 50 µg/ml, chaetomanone A at 10 and100 µg/ml and trichotoxin A50 at 100 µg/ml showed plant canopy of 24.75, 25.25, 24.0, 25.13, and 22.13, respectively which higher significantly plant canopy than prochloraz treatment and inoculated control treatment which showed plant canopy of 19.63 and 17.38 cm, respectively.

The results of 60 days after treatments revealed that disease severity index and plant disease resistance of all treatments presented the same results as the treatments at 45 days. Plant height of all concentrations of bioactive compounds and non-inoculated control presented non-significantly plant height which plant height of treatments of as high as non-inoculated control which plant height of treatments of chaetoglobosin C at 10, 50 and 100 µg/ml, chaetomanone A at 10, 50 and 100 µg/ml, trichotoxin A50 at 10, 50, and 100 µg/ml and non-inoculated control were 62.63, 63.50, 61.75, 60.25, 59.63, 57.50, 55.13, 60.38, 62.0, and 61.13 cm, respectively. While prochloraz treatment and inoculated control treatment showed lower significantly plant height of 41.38 and 38.50 cm, respectively. The results of plant canopy showed that tomatoes which treated with chaetomanone A at 50 µg/ml and trichotoxin A50 at 10 µg/ml showed higher significantly plant canopy than another treatments which were 38.13 and 38.25 cm, respectively. While treatments of chaetoglobosin C at 10, 50 and 100 µg/ml, chaetomanone A at 10 and 100 µg/ml,

trichotoxin A50 at 50 and 100 µg/ml showed non-significantly results with non-inoculated control which plant canopy of 32.13, 33.38, 36.75, 34.13, 33.63, 34.75, 35.75 and 37.63 cm, respectively. Plant canopy of tomatoes which treated with prochloraz and inoculated control showed lower significantly plant canopy of 19.63 and 17.38 cm, respectively. The results of the treatments were demonstrated in Table 4.9-4.10 and Figure 4.58.

Table 4.9 Disease severity index and plant disease resistance of tomato at 30, 45 and 60 days after treatments.

Treatment	30 days		45 days		60 days	
	DSI	PDR	DSI	PDR	DSI	PDR
chaetoglobosin C 10 µg/ml	2.25bc[1]	43.22b	2.0c	53.80b	2.0c	53.80b
chaetoglobosin C 50 µg/ml	2.0bc	48.22ab	1.75cd	58.80ab	1.75cd	58.8ab
chaetoglobosin C 100 µg/ml	2.13bc	45.09b	1.75cd	58.80ab	1.75cd	58.8ab
chaetomanone A 10 µg/ml	1.88bc	54.17ab	1.63cd	61.58ab	1.63cd	61.58ab
chaetomanone A 50 µg/ml	2.0bc	48.22ab	1.88cd	55.68ab	1.88cd	55.68ab
chaetomanone A 100 µg/ml	1.88bc	50.72ab	1.88cd	55.68ab	1.88cd	55.68ab
trichotoxin A50 10 µg/ml	2.13bc	44.05b	1.88cd	55.68ab	1.88cd	55.68ab
trichotoxin A50 50 µg/ml	1.50bc	63.96a	1.50cd	65.15a	1.50cd	65.15a
trichotoxin A50 100 µg/ml	1.88bc	52.38ab	1.88cd	56.75ab	1.88cd	56.75ab
prochloraz	3.0ab	21.94c	3.13b	26.73c	3.13b	26.73c
Inoculated control	3.88a	-	4.25a	-	4.25a	-
Non-inoculated control	1.0c	-	1.0d	-	1.0d	-
CV (%)	32.96	15.92	21.88	10.64	21.88	10.64

[1]Average of four replications (two plants/replication). Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

[2]Plant disease resistance (PDR) = (DSI of inoculated control - DSI of each treatment) / DSI of inoculated control x 100.

Table 4.10 Plant growth parameters of tomato at 30, 45 and 60 days after treatments.

Treatment	30 days		45 days		60 days	
	Height (cm)	Canopy (cm)	Height (cm)	Canopy (cm)	Height (cm)	Canopy (cm)
chaetoglobosin C 10 µg/ml	35.50a[1]	24.63ab	47.88a	24.75abc	62.63a	32.13b
chaetoglobosin C 50 µg/ml	36.0a	27.38ab	48.0a	25.25abc	63.50a	33.38ab
chaetoglobosin C 100 µg/ml	40.25a	31.13a	50.75a	29.38a	61.75a	36.75ab
chaetomanone A 10 µg/ml	37.50a	27.63ab	44.63a	24.0abc	60.25a	34.13ab
chaetomanone A 50 µg/ml	40.25a	31.50a	51.63a	29.0a	59.63a	38.13a
chaetomanone A 100 µg/ml	31.63ab	24.13ab	44.88a	25.13abc	57.50a	33.63ab
trichotoxin A50 10 µg/ml	38.75a	27.50ab	45.50a	27.88ab	55.13a	38.25a
trichotoxin A50 50 µg/ml	31.88ab	24.88ab	47.88a	27.25ab	60.38a	34.75ab
trichotoxin A50 100 µg/ml	31.0ab	22.25bc	42.50a	22.13bc	62.0a	35.75ab
prochloraz	23.13b	16.88cd	30.0b	19.63cd	41.38b	25.75c
Inoculated control	22.75b	15.0d	28.0b	17.38d	38.50b	25.25c
Non-inoculated control	40.0a	27.13ab	50.38a	28.63a	61.13a	37.63ab
CV (%)	18.25	18.28	14.78	14.24	9.40	10.48

[1]Average of four replications (two plants/replication). Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

Figure 4.58 Evaluation of microbial elicitors to induce plant resistance for tomato wilt at 30 days after treatments.

T1 = chaetoglobosin C 10 µg/ml, T2 = chaetoglobosin C 50 µg/ml, T3 = chaetoglobosin C 100 µg/ml, T4 = chaetomanone A 10 µg/ml, T5 = chaetomanone A 50 µg/ml, T6 = chaetomanone A 100 µg/ml, T7 = trichotoxin A50 10 µg/ml, T8 = trichotoxin A50 50 µg/ml, T9 = trichotoxin A50 100 µg/ml, T10 = prochloraz, T11 = Inoculated control and T12 = Non-inoculated control.

4.6 Evaluation of Bio-agent formulations to control Fusarium wilt of tomato *in vivo*

The antagonistic fungi were tested for the abilities as biological control agent to control tomato wilt *in vivo*. Bio-agent formulations which separately formulated as oil formulation of antagonistic fungi namely N0802 (*C. globosum* KMITL-N0802), CLT (*C. lucknowense* CLT), and PC01 (*T. harzianum* PC01) were sprayed every 15 days on tomato seedlings var. Sida compared with prochloraz, inoculated with pathogen and non-inoculated control. Data was collected as growth parameters every 30 days such as disease severity index, plant height (cm), plant canopy (cm). Plant fresh weight (g) and plant dry weight (g) and yield were recorded at harvesting. Result revealed that bio-agent formulations namely N0802, CLT and PC01 gave significantly highest disease reduction of tomato wilt which were 43.90, 41.22 and 41.97 %, respectively and followed by prochoraz treatment (20.39%). That resulted to increase in yield of tomato, bio-agent formulations of N0802, CLT and PC01 were also significantly increased in yield of tomato which were 88.53, 83.74 and 87.24 %, respectively and followed by prochoraz treatment (41.57%). With this, disease severity index (DSI) of tomatoes which treated with bio-agent formulations of developed N0802, CLT, PC01 and prochloraz at 60 days were not significantly different in DSI which were 2.6, 3.0, 2.8, and 3.7, respectively when compared to tomato inoculated with pathogen, the DSI was 4.7 (Table 4.9). Moreover, the tested bio-agent formulations gave significantly higher plant growth parameters than prochloraz and non-treated control. Treated bio-agent formulations of N0802, CLT and PC01 gave non-significantly differ in plant height as 37.40, 35.65 and 40.20 cm, respectively but significantly different when compared to prochloraz and inoculated control which showed plant height as 21.10 and 14.80 cm, respectively. Moreover, it showed that bio-agent formulations of N0802, CLT PC01 and non-inoculated with pathogen could significantly higher plant fresh and dried weights than prochloraz and inoculated control (Table 4.11).

Interestingly, bio-agent formulation treatments of N0802 gave significantly highest in yields as 133.81 g/plant and followed by bio-agent formulations treatments of CLT, PC01 and non-inoculated treatment which were 94.40, 120.32 and 82.47 g/plant, respectively. Prochloraz treatment and inoculated control gave non-significantly differ in yields as 26.27 and 15.35 g/plant, respectively (Table 4.12 and Figure 4.59).

Table 4.11 Disease severity and percentage of disease reduction at 30 and 60 days after treatments.

Treatments[1]	30 days		60 days	
	DSI	DR[3]	DSI	DR
Fol	4.20a[2]	-	4.70a	-
N0802	2.90c	31.27ab	2.60c	43.90a
CLT	3.0bc	29.31ab	2.75c	41.22a
PC01	2.80c	33.65a	2.70c	41.97a
prochloraz	3.40b	20.15b	3.70b	20.39b
control	1.0d	-	1.0d	-
CV (%)	10.05	18.55	7.8	14.77

[1]Fol = inoculated with pathogen only, N0802 = bio-agent formulation of *C. globosum* KMITL-N0802, CLT = bio-agent formulation of *C. lucknowense* CLT, PC01 = bio-agent formulation of *T. harzianum* PC01

[2]Average of four replications (five plants/replication). Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

[3]% disease reduction (DR) = Disease severity index (DSI) of control – Disease severity index of treatment/ Disease severity index of control x 100.

Table 4.12 Plant height and canopy of tomatoes at 30 and 60 days after treatments.

Treatments[1]	Plant height (cm)		Plant canopy (cm)	
	30 days	60 days	30 days	60 days
Fol	15.10c	32.00b	16.35d	27.68b
N0802	18.95c	46.78a	25.65bc	41.10a
CLT	24.20b	41.43ab	30.60ab	32.73ab
PC01	22.85b	46.73a	29.15ab	41.28a
prochloraz	17.80c	35.48ab	19.15cd	28.93b
control	28.55a	49.50a	34.15a	41.00a
CV (%)	11.56	15.18	17.27	15.19

[1]Fol = inoculated with pathogen only, N0802 = bio-agent formulation of *C. globosum* KMITL-N0802, CLT = bio-agent formulation of *C. lucknowense* CLT, PC01 = bio-agent formulation of *T. harzianum* PC01

[2]Average of four replications (five plants/replication). Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 4.13 Growth parameters after treated with Bio-agent formulation.

Treatments[1]	Number of fruit			Fruit weight (g)	Increase in yield[3] (%)	Fruit size (cm)		Plant fresh weigh (g)		Plant dry weight (g)	
	total	per replication	per plant			width	length	stem	root	stem	root
Fol	30	7.5	1.50b	15.35 c[2]	-	1.56 a	1.98 a	20.45 c	1.75 c	3.55 c	0.25 b
N0802	201	50.25	10.05a	133.81 a	88.53a	2.34 c	2.86 d	61.95 a	5.60 a	10.55 a	0.98 a
CLT	152	38	7.50a	94.40 ab	83.74a	2.23 c	2.63 cd	47.90 abc	4.10 ab	7.95 abc	0.73 a
PC01	186	46.5	9.30a	120.32 ab	87.24a	2.34 c	2.86 d	50.35 ab	4.55 ab	11.20 a	0.91 a
prochloraz	50	12.5	2.50b	26.27 c	41.57b	1.84 b	2.23 b	30.20 bc	2.30 ab	4.60 bc	0.30 b
control	137	34.25	6.85a	82.47 b	81.39a	2.28 c	2.53 c	44.50 abc	5.85 a	8.90 ab	0.99 a
CV (%)			38.21	31.98	12.85	25.1	28.6	30.39	39.64	26.95	28.54

[1]Fol = inoculated with pathogen only, N0802 = Bio-agent formulation of *C. globosum* KMITL-N0802, CLT = Bio-agent formulation of *C. lucknowense* CLT, PC01 = Bio-agent formulation of *T. harzianum* PC01.

[2]Average of four replications (five plants/replication). Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

[3]% increase in yield = Yield per plant of treatment – Yield per plant of control/ Yield per plant of treatment x 100.

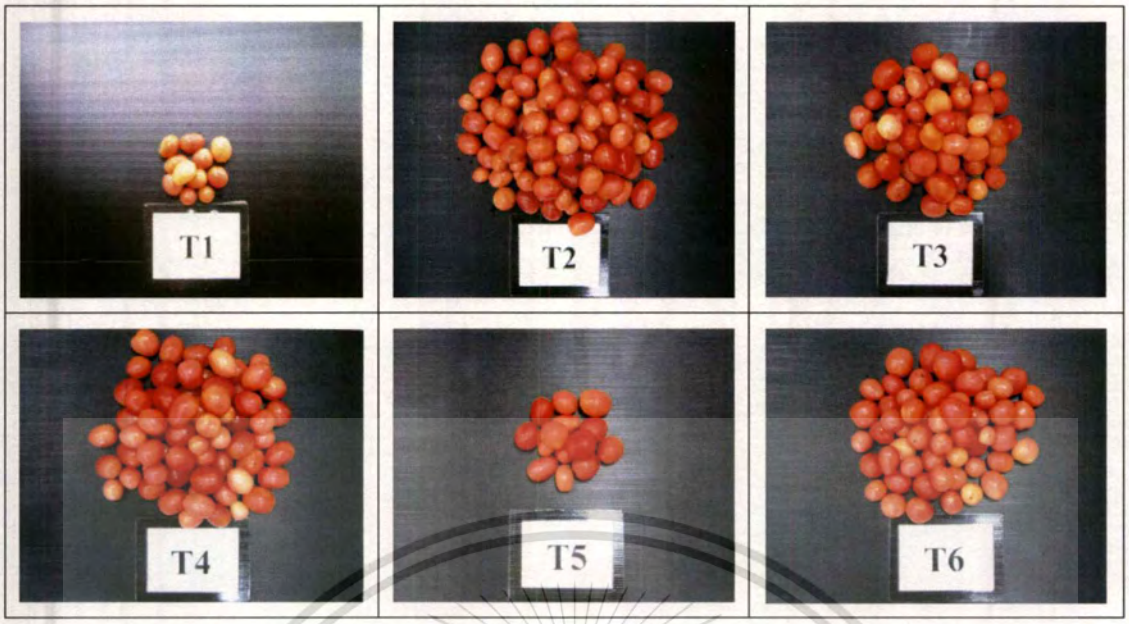


Figure 4.59 Tomato fruits yielded from testing Bio-agent formulation.

T1 = inoculated control, T2 = Bio-agent formulation of *Chaetomium globosum* KMITL-N0802, T3 = Bio- agent formulation *Chaetomium lucknowense* CLT, T4 = Bio-agent formulation of *T. harzianum* PC01, T5 = treated with prochloraz at 20g/20L of water, T6 = non- inoculated control.

CHAPTER 5

DISCUSSION

Fusarium oxysporum f. sp. *lycopersici* NKSC01 was isolated from infested tomato fields in Thailand and proved to be the most aggressive isolate to tomato var. Sida as also reported by Sibounnavong *et al.* (2010). The sequences of *Fusarium* spp. 45 isolates were confirmed morphological identification by using ITS sequences with the length of the complete ITS1, 5.8S and ITS2 including a small portion of 18S rDNA and a small portion of the 28S rDNA. The phylogenetic tree from PAUP analysis presented cluster of the *Fusarium* spp. into five species as *F. incarnatum*, *F. solani*, *F. oxysporum*, *F. chlamydosporum* and *F. redolens*. The result was supported by the report of Nagarajan *et al.* (2004) who stated that the rDNA region analyses have been used successfully to differentiate other *Fusarium* species such as *F. avenaceum, F. arthroporioides,* and *F. tricinctum*. Moreover, the phylogenetic tree clearly demonstrated to confirm the isolate NKSC01 is *F. oxysporum* f.sp. *lycopersici* as a valid identification. As a result, there are many reported confirmation that this region has been validly used to confirm the species of *F. oxysporum* (Paplomatas, 2004; Hirano and Arie, 2006; Kawabe *et al.*, 2005). Moreover, there were many reports using PCR-RFLP with restriction enzymes *Hha*I, *Msp*I, *Hae*III, *Pvu*II, *Bgl*II, *Mse*I and *Alu*I or PCR-RAPD with primers OPF01, OPF02, OPF04, OPF05, OPF06, OPF08, OPF10, OPF11, OPF12, OPF13 and OPF14 to identify *F. oxysporum* into formae speciales and race (Marlartt *et al.* 1996; Bogale *et al.* 2007; Fernandez *et al.* 1997; Assigbetse *et al.* 1994). With these reports, our work should be study further by using PCR-RFLP or PCR-RAPD techniques to confirm formae speciales and/or virulence of each isolate of *F. oxysporum*.

Dual culture test exhibited that *Chaetomium globosum* N0802, *C. lucknowense* CLT and *Trichoderma harzianum* PC01 could control Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*. Based on the results, they exhibited the high properties to inhibit conidial production over 90%. The results were similar to the report of Srinon *et al.* (2006) which stated that *T. harzianum* WS01 showed efficacies to inhibit conidial production of *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *cucumerinum* (wilt of cucumber) over 90%. In this study, the antagonistic substances (crude extracts) of *C. globosum* N0802, *C. lucknowense* CLT and *T. harzianum* PC01 at 1,000 µg/ml could inhibit the conidial production of pathogen over 50%. With this, crude extracts of *T. harzianum* PC01 at 1,000 µg/ml inhibited conidial production over

90%. This results were similar to many works that antagonistic substances from *C. globosum* CG extracted with ethyl acetate and *T. harzianum* PC01 extracted with ethyl acetate at 500 µg/ml could inhibit conidia production of *Colletotrichum gloeosporioides* WMF01 causing anthracnose disease of grape which ED_{50} values were 2 and 7 µg/ml, respectively (Soytong *et al.* 2005). Result in this study, the ED_{50} values of crude extracts of *C. globosum* N0802 and *T. harzianum* PC01 to inhibit spore production of *F. oxysporum* f. sp. *lycopersici* were 157 and 192 µg/ml, respectively. These tested antagonistic fungi showed antibiotic mechanism to inhibit growth of Fusarium wilt pathogen which supported by the report of Soytong (1992) who stated that antagonistic substance from *C. cupreum*, *C. globosum* and *T. harzianum* could inhibit growth and also broken cells of *F. oxysporum* f. sp. *lycopersici*. *C. globosum* produces cell wall hydrolases (such as chitinase and glucanase) and antibiotics (such as chaetoglobosin and chaetomanone), which inhibit fungal plant pathogens (Liu *et al.* 2008). The example of the antagonistic substance of *C. globosum* could degrade cell walls of phytopathogenic fungi was mentioned by Liu *et al.* (2008) who reported that CHI46 enzyme could efficiently degrade cell walls of the *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *S. tritici*, and *Phytophthora sojae*. Besides this, antibiotic substances namely chaetomanone which extracted from *C. globosum* N0802 was also reported to exhibit antitubercular activity caused by *Mycobacterium tuberculosis*, a major human disease (Kanokmedhakul *et al.* 2002). It may be conclude that *C. globosum* KMITL-N0802 exhibited antibiotic mechanism to control *F. oxysporum* f.sp. *lycopersici* NKSC01.

Moreover, the α-tomatine quantification of tomato seedlings treated with microbial elicitors, prochloraz and inoculated control at 10 days and 15 days showed lower quantity than at 5 days after treatments. This result may support the relation between fungal infection and α-tomatine production in tomato seedlings after inoculated with pathogen and sprayed with microbial elicitors. The reason may be supported by the reports of Osbourn *et al.* (1995), Lairini *et al.* (1996) and Lairini and Ruiz-Rubio (1998) who stated that α-tomatine presented in tomato plants and acted as a preformed chemical barrier against phytopathogenic fungi. But *F. oxysporum* f. sp. *lycopersici* was resistant to α-tomatine by producing specific tomatine-detoxifying enzymes known as tomatinase which was able to detoxify α-tomatine into nonfungitoxic forms by cleaving the glycoalkaloid into the tetrasaccharide lycotetraose and tomatidine which less inhibitory to fungal growth, suggesting that α-tomatine detoxification may be important strategy for successful infection of tomato plant. It is noticed that the non-inoculated tomato was also found α-tomatine at 5, 10 and 15 days which surprising that the quantity of tomatine at 10 and 15 days showed

higher α-tomatine quantification than 5 days and even higher than all treated tomato at 15 days. This result may possible due to mistaken technique of inoculation that cutting the root tips before inoculation of non-inoculated tomato. As a result, Doke (1997) stated that physical injuries are also induced resistant and may concern on phytoalexin production through the first signal of oxidative burst.

The bioactive compounds of chaetoglobosin C, chaetomanone A and trichotoxin A50 showed a potent antifungal activity against *F. oxysporum* f. sp. *lycopersici* NKSC01. All tested antifungal substances strongly inhibited mycelia growth and sporulation of pathogen at concentration of 100 µg/ml over 80%. Chaetoglobosin C, chaetomanone A and trichotoxin A50 inhibited the mycelial growth of pathogen with ED_{50} values of 71, 68 and 71 µg/ml, respectively. Meanwhile, the sporulation of pathogen was inhibited by chaetoglobosin C, chaetomanone A and trichotoxin A50 with ED_{50} values of 17, 22 and 18 µg/ml, respectively. The results of the abilities of chaetoglobosin C were also supported by the work of Soytong *et al.* (2005) which was quite different from our results. They reported that chaetoglobosin C could inhibit the growth of *F. oxysporum* caused tomato wilt the ED_{50} value of 2.00 µg/ml. This result showed lower ED_{50} value than our results. Moreover, chaetoglobosin C could also inhibit the spore production of *P. parasitica* causing root and basal rot of tangerine and *C. gloeosporioides* causing anthracnose of tangerine which the ED_{50} value of 35.0 and 0.09 µg/ml, respectively (Soytong *et al.* 2007).

The results of the research findings showed trichotoxin A50 could inhibit mycelium growth and sporulation of *F. oxysporum* f.sp. *lycopersici* with the ED_{50} values of 71 and 18 µg/ml, respectively. The result was similar to previous works of Alkhail (2005) who reported that culture filtrates at concentration 0.5-2.0 % of *T. harzianum* could control the growth of *F. oxysporum* f. sp. *lycopersici* varied from 17.40 to 50.0 % but the antifungal metabolite was not characterize. The reports of Haggag and Mohamed (2007) and El-Hasan *et al.* (2009) mentioned to antifungal metabolites from *T. harzianum* namely trichodermin, gliotoxin and gliovirin could reduce the growth rate of *F. oxysporum* also as viridiofungin A produced from *T. harzianum* isolate T23 could inhibit mycelial growth of *F. moniliforme*. These reports supported our results that trichotoxin A50 which was extracted from *T. harzianum* could exhibit antibiosis activity to inhibit the growth of Fusarium wilt pathogen as same as the other metabolites.

Moreover, trichotoxin A50 could inhibit another pathogenic fungi as the reported of Soytong *et al.* (2005) and Soytong *et al.* (2007) which demonstrated that trichotoxin A50 could inhibit sporulation of *C. gloeosporioides* causing anthracnose of grape, *P. palmivora* causing root

rot of durian and black pepper, *P. parasitica* causing root and basal rot of tangerine and *C. gloeosporioides* causing anthracnose of tangerine. The bioactive compounds, chaetoglobosin C, chaetomanone A and trichotoxin A50 affected the bioactivity to the pathogen cells of *F. oxysporum* f sp. *lycopersici*. It was observed that macroconidia of the tested pathogen showed abnormal cells that cell wall of pathogens collapsed and cytoplasm was clogged inside the cells and somewhat released from the pathogen cells. With this affect, the report of Alkhail (2005) supported our results as he reported that antifungal metabolite of *T. harzianum* could control the growth of *F. oxysporum* f. sp. *lycopersici*. The cell wall of *Fusarium*'s conidia collapsed and cytoplasm was clogged. The similar reaction of some antagonistic substances from strains of *C. globosum* and *C. cochliodes* also showed the same reaction activity of broken the pathogen cells (Chang and Kommedahl, 1968) as Soytong (1992) reported the culture filtrate of *C. cupreum* could clearly demonstrate to broken the pathogen cells of *F. oxysporum* f sp *lycopersici*. With this, it is indicated that chaetoglobosin C, chaetomanone A and trichotoxin A50 may play an important role of lysis and lost of pathogenicity.

The research work exhibited the efficacies of bioactive compounds as a strong inhibitory activity *in vivo* against tomato Fusarium wilt. Inoculated tomato plant which applied with bioactive compounds at 10-100 µg/ml showed higher plant disease immunity (53.80-65.15%) compared with prochloraz (26.73%). With the results, tomato plants with treated with bioactive compounds also showed significantly different in plant height than chemical prochloraz and inoculated control. This result was similar as a work of Soytong *et al.* (2007) who reported that trichotoxin A50 exhibited a potential as plant growth regulators in Chinese cabbage, kale and mungbean. This result was supported by report of Soytong *et al.* (2001) and Suwan *et al.* (2000) which mentioned that chaetoglobosin C extracted from *C. globosum* acts as an induction of plant immunity for disease resistance by inducing a localized and sub-systemic oxidative burst in tomato, tobacco, potato, and carrot. Base on the results, it could conclude that chaetoglobosin C and trichotoxin A50 could elicit the resistant or immunity in tomato and other plants, inhibit the pathogen and acted as plant growth regulators to stimulate plant growth.

This research finding demonstrated that chaetomanone A was firstly extracted from *Chaetomium lucknowense*. But previous report of Kanokmedhakul *et al.* (2002) stated that chaetomanone A was extracted from *C. globosum* strain N0802 and it exhibited antitubercular activity against *Mycobacterium tuberculosis*, a major human disease. But there are no reports on chaetomanone A against plant pathogenic fungi. It is suggested that different strains of *C.*

globosum, C. lucknowense and T. harzianum could differ in antibiotic production implies specific antibiotic production strains. This result suggest that chaetoglobosin C, chaetomanone A and trichotoxin A50 play an important roles in antagonism and antibiosis against *F. oxysporum* f. sp. *lycopersici* causing tomato wilt. Further studies would possible be developed these bioactive compounds as the elicitors to induce phytoalexin for plant resistance.

In vivo testing, Bio-agent formulations N0802 (*C. globosum*), CLT (*C. lucknowense*) and PC01 (*T. harzianum*) were clearly demonstrated that these Bio-agent formulations gave a high efficacies to control Fusarium wilt of tomato caused by *F. oxysporum* f. sp *lycopersici*. The Bio-agent formulations could reduce disease incidence of tomato wilt and leading to increase in yield. The study showed Bio-agent formulation of N0802 could decrease wilt incidence of 44.68% and increase in yield of 88.53%. The Bio-agent formulation of PC01 decreased disease incidence of 50.97% and increased in yield of 87.24%. The same results as a work of Phuwiwat and Soytong (1999) which reported that the same strain of *T. harzianum* PC01 could also promote the growth of Chinese radish and yield. The results similar as report of Larkin and Fravel (1998) who stated that *Trichoderma* spp. and *Gliocladium* spp. could reduce tomato wilt in field trial between 37–75% which were the same rate of PC01 in this study. Besides this, the work of Yiğit and Dikilitas (2007) also supported the similar results which were reported that *T. harzianum* T-22 could reduce disease incidence of tomato Fusarium wilt at 42% and also improve yield and mineral contents of tomatoes.

As a result of Bio-agent formulations from *Chaetomium* sp., N0802 (*C. globosum*) and CLT (*C. lucknowense*) exhibited effective control Fusarium wilt of tomato, that supported by previous work of Soytong *et al.* (2001) who mentioned that the biological products contained with *Chaetomium* spp. (22 strains of *C. cupreum* and *C. globosum*) in biopellet and biopowder formulations which applied to soil could suppress the growth of *F. oxysporum* f. sp. *lycopersici* and reduce infection rate in tomato. Besides this, Soytong *et al.* (2005) stated that *Chaetomium* bioproducts formulated from *C. globosum* and *C. cupreum* as powder formulation could control bud rot and basal stem rot of bottle palms which caused by *Thielaviopsis paradoxa* in the field and reduce disease incidence of 75%.

As the results of research, *C. globosum* KMITL-N0802, *C. lucknowense* CLT and *T. harzianum* PC01 and their antagonistic substances include their bioactive compounds, chaetoglobosin C, chaetomanone A and trochotoxin A50 are effective biological control agents which act in multi mechanism of actions to control Fusarium wilt. Their potential include with

competition, produce antibiotics or substance to inhibit growth of pathogen, induce resistance in tomato plants and decrease disease incidence and they also stimulate plant growth as many reports mentioned to. However, there was not any report with *C. lucknowense* or it's substance to control plant pathogen. The study with this antagonistic fungus should be done further. The formulation of fungal products should be developed to use as biofungicides for safety and effective agriculture.

CHAPTER 6

CONCLUSIONS

Forty-five isolates of *Fusarium* spp. were isolated from infested tomato and rhizosphere soil in eight provinces. Pathogenicity test could separate the forty-five isolates of *Fusarium* into pathogenic and non-pathogenic isolates based on disease virulence. *F. oxysporum* f.sp. *lycopersici* isolate NKSC01 presented to be the most aggressive isolate and were selected to use as pathogen in this study. Phylogenetic analysis revealed five groups of *Fusarium* spp. as follows:- *F. oxysporum*, *F. solani*, *F. incarnatum*, *F. chlamydosporum* and *F. redolens*. The phylogenetic tree showed relationship among the isolates of *F. oxysporum* and pathogenicity test. It divided twelve isolates of *F. oxysporum* into three virulent groups based on disease severity index.

The antagonistic fungi, *Chaetomium globosum* KMITL-N0802, *C. lucknowense* CLT and *Trichoderma harzianum* PC01 could inhibit *F. oxysporum* f.sp. *lycopersici* NKSC01 in dual-culture which mycelial inhibition 71.11%, 88.89% and 90.56%, respectively and conidial production between 92.14%, 92.54% and 99.99%, respectively. Crude extracts of the antagonistic fungi showed potent to inhibit conidial production of the pathogen which crude hexane of *C. globosum* KMITL-N0802, crude hexane of *C. lucknowense* CLT and crude methanol of *T. harzianum* PC01 presented the highest ED_{50} value of 157, 188 and 192 µg/ml, respectively.

In vitro testing bioactive compounds revealed that chaetoglobosin C, chaetomanone A, and trichotoxin A50 showed high potent for mycelial and conidial inhibitions of *F. oxysporum* f.sp. *lycopersici* NKSC01. ED_{50} of mycelial inhibition of chaetoglobosin C, chaetomanone A, and trichotoxin A50 against pathogen were 71, 68 and 71 µg/ml, respectively and ED_{50} of conidial inhibition were 17, 22 and 18 µg/ml. Moreover, macroconidia of pathogen showed lysis of cell wall and abnormal cytoplasm when attacked with bioactive compounds.

Chaetoglobosin C, chaetomanone A, and trichotoxin A50 were expressed as microbial elicitors to induce immunity in tomato plant var. Sida. Thereafter, tomato which treated with bioactive compounds induced plant resistance between 35.18 to 44.97%. The bioactive compounds could elicit α-tomatine between 205.04-254.25 µg/g which higher than chemical fungicide (prochloraz) and inoculated control (131.56 and 77.46 µg/g) at 15 days after treated with pathogen. Meanwhile, bioactive compounds were tested for their abilities as microbial elicitors to induce plant resistance to control tomato wilt *in vivo* revealed that chaetoglobosin C,

chaetomanone A, and trichotoxin A50 at concentration 10, 50 and 100 µg/ml could induce resistance in tomato plant which plant disease resistance between 53.80-65.15% which higher than prochloraz that showed plant disease resistance only 26.73%.

In vivo test, bio-agent formulation namely N0802 from *C. globosum* KMITL-N0802, CLT from *C. lucknowense* CLT and PC01 from *T. harzianum* PC01 were sprayed onto inoculated tomato plants every 2 weeks. The bio-agent formulations could reduce disease incidence between 41.22 to 43.90% which higher than prochloraz (20.39%). Besides, the bio-agent formulations could increase in yield between 83.74 to 88.53%.

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APPENDIX

RESEARCH PUBLICATIONS

Publication:

1. C. Charoenporn, S. Kanokmedhakul, F.C. Lin, S. Poeaim and K.Soytong., 2010. Evaluation of bio-agent formulations to control *Fusarium* wilt of tomato. African Journal of Biotechnology. 9(36): 5836-5844.

Oral Presentations:

1. Charoenporn, C., Kanokmedhakul, S., Lin, F.C. and Soytong, K. 2007. Effects of media and pH levels on the growth of *Fusarium* wilt of tomato and its pathogenicity test. The International Conference on Integration of Science & Technology for Sustainable Development, 26-27 April 2007, KMITL, Bangkok, Thailand.
2. Charoenporn, C., Kanokmedhakul, S., Lin, F.C. and Soytong, K. 2007. A study on *Fusarium* wilt of tomato : fungal morphology, suitable media and pH level for fungal growth and its pathogenicity. International Conference on Engineering, Applied Science, and Technology (ICEAST), 21-23 November 2007, Swiss Hotel Le Concorde, Bangkok, Thailand.
3. Charoenporn, C., Kanokmedhakul, S., Lin, F.C. and Soytong, K. 2007. Studies on Fusarium Wilt of Tomato: Preliminary Test for Morphology, Pathogenicity and its Biological Control. The 2nd Annual Meeting of Thai Mycological Association (TMA) and Mycology Conference in Thailand, 23 June 2007, Chiang Mai University, Chiang Mai, Thailand.
4. Charoenporn, C., Kanokmedhakul, S., Lin, F.C., Poeaim, S. and Soytong, K. 2008. Biological control of tomato wilt using *Chaetomium* spp. The 3rd Annual Meeting of Thai Mycological Association (TMA) and Mycology Conference in Thailand, 11 October 2008, Khon Kaen University, Khon Kaen, Thailand.
5. Charoenporn, C., Kanokmedhakul, S., Lin, F.C. and Soytong, K. 2009. Biological control of tomato Fusarium wilt using bioactive compounds from antagonistic fungi. The 4th Annual Meeting of Thai Mycological Association (TMA) and Mycology Conference in Thailand, 24 October 2009, Maejo University, Chiang Mai, Thailand.

Poster Presentation:

1. Charoenporn, C. and Soytong. K. 2006. Study on Fusarium Wilt of Tomato. The Annual Meeting of Thai Mycological Association (TMA) and Mycology Conference in Thailand, 24-29 October 2006, Faculty of Agricultural Technology, King Mongkut's Institue of Technology Ladkrabang, Bangkok, Thailand.

African Journal of Biotechnology Vol. 9(36), pp. 5836-5844, 6 September, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684-5315 ©2010 Academic Journals

Full Length Research Paper

Evaluation of bio-agent formulations to control *Fusarium* wilt of tomato

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Accepted 18 June, 2010

The ED_{50} value of antagonistic substance from *Chaetomium globosum* N0802 extracted with hexane was 157 µg/ml. This gave the highest inhibition of conidial production of *Fusarium oxysporum* f. sp. *lycopersici* causing tomato wilt var Sida. Crude hexane from *Chaetomium lucknowense* CLT and crude methanol from *Trichoderma harzianum* PC01 gave ED_{50} values of 188 and 192 µg/ml. It clearly demonstrated that antagonistic substances from all tested fungi could be deformed and this could break the conidial cells. The bio-agent formulations namely N0802, CLT and PC01 gave significantly highest disease reduction of tomato wilt which were 44.68, 36.28 and 41.01%, respectively, followed by prochoraz (21.95%). All tested bio-agent formulations could significantly increase the yield of tomato when compared to prochoraz and inoculated control. It is concluded that *C. globosum*, *C. lucknowense* and *T. harzianum* developed as bio-agent formulations namely N0802, CLT and PC01 and showed their abilities to control tomato wilt.

Key words: *Fusarium oxysporum* f. sp. *lycopersici*, *Chaetomium globosum*, *Chaetomium lucknowense*, *Trichoderma harzianum*, bio-agent formulations.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the world's most cultivated vegetable crop. Fusarium wilt is one of the most serious disease in tomato throughout the world, especially in upland. This disease is caused by *Fusarium oxysporum* f. sp. *lycopersici* (Sacc.) leading to serious economic losses (Snyder and Hansen, 1940). It becomes one of the most prevalent and damaging disease wherever tomatoes are grown intensively because the pathogen can persist indefinitely in infested soils (Agrios, 1997). The excessive misuse of a wide range of fungicides has led to it being harmful to the environment and increases the resistant pathogen populations (Özgönen et al., 2001). *F. oxysporum* f. sp. *lycopersici* becomes resistant to those chemical fungicides. For this reason, alternative methods to control the disease had been studied with emphasis on biological control using fungi or bacteria to reduce fungicide application and decrease cost of production. Biological control has the potential to manage this disease which occurred through different mechanisms such as antibiosis, competition, suppression,

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Abbreviations: **DSI,** Disease severity index; **ITS,** internal transcribed spacer; **PDA,** potato dextrose agar; **PDB,** potato-dextrose broth; **PB,** phosphate buffer; **PCR,** polymerase chain reaction; **DMSO,** dimethyl sulfoxide.

direct parasitism, induced resistance, hypovirulence and predation. The antagonistic activity has often been associated with production of secondary metabolites (Haggag and Mohamed, 2007; Larkin and Fravel, 1998). There were many reports of biological control agents to control Fusarium wilt pathogen such as *Trichoderma harzianum*, *Pythium oligandrum*, *Achromobacter xylosoxydans*, *Penicillium oxalicum* and non-pathogenic *F. oxysporum* (Mohamed and Haggag 2006; Floch et al., 2003; De Cal et al., 2000; Moretti et al., 2008; Silva and Bettiol, 2005). Soytong (1992) and Kanokmedhakul et al. (1993) reported that crude extract of *Chaetomium cupreum* KMITL-N 4320 inhibited spore production of *F. oxysporum* f. sp. *lycopersici* at 85.14%. Moreover, there are many reports on bioactive com-pounds which were extracted from antagonistic fungi to inhibit Fusarium wilt of tomato pathogen (Kanokmedhakul et al., 2003; Kanokmedhakul et al., 2006). These bioactive compounds such as Trichotoxin A50 extracted from *T. harzianum* PC01, and Chaetoglobosin C extracted from *Chaetomium globosum* KMITL-N0802 have been reported to elicit the resistant or immunity in plant (Soytong et al., 2001). The research project was to eva-luate the potential of antagonistic fungi, *C. globosum* KMITL-N0802, *Chaetomium lucknowense* CLT and *T. harzianum* PC01 which are formulated as biological fungicides to control Fusarium wilt of tomato.

MATERIALS AND METHODS

Isolation of pathogen

The diseased samples of tomato wilt were collected from infested fields in Bangkok, Pathumthani, Nakhon Ratchasima, Buriram, Nongkhai, Sakonnakhon, Khon Kaen, and Mukdahan provinces, Thailand. *F. oxysporum* f. sp. *lycopersici* was isolated from diseased plant using tissue transplanting method according to the method of Agrios (1997). Single spore isolation was performed on each isolate to pure culture on potato dextrose agar (PDA).

Pathogenicity test

To confirm the identification of species and form a speciales of *Fusarium* isolates, pathogenicity test was performed using root-dipped method (Marlatt et al., 1996) with conidial suspension of pathogen 2 × 10^6 conidia/ml. Disease severity index (DSI) was scored at 21 days after inoculation based on the modified disease severity scale of Silva and Bettiol (2005). They were as follows: 1 = no symptom; 2 = plant showed yellowing leaves and wilting 1 - 20%, 3 = plant showed yellowing leaves and wilting 21 - 40%, 4 = plant showed yellowing leaves and wilting 41 - 60%, 5 = plant showed yellowing leaves and wilting 61 - 80% and 6 = plant showed yellowing leaves and wilting 81-100% or die. Pathogenicity test was conducted twice for each isolate. All tested isolates were recorded for non-pathogenic and pathogenic isolates. Virulent group was categorized according to DSI as non-pathogenic (DSI =1), low (DSI ≤ 3.50), moderate (DSI > 3.50 - 4.50), and high (DSI > 4.50). The most aggressive isolate was selected for further experiment.

Molecular and phylogenetic analysis

Since there have been variations and doubts in morphological characters, rDNA sequencing analysis of the internal transcribed spacer (ITS) regions were performed to determine the phylogenetic relationship among isolates of *Fusarium* species.

DNA extraction

Stationary cultures were grown in potato-dextrose broth (PDB) for 3 days at room temperature (28 - 32°C). DNA extraction was prepared as the protocol of GF-1 Plant DNA Extraction Kit (Vivantis Co., Ltd., Selangor DE, Malaysia). 0.2 g of fresh weight mycelium was ground to fine powder with mortar and pestle using liquid nitrogen and mixed with 280 µl of lysis buffer. Protinase K (20 mg/ml) was added and mixed before being incubated at 60°C for 1 h in a water-bath. The mixture was kept on ice for 3 min and then centrifuged for 5 min at 10,000 g. The supernatant was added with 20 µl of RNase A (20 mg/ml) and was incubated at 37°C for 5 min. The samples were homogenized by adding equal volumes of a mixture of phosphate buffer (PB) (600 µl) and were incubated for a further 10 min at 60°C. DNA was precipitated by adding 200 µl of absolute ethanol and then the sample was transferred into a column and centrifuged at 10,000 g for 1 min. DNA pellets were washed with 750 µl wash buffer and the procedure repeated again. DNA was eluted by adding 50 µl of 10 mM Tris-HCL, pH 8.5 and centrifuged for 1 min at 10,000 g.

Polymerase chain reaction (PCR) and DNA sequencing

The region of the ribosomal, including a small portion of 18S rDNA, ITS 1, 5.8S rDNA, ITS2, and a small portion of the 28S rDNA, was amplified by PCR. The primers used were, PN3: 5´-CGTTGGT GAACCAGCGGAGGGATC-3´ and PN16: 5´-TCCCTTTCAACAA TTTCACG-3´, as described by Neuvéglise et al. (1994). PCR reactions were conducted using modified method of Yasushi and Tsutomu (2006) in a 25 µl reaction mixture containing 200 ng of DNA template, 4 µl (1.25 mM) dNTPs, 1 µl of each primer (20 *pmol*/µl), 1.5 µl of $MgCl_2$, 0.2 µl of *Taq* DNA polymerase (Vivantis Co., Ltd., Malaysia) and 2.5 µl of 10X PCR buffer. Amp-lification was performed using a thermal cycler. A cycle was composed of an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 2 min, annealing at 55°C for 2 min and extension at 72°C for 3 min. 5 µl of PCR products were electrophoresed in a 1.5% agarose gel in 1xTAE buffer, which was stained with ethidium bromide for observation of the amplicons. PCR products were sent for purification and sequence at Tech Dragon Limited, Hong Kong.

Molecular phylogeny analysis

DNA sequences of tested *Fusarium* sp. were edited and aligned with BioEdit, version 7.0.5 program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Sequences were multiple aligned with Clustal X version 1.83 before the analysis was performed using maximum parsimony methods with PAUP 4.0b (Swofford, 1998). For maximum parsimony analyses, bootstraps of 1,000 replicates were performed to examine the relative of each isolate. Maximum parsimony trees were calculated via fast step-wise addition with the representative isolates of *Fusarium* sp. from GenBank (http://www.ncbi.nlm.nih.gov) as shown in Table 1 and *Verticillium dahliae* was used for analysis as outgroup. Genetic relative among *Fusarium*

Table 1. Sequences of *Fusarium* sp. from GenBank used in this study.

Fusarium species	Locality	ITS GenBank accession No.
F. oxysporum f.sp. *lycopersici*	India	EU214564.1
F. incarnatum	Japan	AY633745.1
F. chlamydosporum	P.R. China	AB369435.1
F. redolens	P.R. China	FJ441013.1
F. solani	USA	AF161222.1
F. sporotrichioides	P.R. China	FJ238107.1

sp. isolates in ITS regions of rDNA seq-uences were determined as a cluster in the phylogenetic tree.

Dual culture test

The antagonistic fungi were tested to inhibit the growth of *F. oxysporum* f. sp. *lycopersici* by using dual culture test. The antagonistic fungi e.g. *C. globosum* N0802, *C. lucknowense* CLT and *T. harzianum* PC01 were provided by Assoc. Prof. Dr. Kasem Soytong of King Mongkut of the Institute of Technology Ladkrabang, Thailand. Dual culture test was conducted using the method of Soytong (1992). The experiment was designed in completely randomized design (CRD) with four replications. The most aggressive isolate of *F. oxysporum* f. sp. *lycopersici* was used in the experiment. The tested dual culture plates were incubated at room temperature (28-32°C). The data were collected as colony diameter and conidial number of pathogenic fungus. The colony diameter and conidia of pathogen were measured and calculated using the following formula:

% Inhibition = [(colony diameter or conidial number of pathogen in control – colony diameter or conidial number of pathogen in dual culture plate) / (colony diameter or conidial number of pathogen in control)] x 100.

The experiment was repeated two times.

Testing on antagonistic substances to inhibit *F. oxysporum* f. sp. lycopersici

The crude extracts from antagonistic fungi were performed using the method of Kanokmedhakul et al. (2006). Antagonistic fungi (*C. globosum* N0802, *C. lucknowense* CLT, and *T. harzianum* PC01) were cultured in PDB at room temperature (28 - 30°C) for 30 days. Mycelial mats were removed from PDB, filtered through cheesecloth and air-dried overnight. Dried mycelial mats were ground and extracted with 200 ml hexane and shaken for 24 h at room temperature. The ground mycelia were separated by filtration through Whatman No. 4 filter paper. The marc was extracted again with hexane using the method described above. Then the filtrates were evaporated in *vacuo* to yield the crude extract. The marc was further extracted with ethyl acetate (EtOAc) and methanol (MeOH) respectively using the same procedure as hexane. The crude extracts of antagonistic fungi were tested for inhibition of the most aggressive isolate of *F. oxysporum* f. sp. *lycopersici* obtained from the previous experiment. The experiment was conducted by using 3x6 factorial in CRD with four replications. Factor A represented crude extracts which consisted of crude hexane, crude ethyl acetate and crude methanol and factor B represented concentrations of 0, 10, 50, 100, 500, and 1,000 µg/ml. Each crude extract was dissolved in 2% dimethyl sulfoxide (DMSO), and then mixed into PDA before autoclaving at 121°C, 15 lbs/inch^2 for 30 min. The tested pathogen was cultured on PDA and incubated at room temperature for 5 days; then colony margin was cut by 3 mm diameter sterilized cork borer. The agar plug of pathogen was transferred to the middle of PDA plate (5.5 cm diameter) in each concentration and incubated at room temperature (28 - 30°C) for 4 days. Data were collected as colony diameter and number of conidia. Percentage of inhibition was computed as described above. The effective dose (ED_{50}) was computed by using inhibitory probit analysis.

Testing bio-agent formulations to control *Fusarium* wilt of tomato *in vivo*

Preparation of bio-agent formulations

Bio-agent formulations were separately formulated as oil formulation according to the method of Soytong (2001) by using spores of antagonistic fungi namely N0802 (*C. globosum*), CLT (*C. lucknowense*), and PC01 (*T. harzianum*). Each antagonistic fungus was cultured in PDB and incubated at room temperature (28 ± 32°C) for 30 days. The culture was filtered to get spore masses, and then put in the electrical mixer and each bio-agent formulation was adjusted to 2.5 × 10^6 spores/ml before been added in sterilized palm's oil. These bio-agent formulations were periodically checked for their shelf life at every month for 1 year.

Testing bio-agent formulations to control Fusarium wilt of tomato

Bio-agent formulations of N0802, CLT and PC01 were tested for their abilities to control tomato wilt caused by *F. oxysporum* f. sp *lycopersici in vivo*. Tomato seedlings var. Sida at 30 days old were inoculated with conidial suspension of *F. oxysporum* f. sp. *lycopersici* at concentration of 2 × 10^6 conidia/ml by dipping root for 15 min and transplanting them into plastic pot containing sterilized mix soil (soil: sand: compost, 4:1:1). The mix soil was sterilized at 121°C, 15 lbs/inch^2 for 1 h in two consecutive days. Randomized completely block design (RCBD) was performed with four replications. Treatments were designed as follows: Bio-agent formulations namely N0802, (T_1); CLT (T_2); Bio-agent formulation namely PC01 (T_3); Chemical fungicide (prochoraz 50% WP) (T_4); inoculated with pathogen and non-treated bio-agent formulation (T_5); and non-inoculated control (T_6). Bio-agent formulations were separately applied in each treatment at the rate of 10 ml/ 20 L of water and prochoraz 50% WP chemical fungicide was applied at the rate of 20 g/ 20 L of water at every 2 weeks by spraying rhizosphere around soil and above plants. Data were collected as DSI, fresh plant and dry weights (g), and fruit weight (g) at 30 days and 60 days. DSI was scaled as previous experiment. Percentage of disease reduction was analyzed using the formula:

% disease reduction = [(Disease severity index of control – Disease severity index of treatment)/ (Disease severity index of control)] x 100.

Fresh plant and dry weights (g) and fruit weight (g) were recorded at harvest day. Percent increase in yield was analyzed using the formula:

[(Yield per plant in treatment – yield per plant in control)/ (yield per plant in treatment)] x 100.

All data were subjected to analysis of variance (ANOVA). Treatment means were statistically compared with Duncan's new multiple range test (DMRT) at P≤0.05 to separate means. The experiment was repeated two times.

RESULTS

Isolation of pathogen and pathogenicity test

The 45 isolates were yielded and confirmed species by using morphological characters and molecular phylogeny. 12 isolates of SSoC04, BRC03, SSoC03, KSoC02, NKRC09, KK2, NKSC01, NKSC02, NKRC02, SRC02, NKRC04 and NSC01 proved to be *F. oxysporum* f.sp. *lycopersici.* These isolates were tested for pathogenicity which clearly showed that all isolates were pathogenic to tomato var. sida. The 15 isolates were proved to be *F. solani* as follows: - MSC02, SSoC02, BRC02, BRC01, NSoC01, NSC09, BKFC03, MSoC03, BKRC02, MSoC02, MSC02, KSoC01, BKSC02 and KSoC04. Out of these, 14 isolates were showed to be *Fusarium incarnatum*; they were: PSC01, PSC04, BKFC12, BKFC06, BKFC04, MSoC01, NSC07, NSC02, NRC04, PSC03, PSC02, KSoC03, PSC05 and BKFC01. With this, 2 isolates were showed to be *Fusarium chlamydosporum* as NSoC04 and BKFC07 and the other 2 isolates of NKRC11 and NKSoC01 were confirmed as *Fusarium redolens.*

Disease severity index was recorded and virulent group was categorized from pathogenicity test on tomato seedlings var. *sida* which were inoculated with conidial suspension of *Fusarium* sp. in each isolate at 2×10^6 conidia/ml. The results revealed that tomato seedlings did not show any wilt symptom after inoculation with isolates of *F. incarnatum, F. chlamydosporum, Fusarium solani, Fusarium redolens* and *Fusarium sporotrichiodes.* Twelve isolates were found to be *F. oxysporum* and expressed wilting symptom, they were then categorized into 3 virulent groups. High virulent group was shown in the isolates NKSC01, NKSC02, and KK2 presented to be the most aggressive isolates whose DSI were 6.00, 5.88, and 5.75, respectively. While, the moderate virulent group were the isolates SRC02, NKRC04, NSC01, and NKRC02 whose DSI were 4.38, 4.38, 4.31, and 4.13, respectively. Moreover, low virulent group was the isolates KSoC02, NKRC09, BRC03, SSoC03, and SSoC04 whose DSI were 3.38, 3.38, 3.19, 3.13, and 3.06, respectively. The isolate NKSC01 was classified as *F. oxysporum* f.sp. *lycopersici* according to molecular study and then selec-ted for testing in further experiment (Table 2).

Molecular phylogeny analysis

Forty-five isolates of *Fusarium* spp. were identified by morphological characters but some isolates did not show clear results under the compound microscope. Therefore, the sequences were studied to confirm morphological identification by using ITS sequences with the length of the complete ITS1, 5.8S and ITS2 including a small portion of 18S rDNA and a small portion of the 28S rDNA. This was clearly demonstrated to identify and confirm the species of *Fusarium* spp. as a valid identification. The phylogenetic tree presented a cluster of the *Fusarium* species into 5 major groups (Figure 1). The Group I belonged to *F. solani* which consisted of isolates BKFC03, BKRC02, BKSC02, KSoC01, KSoC04, MRC02, MSC04, MSoC02, MSoC03, NSC09, NSoC01, BRC01, BRC02, MSC02, SSoC02. Group II was identified as *F. incarnatum* which belonged to the isolates BKFC01, BKFC04, BKFC06, BKFC12, KSoC03, MSoC01, NRC04, NSC02, NSC07, PSC01, PSC02, PSC03, PSC04, and PSC05. While, group III was *F. chlamydosporum* which were classified as BKFC07 and NSoC04. Group IV was confirmed to be *F. oxysporum* which consisted of NKSC01, NKSC02, KK2, NKRC02, NKRC04, SRC02, NSC01, BRC03, KSoC02, SSoC03, SSoC04, and NKRC09. Group V was classified as *F. redolens* which was arranged in the isolates NKRC11 and NKSoC01.

Dual culture test

The inhibition on mycelial growth and conidial production of *F. oxysporum* f. sp. *lycopersici* NKSC01 in dual culture test are shown in Table 3. *T. harzianum* PC01 and *C. lucknowense* CLT gave higher significant inhibition of the mycelial growth of the pathogen than *C. globosum* N0802 which were 90.56, 88.89 and 71.11%, respectively. But, all tested antagonists were not significantly inhibited, the conidia production were 99.99, 92.54 and 92.14%, respectively.

Testing antagonistic substances to inhibit *F. oxysporum* f. sp. *lycopersici*

Crude extracts of tested antagonists could inhibit conidial production of the pathogen at the concentrations ranged from 10 to 1,000µg/ml as shown in Table 4. Crude extracts of tested antagonists showed the highest properties

Table 2. Isolates of *Fusarium* sp. and their pathogenicity results.

Provinces	Isolates	DSI[1]	Virulent group[3]	Provinces	Isolates	DSI	Virulent group
Nong Khai	NKSC01	6.00a[2]	H	Burirum	BRC01	1.00d	NP
	NKSC02	5.88a	H		BRC02	1.00d	NP
	NKRC02	4.13b	M		BRC03	3.19c	L
	NKRC04	4.38b	M	Bangkok	BKFC01	1.00d	NP
	NKRC09	3.38c	L		BKFC03	1.00d	NP
	NKRC11	1.00d	NP		BKFC04	1.00d	NP
	NKSoC01	1.00d	NP		BKFC06	1.00d	NP
Sakon Nakhon	SRC02	4.38b	M		BKFC07	1.00d	NP
	SSoC02	1.00d	NP		BKFC12	1.00d	NP
	SSoC03	3.13c	L		BKRC02	1.00d	NP
	SSoC04	3.06c	L		BKSC02	1.00d	NP
Khon Kaen	KK2	5.75a	H	Pathumthani	PSC01	1.00d	NP
	KSoC01	1.00d	NP		PSC02	1.00d	NP
	KSoC02	3.38c	L		PSC03	1.00d	NP
	KSoC03	1.00d	NP		PSC04	1.00d	NP
	KSoC04	1.00d	NP		PSC05	1.00d	NP
Nakhon	NRC04	1.00d	NP	Mukdahan	MRC02	1.00d	NP
Ratchasima	NSC01	4.31b	M		MSC02	1.00d	NP
	NSC02	1.00d	NP		MSC04	1.00d	NP
	NSC07	1.00d	NP		MSoC01	1.00d	NP
	NSC09	1.00d	NP		MSoC02	1.00d	NP
	NSoC01	1.00d	NP		MSoC03	1.00d	NP
	NSoC04	1.00d	NP		Control	1.00d	

[1]Disease severity index (DSI) was scored at 21 days after inoculation. 1= No symptom; 2 = plant showed yellowing of leaves and wilting 1 - 20%, 3 = plant showed yellowing of leaves and wilting 21 - 40%, 4 = plant showed yellowing of leaves and wilting 41 - 60%, 5 = plant showed yellowing of leaves and wilting 61 - 80%, and 6 = plant showed yellowing of leaves and wilting or die 81 - 100%.
[2]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.01.[3] Virulent group of the isolates was determined according to DSI; NP = Non-pathogenic, L = low, M = moderate, H = high.

to inhibit conidial production of *F. oxysporum* f. sp. *Lycopersici* NKSC01 at 1,000µg/ml. The highest conidial inhibition was presented by using crude hexane and crude ethyl acetate of *C. globosum*, crude hexane, crude ethyl acetate and crude methanol of *C. lucknowense*, crude ethyl acetate and crude methanol of *T. harzianum* which were 63.13, 62.58, 74.85, 69.55, 72.62, 96.93 and 97.50%, respectively. Crude extract of *C. globosum* N0802 which was extracted with hexane showed the highest inhibition of conidial production of the pathogen in which ED_{50} value was 157 µg/ml while crude ethyl acetate and crude methanol presented their abilities to inhibit conidial production at the ED_{50} values 339 and 302 µg/ml, respectively. Crude hexane of *C. lucknowense* CLT gave the highest inhibition of ED_{50} value which was 188 µg/ml followed by crude ethyl acetate and crude methanol in which the ED_{50} values were 209 and 212 µg/ml, respectively. Crude extracts of *T. harzianum* PC01 which was extracted from methanol showed the highest inhibition of conidial production of which the ED_{50} value was 192µg/ml while crude ethyl acetate and crude hexane were 232 and 861 µg/ml, respectively.

Testing bio-agent formulations to control *Fusarium* wilt of tomato *in vivo*

Result showed that bio-agent formulations namely N0802, CLT and PC01 gave significantly, high disease reduction of tomato wilt which were 44.68, 36.28 and 41.01 %, respectively, followed by prochoraz treatment (21.95%). This resulted to increase in yield of tomato, bio-agent formulations of N0802, CLT and PC01. There were also significant increased in yield of tomato which were 88.53, 83.74 and 87.24%, respectively, followed by prochoraz treatment (41.57%). With this, disease severity index (DSI) of tomatoes which were treated with bio-agent formulations of developed N0802, CLT, PC01 and

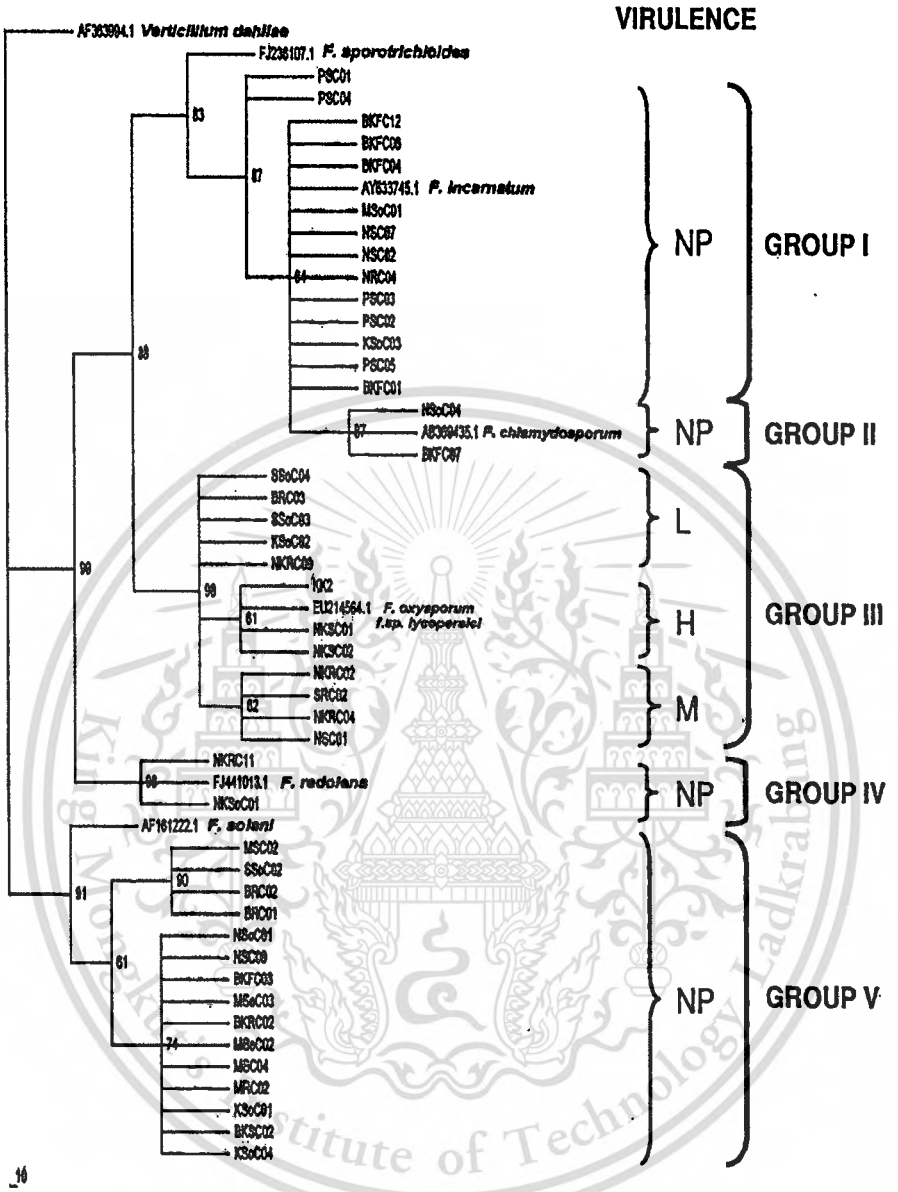

Figure 1. Phylogenetic relationships of *Fusarium* sp. inferred by the parsimony analysis of rDNA sequences. Phylogenetic tree was obtained from analysis by the parsimony method using the PAUP*4.0b program. *Verticillium dahliae* was used as an outgroup. The numbers above the lines represent the 1000 replicates parsimony bootstrap values. Virulence group; NP = non pathogenic, L = low virulent, M = moderate virulent, H = high virulent.

prochloraz at 60 days were not significantly different in DSI which were 2.6, 3.0, 2.8, and 3.7, respectively when compared to tomato inoculated with pathogen, the DSI was 4.7 (Table 5). Moreover, the tested bio-agent formulations gave significantly higher plant growth parameters than prochloraz and non-treated control. Treated

Table 3. Mycelial and conidial inhibition of antagonistic fungi against *Fusarium oxysporum* f.sp. *lycopersici* isolate NKSC01 in dual culture test at 30 days.

Antagonistic fungi	Mycelial inhibition[1] (%)	Conidial inhibition (%)
Chaetomium globosum N0802	71.11b[1]	92.14a
Chaetomium lucknowense CLT	88.89a	92.54a
Trichoderma harzianum PC01	90.56a	99.99a

[1]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 4. Testing antagonistic substances at concentration of 1,000 µg/ml to inhibit *Fusarium oxysporum* f.sp. *lycopersici* isolate NKSC01 at 7 days.

Antagonistic fungi	Crude extract	Conidial inhibition[1] (%)	ED_{50} (µg/ml)
Chaetomium globosum N0802	CG/Hexane	63.13c	157
	CG/Ethyl acetate	62.58c	339
	CG/Methanol	56.57d	302
Chaetomium lucknowense CLT	CL/Hexane	74.85b	188
	CL/ Ethyl acetate	69.55b	209
	CL/Methanol	72.62b	212
Trichoderma harzianum PC01	Thz/Hexane	52.06d	861
	Thz/Ethyl acetate	96.93a	232
	Thz/Methanol	97.50a	192

[1]Average of four replications. Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05.

Table 5. Testing bio-agent formulations to control Fusarium wilt of tomato *in vivo* for 60 days.

Treatments[1]	DSI	DR[3] (%)	Plant height (cm)	Plant fresh weight (g)	Plant dry weight (g)	Yield/plant (g)	Increase in yield[4] (%)
N0802	2.60b[2]	44.68a	37.40b	67.55a	11.53a	133.81a	88.53a
CLT	3.00b	36.28a	35.65b	54.90ab	8.68a	94.40ab	83.74a
PC01	2.80b	41.01a	40.20ab	54.90ab	12.11a	120.32ab	87.24a
prochoraz	3.70ab	21.95b	21.10c	32.5bc	4.90b	26.27c	41.57b
Fol	4.70a	-	14.80c	22.20c	3.80b	15.35c	-
No-Fol	1.00c	-	49.50a	50.35ab	9.89a	82.47b	81.39a

[1] N0802 = *C. globosum* N0802, CLT = *C. lucknowense* CLT, PC01 = *T. harzianum* PC01, Fol = inoculated with *F. oxysporum* f sp *lycopersici* only, No-Fol= non-inoculated with pathogen and non-treated bio-agent formulation. [2]Average of four replications (5 plants/rep.). Means with the same common letters in each column are not significantly different according to Duncan's multiple range test at P = 0.05. [3]% disease reduction (DR) = disease severity index (DSI) of control – disease severity index of treatment/ disease severity index of control x 100. [4]% increase in yield = Yield per plant of treatment – Yield per plant of control/ Yield per plant of treatment x 100.

bio-agent formulations of N0802, CLT and PC01 gave non-significant difference in plant height as 37.40, 35.65 and 40.20 cm, respectively but there was significant difference when compared to prochloraz and inoculated control which showed plant height as 21.10 and 14.80cm, respectively. Moreover, it showed that bio-agent formulations of N0802, CLT PC01 and non-inoculated with pathogen could be significantly higher in fresh plant and dried weights than prochloraz and inoculated control. Interestingly, bio-agent formulation treatments of N0802 gave significantly high yields as 133.81 g/plant, followed by bio-agent formulations treatments of CLT, PC01 and non-inoculated treatment which were 94.40, 120.32 and 82.47 g/plant, respectively. Prochloraz treatment and inoculated control gave non-significant differrence in yields as 26.27 and 15.35 g/plant, respectively.

DISCUSSION

F. oxysporum f. sp. *lycopersici* NKSC01 was isolated from infested tomato fields in Thailand and it proved to be

the most aggressive isolate of tomato var. Sida as confirmed by Sibounnavong et al. (2010). The sequences were confirmed through morphological identification by using ITS sequences with the length of the complete ITS1, 5.8S and ITS2 including a small portion of 18S rDNA and a small portion of the 28S rDNA. It was clearly demonstrated that the isolate NKSC01 is *F. oxysporum* f.sp. *lycopersici* with a valid identification. There are many reports showing that this technique has been validly used to confirm the species of *F. oxysporum* (Paplomatas, 2004; Hirano and Arie, 2006; Kawabe et al., 2005).

Dual culture test showed that *C. globosum* N0802, *C. lucknowense* CLT and *T. harzianum* PC01 could control Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici*. Based on the results, they exhibited high properties to inhibit conidial production over 90%. The results were similar to the report of Srinon et al. (2006) who reported that *T. harzianum* WS01 showed efficacies of more than 90% to inhibit conidial production of *F. oxysporum* f. sp. *lycopersici and F. oxysporum* f. sp. *Cucumerinum* (wilt of cucumber). In this study, the antagonistic substances (crude extracts) of *C. globosum* N0802, *C. lucknowense* CLT and *T. harzianum* PC01 at 1,000 µg/ml could inhibit conidial production of pathogen over 50%. With this, crude extracts of *T. harzianum* PC01 at 1,000 µg/ml inhibited conidial production over 90%. These results are similar to many works that showed that antagonistic substances from *C. globosum* CG extracted with ethyl acetate and *T. harzianum* PC01 extracted with ethyl acetate at 500 µg/ml could inhibit conidia production of *Colletotrichum gloeosporioides* WMF01 causing anthracnose disease of grape in which ED_{50} values were 2 and 7 µg/ml, respectively (Soytong et al., 2005). The result of this study showed that the ED_{50} values of crude extracts of *C. globosum* N0802 and *T. harzianum* PC01 were 157 and 192 µg/ml, respectively with effective inhibition to Fusarium wilt pathogen. These tested antagonistic fungi showed antibiotic mechanism to inhibit growth of Fusarium wilt pathogen which was supported by Soytong (1992) who stated that antagonistic substance from *C. cupreum*, *C. globosum* and *T. harzianum* could inhibit growth and also break the cells of *F. oxysporum* f. sp. *lycopersici*. Moreover, Park et al. (2005) stated that liquid culture of *C. globosum* F0142 could suppress the development of disease more than 80% and can exhibit antifungal activity against *Phytophthora infestans* in tomato at moderate level *in vivo*. *C. globosum* N0802 was also reported to produce antibiotic substances namely chaetomanone that exhibited antitubercular activity against *Mycobacterium tuberculosis*, a major human disease (Kanokmedhakul et al., 2002). Besides, Suwan et al. (2000) has reported that *T. harzianum* PC01 can also be used to produced trichotoxin A50, an antibiotic polypopeptides, which could inhibit the pathogen, stimulate plant growth and induce plant immunity. Moreover, Haggag and Mohamed (2007) reported that *T. harzianum* salt-tolerant mutants produced antifungal metabolites (trichodermin, gliotoxin and gliovirin) as antibiotics that could reduce the growth rate of *F. oxysporum*. These results were supported by the work of Yiğit and Dikilitas (2007) which stated that *T. harzianum* has multi mechanism of actions for controlling plant pathogens that is mycoparasitism via production of chitinase, β-1-3 glucanase, β-1-4 glucanase, antibiotic, competition, induced resistance and inactivation of enz-yme produced by pathogen in the process of infection.

In vivo testing, bio-agent formulations N0802 (*C. globosum*), CLT (*C. lucknowense*) and PC01 (*T. harzianum*) clearly demonstrated that these bio-agent formulations gave a highly effective control of Fusarium wilt of tomato caused by *F. oxysporum* f. sp *lycopersici*. The bio-agent formulations could reduce disease incidence of tomato wilt, leading to increase in yield. The study showed that bio-agent formulation of N0802 could decrease wilt incidence of 44.68% and increases the yield by 88.53%. The bio-agent formulation of PC01 decreased disease incidence of 50.97% and increased the yield by 87.24%. This result is similarly reported by Soytong (1992) who stated that registered bio-fungicide formulated from *C. cupreum* could decrease disease incidence of tomato wilt and also increase its yield. The result from bio-agent formulation of PC01 in this study is also supported by the work of Yiğit and Dikilitas (2007). It was reported that *T. harzianum* T-22 could reduce disease incidence of tomato Fusarium wilt at 42% and also improve yield and mineral contents of tomatoes.

As a result of bio-agent formulations N0802 (*C. globosum*), CLT (*C. lucknowense*) supported the previous work of Soytong et al. (2005) which showed that *Chaetomium* bio-products formulated from *C. globosum* and *C. cupreum* as powder formulation could control bud rot and basal stem rot of bottle palms caused by *Thielaviopsis paradoxa* in the field and reduce disease incidence by 75%. These new bio-agent formulations which act as concentration suspension in oil form were of different formulation but are effective in controlling Fusarium wilt of tomato as seen in the report of Soytong et al. (2001) who showed that the biological products consist of *Chaetomium* sp. (22 strains of *C. cupreum* and *C. globosum*) in biopellet and biopowder formulations which when applied to the soil could suppress the growth of *F. oxysporum* f. sp. *lycopersici* and reduce infection rate in tomato.

ACKNOWLEDGMENTS

This research was a part of Ph.D. thesis and was supported by Nakhon Ratchasima Rajabhat University. We thank Mr. Sowat Mardwangsaeng and Mrs. Sukrittaya Dannarong for their help in collecting samples and Ms.

Kanchalika Rattanacherdchai for her help with this experiment.

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King Mongkut's Institute of Technology Ladkrabang

Proceedings of The International Conference on Integration of Science & Technology for Sustainable Development (ICIST)

"Biological Diversity, Food and Agricultural Technology"

26 - 27 April, 2007

Held at KMITL, Bangkok, Thailand.

International Conference on Integration of Science & Technology
for Sustainable Development, Bangkok, Thailand 26 – 27 April 2007, 143-148.

Effects of media and pH levels on the growth of *Fusarium* wilt of tomato and its pathogenicity test

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ABSTRACT

Fusarium oxysporum f.sp. *lycopersici* was isolated from infected fruits, leaves, and stems of tomato plants where collected samples from infested areas. Four isolates of *F. oxysporum* f.sp. *lycopersici* were studied for the growth on agar and liquid media in eight levels of pH (3-10) and tested for its pathogenicity. The fungal isolate BFC01, BFC04, PLC01, and PSC03 grew very fast on both types of media in 5-7 days. The optimum media were PDA at pH 5-8. For its pathogenicity test, disease severity rating of four isolates were between 2.95-3.78. All tested isolates were proved to be pathogenic to tomato seedlings.

Keywords

Fusarium oxysporum f. sp *lycopersici, pathogenicity*

1. INTRODUCTION

Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp *lycopersici* (Sacc.) Snyder & Hansen, which causes economic losses especially on susceptible varieties and under favorable weather condition. Fusarium wilt of tomato is the most destructive in warm climate and sandy soil of temperate regions. It becomes one of the most prevalent and damaging diseases wherever tomatoes are grown intensively because the pathogen can persist indefinitely in infested soils. Its symptoms also include strong downward bending of petioles, yellowing, wilting and dying of the lower leaves, often on one side of the plant. Root necrosis is often extensive. After a few weeks, browning of the vascular system may be seen by cutting the stem open with a knife. This brown discoloration inside the stem may extend from the roots of the plant to the top. Plant growth is stunted and, under warm conditions, the plant may die[1]. Mycelium of *F. oxysporum* grew within the range of pH 2-12 and pH 6 was the most suitable for the growth of all species of *Fusarium*, while a highly acidic medium was unsuitable for sporulation of all species[5]. The list of essential nutrient elements for the growth, sporulation and virulence of *F. oxysporum* includes carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, magnesium, iron, manganese, molybdenum, and zinc. Study on suitable condition for these fungal growth were done by comparing with type of media in different pH levels and to test for pathogenicity of these four *Fusarium* isolates.

2. MATERIALS AND METHODS

2.1 Fungal culture

Four isolates of *F. oxysporum* f.sp. *lycopersici* were collected from infested tomato fields in Bangkok and Pathumthani. Fungal isolations were done by moisted chamber techniques for ripen fruits and tissue transplanted technique for leaves and stems of disease plants. Segments of leaves and stems were surface sterilized by 70% ethanol for 5 minutes and washed with sterile water three times. Tissues were plated on water agar (WA) medium and incubated for 2 days at room temperature. Hyphal tip of fungi were cut and moved into potato dextrose agar (PDA) plates. Single spore isolates were obtained and the culture characteristics and micromorphology were investigated under compound microscope.

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2.2 The growth of fungi on agar media and pH level

The experiment was designed to factorial in Completely Randomized Design (CRD) with four replications, factor A was the type of media as follows:- potato dextrose agar (PDA), potato dextrose yeast extract agar (PDYA), and czapek dox agar (CZA) and factor B was pH level of media as follows:- pH 3, 4, 5, 6, 7, 8, 9, and 10 which were adjusted by hydrochloric acid (HCl) and sodium hydroxide (NaOH). All four isolates of *F. oxysporum* f.sp. *lycopersici* were grown on PDA for 7 days, then agar plug was cut at pheripheral of colony by cork borer, and transferred to the middle plate in each tested media and pH level. The inoculated plates were incubated at room temperature for 7 days before data collection as colony diameter (cm) and number of conidia. Data were computed analysis of variance (ANOVA) by SPSS statistic analysis program version 11. Means were compared with Duncan's New Multiple Range Test (DMRT) at P=0.05.

2.3 The growth of fungi on liquid media and pH level

The experiment was designed to factorial in Completely Randomized Design (CRD) with four replications, factor A was liquid media as follows:- potato dextrose broth (PDB), potato dextrose yeast extract broth (PDYB), and czapek dox broth (CZB) and factor B was pH level of media as follows:- pH 3, 4, 5, 6, 7, 8, 9, and 10 which were adjusted by hydrochloric acid (HCl) and sodium hydroxide (NaOH). For mycelial growth and sporulation; the samples were cultured in liquid media in stationary condition at room temperature. After 5 days, conidial concentrations were evaluated by counting under microscope using haemacytometer. Fungal mycelium was taken off from liquid media and filtrated through four layers cheesecloth then dried with natural air at 28-32°c for 48 hrs and weighed for growth rate compared within four isolates. Data were computed analysis of variance (ANOVA) by SPSS statistic analysis program version 11. Means were compared with Duncan's New Multiple Range Test (DMRT) at P=0.05.

2.4 Pathogenicity test

To confirm the forma specialis of all tested isolates of *F. oxysporum* f.sp. *lycopersici*, pathogenicity tests were performed using 15-day tomato seedlings (variety *sida-somtam*) in greenhouse. Fungal isolates were grown on PDA for 7 days at room temperature then, the fungal mycelium were ground and filtrated through cheesecloth to obtain conidial suspension, then adjusted to 3 concentrations; $1x10^5$, $1x10^6$ and $1x10^7$ conidia/ml. Seedlings were grown in sandy-loam soil for 15 days. The root-dipped method was used for inoculation[2]. Seedlings were removed soil from the roots and washed with tab water to removed excess soil. The roots tip of sixteen seedlings were cut with scissors for 5 mm and then dipped into each conidial suspension for 5 minutes and then transplanted in 8 cm diameter plastic pots that contained a sterile planted soil. For the control, seedling roots were dipped into sterile water without inoculum. Inoculated plants were placed in a greenhouse (25-32° c). External symptoms and disease severity using a disease severity index (DSI) were scored at 21days after inoculation, on the modified following scale of Silva, and Bettiol[3]:- 0= no symptom; 1= symptom on leave 1-20%, lower leaf yellow; 2= symptom on leave 21-40%, plant showed yellowing or wilting of two leaves and; 3= symptom on leave 41-60%, plant showed yellowing or wilting of more two leaves ;4= symptom on leave 61-80%, plant showed vessel browning nearly to the leader shoot, with the most leaves wilted except the leader shoot; and 5= symptom on leave 81-100%, plant showed wilted of leaves up to the shoot or died. The disease severity was analyzed using a factorial experiment in CRD and computed analysis of variance (ANOVA). The mean comparison was conducted by using Duncan's New Multiple Range Test (DMRT) at P=0.05. The experiment was repeated four times.

3. RESULTS AND DISCUSSION

The growth of fungi on different media and pH level

All isolates of fungi were measured the colony diameter on three agar media which revealed over 60 mm except for pH 3 media (data not shown). All isolates produced white to pink colony and abundant aerial mycelia on PDA at pH 4-10, the same result as PDYA and CZA at pH 5-10 (Fig 1). Study on conidia concentration of 3 media with 8 levels of pH, all isolates produced abundant of both macro-conidia and micro-conidia on 3 media at pH 5, 6, 7, and 8 (Table 1).

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Dry weight of fungal mycelium gave significantly different among the type of media and pH levels. The data showed that dry weight of all tested media at pH 6,and 7 gave the highest value, especially in isolate PLC01 and PSC03 (Table 2). Conidia concentration of fungi cultured on liquid media showed that all isolates produced both macro-conidia and micro-conidia abundantly in all tested media at pH 4-8 (Table 3).

Our results were similar to the work of Susan[5] who reported that *F. oxysporum* f.sp. *cubense* grew most rapidly at pH 6 or pH 7 in some isolates. All isolates grew at pH 4 but the growth rate was lower than at pH 5, 6, and 7. This result was also the same as Srobar[4] who reported that pH 6 was the most suitable for the growth of all species of *Fusarium* while a highly acidic medium was unsuitable for sporulation of all species.

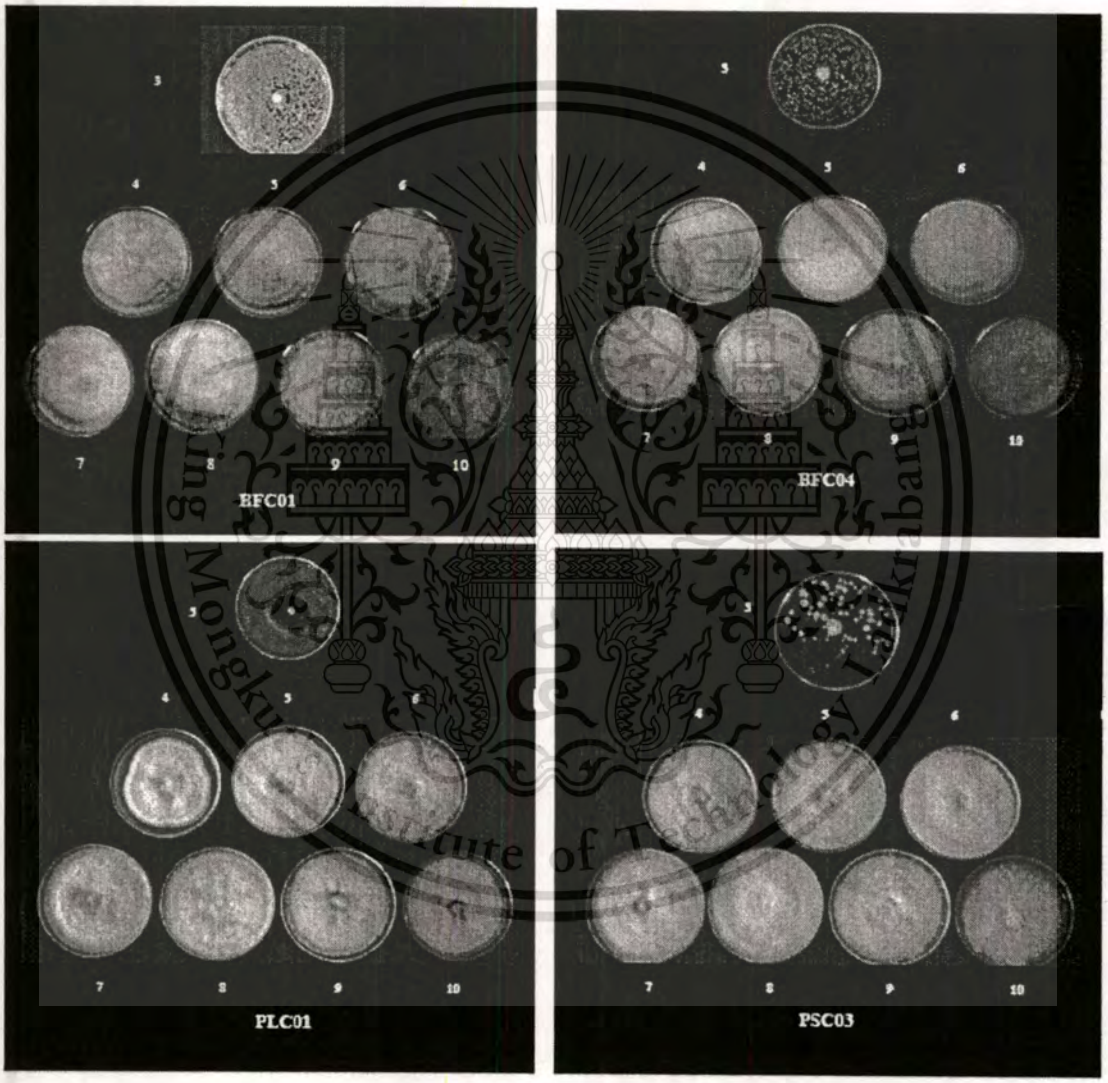

Fig 1 Colony of *Fusarium oxysporum* f.sp. *lycopersici* cultured on PDA at pH 3-10 for 7 days.

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Table 1 Conidia of *Fusarium oxysporum* on agar media at pH 3 -10 for 7 days.

Isolate	Media	Conidia concentration (x10^6 conidia/ml)								
		pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	CV (%)
BFC01	PDA	0.64e[1/]	6.85c	8.94b	11.98a	8.45b	4.30d	3.38d	4.87cd	23.57
	PDYA	0.91e	6.79d	16.95b	10.42c	24.49a	8.06d	7.05d	8.48d	11.17
	CZA	0.82e	10.72d	4.09e	36.08a	20.89c	19.88c	28.31b	8.21d	28.68
BFC04	PDA	0.71d	2.89bc	9.97a	3.30bc	3.86bc	4.01b	2.66c	3.11bc	19.75
	PDYA	0.75e	3.34c	3.71bc	3.67bc	4.54ab	4.88a	1.91d	4.28abc	18.80
	CZA	3.90a	0.60c	1.95b	4.05a	1.76b	1.72b	1.80b	1.65b	23.66
PLC01	PDA	0.56e	4.39c	10.18a	9.25a	9.00a	6.86b	3.30d	2.03e	18.47
	PDYA	0.71d	3.26c	8.29a	5.89b	5.74b	5.38b	3.04c	3.04c	16.10
	CZA	0.64c	2.48b	4.46a	3.94a	3.60a	3.68a	2.55b	2.32b	22.09
PSC03	PDA	1.16e	5.85b	4.54c	7.20a	6.79a	4.88c	4.12c	3.11d	12.57
	PDYA	1.16e	2.85d	3.60cd	6.15b	7.61a	6.00b	4.46c	2.85d	20.06
	CZA	0.90e	4.58c	7.01a	6.83ab	5.59bc	4.65c	3.15d	2.25de	21.28

[1/] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

Table 2 Dry weight of fungal mycelium cultured in liquid media for 5 days.

Isolate	Media	Dry weight of fungal mycelium (mg)								
		pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	CV (%)
BFC01	PDB	17.50c[1/]	56.00b	72.00a	66.00a	72.25a	60.75ab	58.75ab	58.75ab	17.95
	PDYB	13.00c	63.50b	79.25a	73.75ab	70.75ab	73.75ab	73.00ab	62.50b	11.08
	CZB	61.25ab	72.00a	60.50ab	68.75ab	71.50a	69.50ab	64.50ab	57.25b	11.73
BFC04	PDB	46.00c	59.25bc	74.25ab	72.25ab	87.00a	60.50bc	60.75bc	76.75ab	16.86
	PDYB	28.00b	71.25a	81.75a	66.25a	81.25a	85.75a	79.75a	67.00a	19.78
	CZB	59.50abc	61.50abc	44.25c	61.25abc	54.25bc	67.50ab	55.50bc	79.75a	21.47
PLC01	PDB	57.50d	106.00abc	114.50a	110.00ab	111.00ab	113.50ab	103.75bc	99.50c	6.20
	PDYB	61.50c	106.00b	94.75b	104.50b	119.75a	104.50b	121.75a	102.75b	8.09
	CZB	95.75bc	119.50a	79.25d	105.00ab	115.00a	85.25cd	104.50ab	107.00ab	9.54
PSC03	PDB	59.00c	99.00ab	94.50ab	100.50ab	116.25a	82.00bc	89.50b	81.75bc	17.14
	PDYB	53.00d	104.25bc	124.25a	117.00ab	102.75bc	118.75ab	54.25d	89.75c	12.59
	CZB	81.25a	78.00a	58.00bc	78.50a	61.00b	66.50ab	42.75cd	34.25d	17.53

[1/] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

Table 3 Conidia of *Fusarium oxysporum* on 3 liquid media at pH 3 -10 for 5 days.

Isolate	Media	Conidia concentration (x10^4 conidia/ml)								
		pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	CV (%)
BFC01	PDB	2.50c[1/]	13.88a	3.44bc	5.31b	11.31a	5.62b	3.06bc	2.94bc	27.85
	PDYB	4.31b	8.44a	3.38bc	2.50c	4.81b	4.81b	2.19c	2.38c	23.33
	CZB	4.00d	13.44a	9.12b	8.44bc	3.94d	8.25bc	6.06cd	5.38d	21.92
BFC04	PDB	1.00e	2.31d	6.31a	5.00bc	5.44b	4.56c	1.12e	1.12e	16.44
	PDYB	2.62d	4.06c	4.50bc	5.12ab	5.81a	5.19ab	2.69d	2.00d	12.70
	CZB	6.75d	18.00b	14.19c	20.50a	19.81a	17.94b	12.69c	16.94b	7.45
PLC01	PDB	2.00e	5.44d	6.44c	8.25b	8.69b	10.19a	4.88d	4.88d	10.26
	PDYB	1.88d	3.75e	8.31b	8.75b	10.50a	9.1ab	2.50d	2.19d	11.89
	CZB	2.31c	4.94b	8.38a	9.38a	8.25a	2.69c	2.50c	2.31c	16.61
PSC03	PDB	2.84c	5.88ab	4.56b	7.06a	5.56b	4.69b	2.69c	1.88c	19.50
	PDYB	2.75d	5.94c	9.25b	10.62a	9.31b	8.81b	1.75de	1.44e	11.81
	CZB	2.31e	6.50d	7.75c	11.06a	9.31b	9.06b	5.50d	3.06e	11.39

[1/] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

Pathogenicity test

All tested isolates proved to be pathogenic isolates to cause wilting on tomato seedlings. There were not significantly different in disease severity between the four isolates at all conidia concentrations (Table 4).

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Table 4 Disease severity of tomato wilt.

Isolates	Disease severity[1]		
	Conidia concentration (conidia/ml)		
	$1x10^5$	$1x10^6$	$1x10^7$
BFC01	3.45a[2]	3.57a	3.70a
BFC04	3.05a	3.00a	3.78a
PLC01	2.95a	3.40a	3.78a
PSC03	3.10a	3.27a	3.65a
control	0.50b	0.25b	0.00b

[1] Disease severity – rating: 0=no symptom to 5= symptom on leave 81-100%, plant showed wilted leaved up to the leader shoot or died

[2] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

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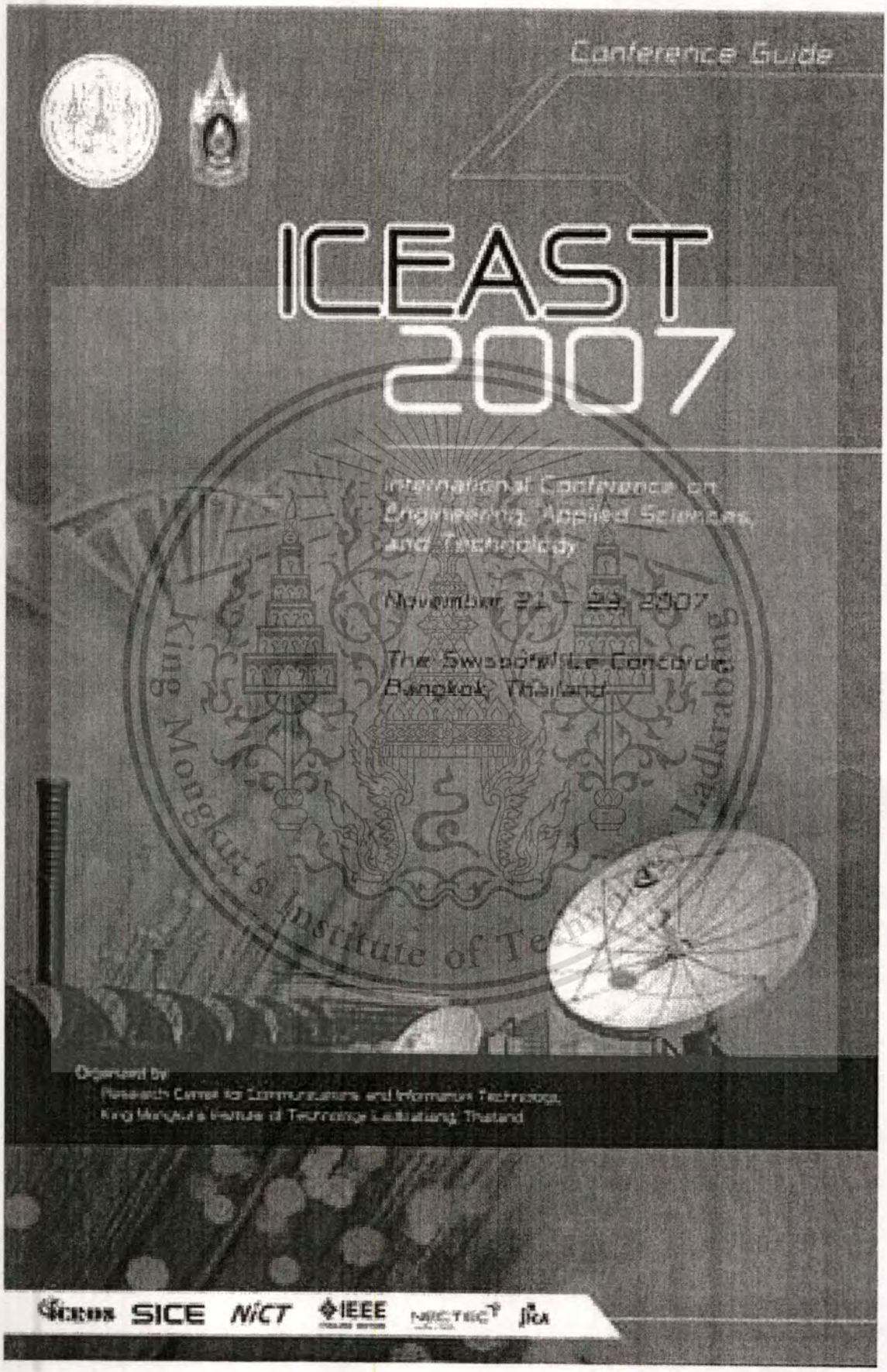

A study on Fusarium wilt of tomato : fungal morphology, suitable media and pH level for fungal growth and its pathogenicity test

Chamaiporn Charoenporn[1], Somdej Kanokmedhakul[2], Fucheng Lin[3], and Kasem Soytong[1]
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Abstract—*Fusarium oxysporum* f.sp. *lycopersici* was isolated from infected fruits, leaves, and stems of tomato plants where collected samples from infested areas. Four isolates of *F. oxysporum* f.sp. *lycopersici* were studied for morphology, the growth on agar and liquid media in eight levels of pH (3-10) and tested for its pathogenicity. The fungal isolate BFC01, BFC04, PLC01, and PSC03 have similar characteristic; macro-conidia were falcate, 3-6 septate, hyaline, sparse to abundant, 2.6-4.8 x 14.1-34.2 µm, micro-conidia were ellipsoid, oval or allantoid, 0-1 septate, hyaline, 1.9-4.9 x 6.8-23.3 µm, chlamydospore formed intercalary and terminal, smooth walled. The fungi grew very fast on both types of media in 5-7 days. The suitable media were PDA at pH 5-8. For its pathogenicity test, disease severity rating of four isolates were between 2.95-3.78. All tested isolates were proved to be pathogenic to tomato seedlings.

Keywords—*Fusarium oxysporum* f. sp *lycopersici*, morphology, pathogenicity

I. INTRODUCTION

Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp *lycopersici* (Sacc.) Snyder & Hansen, which widely distributed in soil and on organic substrates and causes economic losses especially on susceptible varieties under favorable weather condition. 28°c soil temperature, low soil moisture, short day length, low in nitrogen and phosphorus and high in potassium, and low pH. The fungi survived winter in the mycelium or chlamydospore state [2]. Fusarium wilt of tomato is the most destructive in warm climate and sandy soil of temperate regions. It becomes one of the most prevalent and damaging diseases wherever tomatoes are grown intensively because the pathogen can persist indefinitely in infested soils. Its symptoms also include strong downward bending of petioles, yellowing, wilting and dying of the lower leaves, often on one side of the plant. Root necrosis is often extensive. After a few weeks, browning of the vascular system may be seen by cutting the stem open with a knife. This brown discoloration inside the stem may extend from the roots of the plant to the top. Plant growth is stunted and, under warm conditions, the plant may die [1]. Mycelium of *F. oxysporum* grew within the range of pH 2-12 and pH 6 was the most suitable for the growth of all species of *Fusarium*, while a highly acidic medium was unsuitable for sporulation of all species [6]. The list of essential nutrient elements for the growth, sporulation and virulence of *F. oxysporum* includes carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, potassium, magnesium, iron, manganese, molybdenum, and zinc. Study on morphology, suitable condition for these fungal growth were done by comparing with type of media in different pH levels and to test for pathogenicity of these four *Fusarium* isolates.

II. METHODOLOGY

A. Fungal culture and morphology study

F. oxysporum f.sp. *lycopersici* were collected from infested tomato fields in Bangkok and Pathumthani. Fungal isolations were done by moisted chamber techniques for ripen fruits and tissue transplanting technique for leaves and stems of disease plants. Segments of leaves and stems were surface sterilized by 70% ethanol for 5 minutes and washed with sterile water three times. Tissues were plated on water agar (WA) medium and incubated for 2 days at room temperature. Hyphal tip of fungi were cut and moved into potato dextrose agar (PDA) plates. Single spore isolates were obtained and the culture characteristics and fungal morphology (macro-conidia, micro-conidia, and chlamydospore characteristics) were investigated under compound microscope and compared between four isolates.

B. The growth of fungi on agar media and pH level

The experiment was designed to factorial experiment in Completely Randomized Design (CRD) with four replications, factor A was the type of media as follows:- potato dextrose agar (PDA), potato dextrose yeast extract agar (PDYA), and czapek dox agar (CZA) and factor B was pH level of media as follows:- pH 3,4,5,6,7,8,9,and 10 which were adjusted by hydrochloric acid (HCl) and sodium hydroxide (NaOH).

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All four isolates of *F. oxysporum* f.sp. *lycopersici* were grown on PDA for 7 days, then agar plug was cut at pheripheral of colony by cork borer, and transferred to the middle plate in each tested media and pH level. The inoculated plates were incubated at room temperature for 7 days before data collection as colony diameter (cm) and number of conidia. Data were computed analysis of variance (ANOVA) by SPSS statistic analysis program version 11. Means were compared with Duncan's New Multiple Range Test (DMRT) at P=0.05.

C. The growth of fungi on liquid media and pH level

The experiment was designed to factorial experiment in Completely Randomized Design (CRD) with four replications, factor A was liquid media as follows:- potato dextrose broth (PDB), potato dextrose yeast extract broth (PDYB), and czapek dox broth (CZB) and factor B was pH level of media as follows:- pH 3,4,5,6,7,8,9,and 10 which were adjusted by hydrochloric acid (HCl) and sodium hydroxide (NaOH). For mycelial growth and sporulation; the samples were cultured in liquid media in stationary condition at room temperature. After 5 days, conidial concentrations were evaluated by counting under microscope using haemacytometer. Fungal mycelium was taken off from liquid media and filtrated through four layers cheesecloth then dried with natural air at 28-32°c for 48 hrs and weighed for growth rate compared within four isolates. Data were computed analysis of variance (ANOVA) by SPSS statistic analysis program version 11. Means were compared with Duncan's New Multiple Range Test (DMRT) at P=0.05.

D. Pathogenicity test

To confirm the forma specialis of all tested isolates of *F. oxysporum* f.sp. *lycopersici*, pathogenicity tests were performed using 15-day tomato seedlings (variety *sida-somtam*) in greenhouse. Fungal isolates were grown on PDA for 7 days at room temperature then, the fungal mycelium were ground and filtrated through cheesecloth to obtain conidial suspension, then adjusted to 3 concentrations; $1x10^5$, $1x10^6$ and $1x10^7$ conidia/ml. Seedlings were grown in sandy-loam soil for 15 days. The root-dipped method was used for inoculation [3]. Seedlings were removed soil from the roots and washed with tab water to removed excess soil. The roots tip of sixteen seedlings were cut with scissors for 5 mm and then dipped into each conidial suspension for 5 minutes and then transplanted in 8 cm diameter plastic pots that contained a sterile planted soil. For the control, seedling roots were dipped into sterile water without inoculum. Inoculated plants were placed in a greenhouse (25-32° c). External symptoms and disease severity using a disease severity index (DSI) were scored at 21days after inoculation, on the modified following scale of Silva, and Bettiol [5]:- 0= no symptom; 1= symptom on leaves 1-20%, lower leaf yellow; 2= symptom on leaves 21-40%, plant showed yellowing or wilting of two leaves and; 3= symptom on leaves 41-60%, plant showed yellowing or wilting of more two leaves ;4= symptom on leaves 61-80%, plant showed vessel browning nearly to the leader shoot, with the most leaves wilted except the leader shoot; and 5= symptom on leaves 81-100%, plant showed wilted of leaves up to the shoot or died. The disease severity was analyzed using a factorial experiment in CRD and computed analysis of variance (ANOVA). The mean comparison was computed by using Duncan's New Multiple Range Test (DMRT) at P=0.05. The experiment was repeated four times.

III. RESULTS AND DISCUSSION

A. The fungal morphology

Four isolates of *Fusarium oxysporum* f. sp *lycopersici*, BFC01, BFC04, PLC01, and PSC03 showed similar charachteristics; colony, macro-conidia and micro-conidia, and chlamydospores. The results showed the details in Table 1 and Fig. 1.

B. The growth of fungi on different media and pH level

All isolates of fungi were measured the colony diameter on three agar media which revealed over 60 mm except for pH 3 media (data not shown). All isolates produced white to pink colony and abundant aerial mycelia on PDA at pH 4-10, the same result as PDYA and CZA at pH 5-10 . Study on conidia concentration of 3 media with 8 levels of pH, all isolates produced abundant of both macro-conidia and micro-conidia on 3 media at pH 5, 6, 7, and 8 (Table 2).

Dry weight of fungal mycelium gave significantly different among the type of media and pH levels. The data showed that dry weight of all tested media at pH 6,and 7 gave the highest value, especially in isolate PLC01 and PSC03 (Table 3). Conidia concentration of fungi cultured on liquid media showed that all isolates produced both macro-conidia and micro-conidia abundantly in all tested media at pH 4-8 (Table 4).

Our results were similar to the work of Susan [6] who reported that *F. oxysporum* f.sp. *cubense* grew most rapidly at pH 6 or pH 7 in some isolates. All isolates grew at pH 4 but the growth rate was lower than at pH 5,6, and 7. This result was also the same as Srobar [4] who reported that pH 6 was the most suitable for the growth of all species of *Fusarium* while a highly acidic medium was unsuitable for sporulation of all species.

C. Pathogenicity test

All tested isolates proved to be pathogenic isolates to cause wilting on tomato seedlings (var. sida-somtam) at level 2.95-3.78. There were not significantly different in disease severity between the four isolates at all conidia concentrations (Table 5).

TABLE I

MORPHOLOGY OF *FUSARIUM OXYSPORUM* f.sp. *LYCOPERSICI* ISOLATE BFC01, BFC04, PLC01, AND PSC03.

Isolate	Colony	Macro-conidia	Micro-conidia	Chlamydospore
BFC01	White, felted to floccose	Falcate, 3-6 septate, 3.2-4.3 x 14.1-25.5	ellipsoid or oval,0-1 septate, hyaline, 2.9-4.5 x 6.8-17.5	Intercalary or terminal, smooth walled
BFC04	White or pinkish white, felted to floccose	Falcate , 3-5 septate 2.6-4.8 x 16.0-31.1 µm	ellipsoid or oval,0-1 septate, hyaline,1.9-3.4 x 9.5-23.3 µm	Intercalary or terminal, smooth walled
PLC01	Pinkish white, felted to floccose	Falcate , 3-4 septate, 2.9-4.6 x 15.7-34.2 µm	Oval to allantoid,0 septate, hyaline, 3.4-4.9 x 6.5-13.4 µm	Intercalary or terminal, smooth walled
PSC03	White, felted to floccose	Falcate, 3-4 septate, 2.6-4.7 x 17.3-30.2 µm	ellipsoid or oval,0-1 septate, hyaline, 2.3-3.7 x 11.6-20.2 µm	Intercalary or terminal, smooth walled

TABLE II

CONIDIA OF *FUSARIUM OXYSPORUM* ON AGAR MEDIA AT pH 3-10 FOR 7 DAYS

Isolate	Media	Conidia concentration (x10^6 conidia/ml)								
		pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	CV (%)
BFC01	PDA	0.64e[1]	6.85c	8.94b	11.98a	8.45b	4.30d	3.38d	4.87cd	23.57
	PDYA	0.91e	6.79d	16.95b	10.42c	24.49a	8.06d	7.05d	8.48d	11.17
	CZA	0.82e	10.72d	4.09e	36.08a	20.89c	19.88c	28.31b	8.21d	28.68
BFC04	PDA	0.71d	2.89bc	9.97a	3.30bc	3.86bc	4.01b	2.66c	3.11bc	19.75
	PDYA	0.75e	3.34c	3.71bc	3.67bc	4.54ab	4.88a	1.91d	4.28abc	18.80
	CZA	3.90a	0.60e	1.95b	4.05a	1.76b	1.72b	1.80b	1.65b	23.66
PLC01	PDA	0.56e	4.39c	10.18a	9.25a	9.00a	6.86b	3.30d	2.03e	18.47
	PDYA	0.71d	3.26c	8.29a	5.89b	5.74b	5.38b	3.04c	3.04c	16.10
	CZA	0.64c	2.48b	4.46a	3.94a	3.60a	3.68a	2.55b	2.32b	22.09
PSC03	PDA	1.16e	5.85b	4.54c	7.20a	6.79a	4.88c	4.12c	3.11d	12.57
	PDYA	1.16e	2.85d	3.60cd	6.15b	7.61a	6.00b	4.46c	2.85d	20.06
	CZA	0.90e	4.58c	7.01a	6.83ab	5.59bc	4.65c	3.15d	2.25de	21.28

[1] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

TABLE III

DRY WEIGHT OF FUNGAL MYCELIUM CULTURE IN LIQUID MEDIA FOR 5 DAYS

Isolate	Media	Dry weight of fungal mycelium (mg)								
		pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	CV (%)
BFC01	PDB	17.50c[1]	56.00b	72.00a	66.00a	72.25a	60.75ab	58.75ab	58.75ab	17.95
	PDYB	13.00e	63.50b	79.25a	73.75ab	70.75ab	73.75ab	73.00ab	62.50b	11.08
	CZB	61.25ab	72.00a	60.50ab	68.75ab	71.50a	69.50ab	64.50ab	57.25b	11.73
BFC04	PDB	46.00c	59.25bc	74.25ab	72.25ab	87.00a	60.50bc	60.75bc	76.75ab	16.86
	PDYB	28.00b	71.25a	81.75a	66.25a	81.25a	85.75a	79.75a	67.00a	19.78
	CZB	59.50abc	61.50abc	44.25c	61.25abc	54.25bc	67.50ab	55.50bc	79.75a	21.47
PLC01	PDB	57.50d	106.00abc	114.50a	110.00ab	111.00ab	113.50ab	103.75bc	99.50c	6.20
	PDYB	61.50e	106.00b	94.75b	104.50b	119.75a	104.50b	121.75a	102.75b	8.09
	CZB	95.75bc	119.50a	79.25d	105.00ab	115.00a	85.25cd	104.50ab	107.00ab	9.54
PSC03	PDB	59.00c	99.00ab	94.50ab	100.50ab	116.25a	82.00bc	89.50b	81.75bc	17.14
	PDYB	53.00d	104.25bc	124.25a	117.00ab	102.75bc	118.75ab	54.25d	89.75c	12.59
	CZB	81.25a	78.00a	58.00bc	78.50a	61.00b	66.50ab	42.75cd	34.25d	17.53

[1] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

TABLE IV

CONIDIA OF *FUSARIUM OXYSPORUM* ON 3 LIQUID MEDIA AT PH 3-10 FOR 5 DAYS

Isolate	Media	Conidia concentration (x10^6 conidia/ml)								
		pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	CV (%)
BFC01	PDB	2.50c[1]	13.88a	3.44bc	5.31b	11.81a	5.62b	3.06bc	2.94bc	27.85
	PDYB	4.31b	8.44a	3.38bc	2.50c	4.81b	4.81b	2.19c	2.38c	23.33
	CZB	4.00d	13.44a	9.12b	8.44bc	3.94d	8.25bc	6.06cd	5.38d	21.92
BFC04	PDB	1.00e	2.31d	6.31a	5.00bc	5.44b	4.56c	1.12e	1.12e	16.44
	PDYB	2.62d	4.06c	4.50bc	5.12ab	5.81a	5.19ab	2.69d	2.00d	12.70
	CZB	6.75d	18.00b	14.19c	20.50a	19.81a	17.94b	12.69c	16.94b	7.45
PLC01	PDB	2.00e	5.44d	6.44c	8.25b	8.69b	10.19a	4.88d	4.88d	10.26
	PDYB	1.88d	3.75c	8.31b	8.75b	10.50a	9.1ab	2.50d	2.19d	11.89
	CZB	2.31c	4.94b	8.38a	9.38a	8.25a	2.69c	2.50c	2.31c	16.61
PSC03	PDB	2.84c	5.88ab	4.56b	7.06a	5.56b	4.69b	2.69c	1.88c	19.50
	PDYB	2.75d	5.94c	9.25b	10.62a	9.31b	8.81b	1.75de	1.44e	11.81
	CZB	2.31e	6.50d	7.75c	11.06a	9.31b	9.06b	5.50d	3.06e	11.39

[1] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

TABLE V
DISEASE SEVERITY OF TOMATO WILT FOR PATHOGENICITY

Isolates	Disease severity[1/]		
	Conidia concentration (conidia/ml)		
	$1x10^5$	$1x10^6$	$1x10^7$
BFC01	3.45a[2/]	3.57a	3.70a
BFC04	3.05a	3.00a	3.78a
PLC01	2.95a	3.40a	3.78a
PSC03	3.10a	3.27a	3.65a
control	0.50b	0.25b	0.00b

[1/] Disease severity – rating: 0=no symptom to 5= symptom on leave 81-100%, plant showed wilted leaved up to the leader shoot or died

[2/] Mean of four replications. Mean followed by a common letter are not significantly different when compared to Duncan's New Multiple Range Test at P=0.05

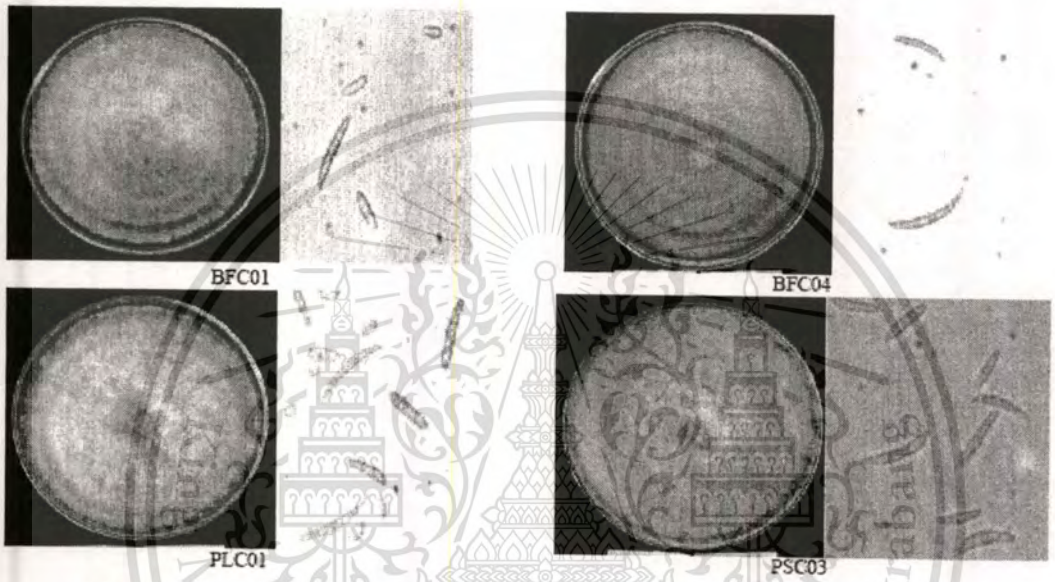

Fig. 1 Colony and conidia of *Fusarium oxysporum* f. sp *lycopersici* isolate BFC01, BFC04, PLC01, and PSC03.

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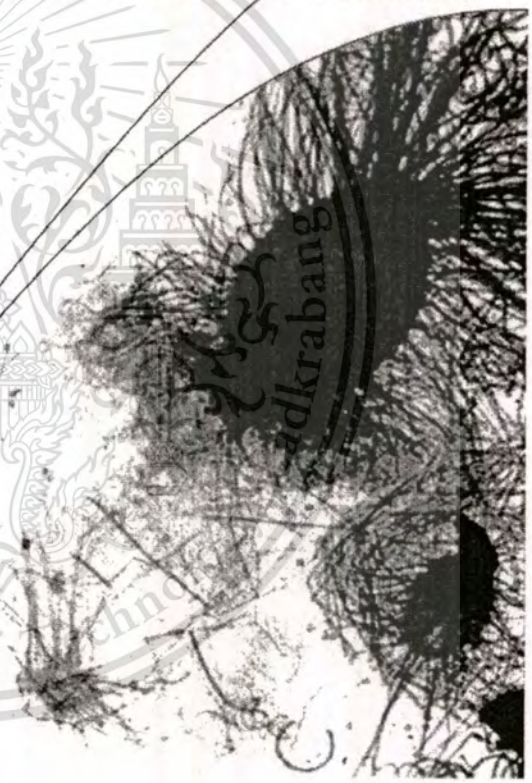
THE ANNUAL MEETING OF THAI MYCOLOGICAL ASSOCIATION AND MYCOLOGY CONFERENCE IN THAILAND

AT FACULTY OF AGRICULTURE, CHIANG MAI UNIVERSITY, 23 June 2007

THEME: BUILDING UP MYCOLOGY IN THAILAND



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The 2nd Annual Meeting of Thai Mycological Association and
Mycology Conference in Thailand. 23 June 2007, p. 21.

Studies on Fusarium Wilt of Tomato: Preliminary Test for Morphology, Pathogenicity and its Biological Control

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Abstract: *Fusarium oxysporum* f. sp. *lycopersici* was isolated from infected fruits, leaves, stems of tomato plants and rhizospere soil where collected samples from infested areas in Nakhon Ratchasima, Mukdahan, Burirum, Pathumthani and Bangkok provinces. Thirty-six isolates of *F. oxysporum* were studied for morphology, pathogenicity tests. Results showed that the most virulent isolate of *F. oxysporum* f. sp. *lycopersici* causing tomato wilt was isolate No. PSC03. The description of isolate No. PSC03 are as follows: colony on potato sucrose agar (PSA) appears white or pinkish white to orange, aerial mycelium, felted to floccose. Macro-conidia are hyaline, fusiform or falcate shape, moderately curved, 3-6 septate, 2.6-4.7 x 17.3-30.2 µm. Micro-conidia have 0-1 septate, ellipsoid to cylindrical shape, hyaline, with conidia size of 2.3-3.7 x 11.6-20.2 µm. Chlamydospores form terminal or intercalary in hyphae, solitary or in chain, smooth to roughened wall. Pathogenicity was tested for 36 isolates to prove their pathogenic isolates. Dual culture antagonistic test between the tested pathogen and the selected antagonistic fungi showed that *Trichoderma koningii* No. MA103 was significantly inhibited spore production of *F. oxysporum* f.sp. *lycopersici* as 55.29% when compared to *Penicillium islandicum* isolate No. NA604 which inhibited spore production as 42.31%. It is suggested that further screening for the other antagonistic fungi must be done to screen the most active biological control agents against *F. oxysporum* f. sp. *lycopersici*.

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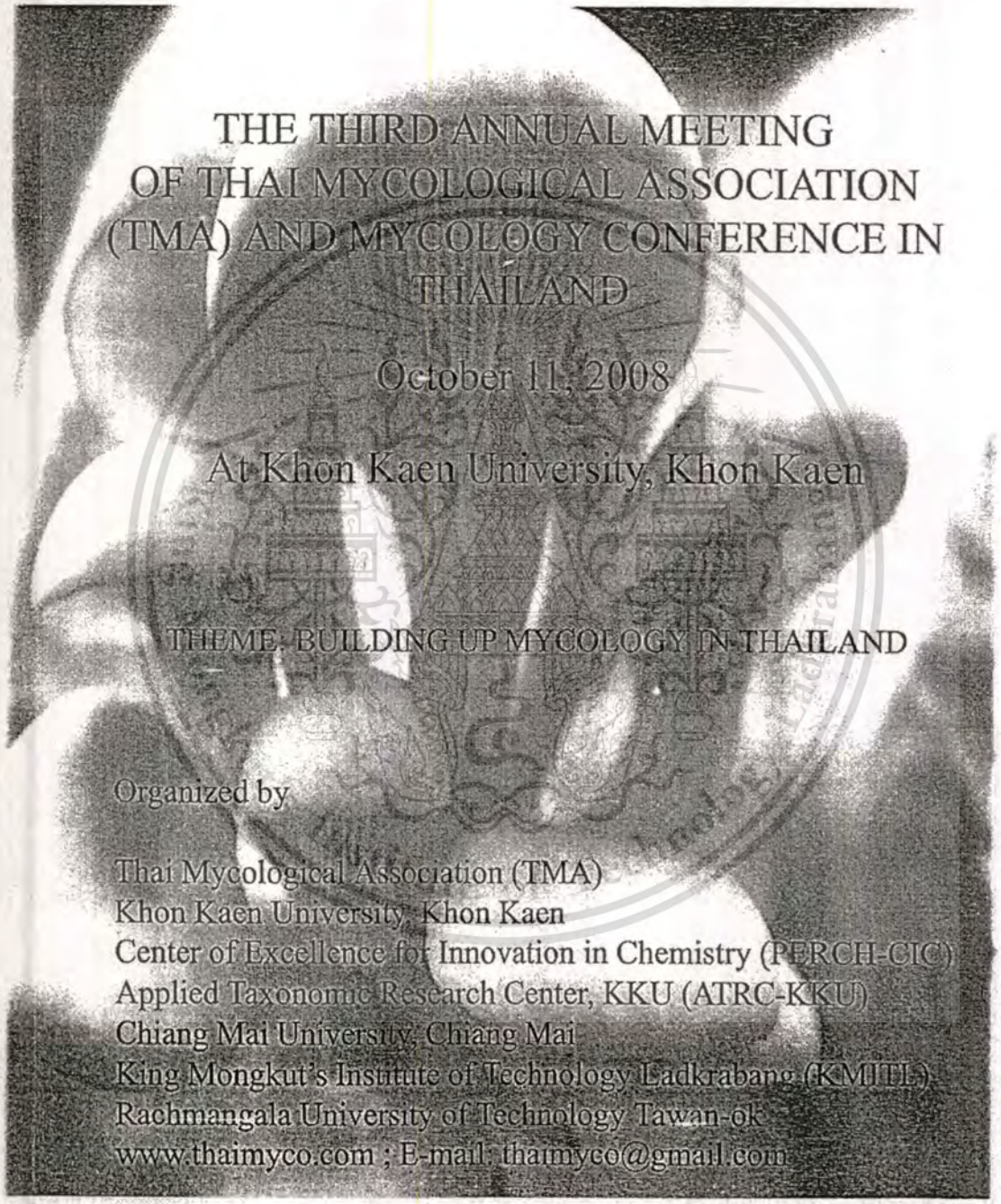

THE THIRD ANNUAL MEETING OF THAI MYCOLOGICAL ASSOCIATION (TMA) AND MYCOLOGY CONFERENCE IN THAILAND

October 11, 2008

At Khon Kaen University, Khon Kaen

THEME: BUILDING UP MYCOLOGY IN THAILAND

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The 3rd Annual Meeting of Thai Mycological Association and
Mycology Conference in Thailand. 11 October 2008. p. 23

Biological control of tomato wilt using *Chaetomium* spp.

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Abstract: *Fusarium oxysporum* f.sp. *lycopersici* was isolated from infected fruits, leaves, stems of tomato plants and rhizospere soil where collected samples from infested areas in Nakhon Ratchasima, Mukdahan, Burirum, Pathumthani, Bangkok, Nong Khai, Sakon Nakhon, and Khon Kaen provinces. Eighty eight isolates of *F. oxysporum* were studied for morphology, pathogenicity tests. The most virulent isolate of *F. oxysporum* f.sp. *lycopersici* causing tomato wilt was isolate No. NKSC01. The description of isolate No. NKSC01 are as follows: colony on potato sucrose agar (PSA) appears white with purple tinge, felt mycelium. Macro-conidia are hyaline, fusiform or falcate shape, moderately curved, 3-4 septate, 2.6-4.7 x 17.3-30.2 µm. Micro-conidia have 0-1 septate, ellipsoid to cylindrical shape, hyaline, with conidia size of 2.3-3.7 x 11.6-20.2 µm. Chlamydospores form terminal or intercalary in hyphae, solitary, smooth wall. Disease severity index on pathogenicity tests of *F. oxysporum* f.sp. *lycopersici* NKSC01 was 6.00. Dual culture test with antagonistic fungi, *Chaetomium globosum* KMITL-N0802, and *C. lucknowense* CLT was not significantly inhibited spore production of *F. oxysporum* f.sp. *lycopersici* as 92.14% and 92.54%, respectively. Crude extracts which extracted with hexane, ethyl acetate, and methanol from *C. globosum* KMITL-N0802, and *C. lucknowense* CLT were tested for growth inhibition of *F. oxysporum* f.sp. *lycopersici* NKSC01. The crude extract from *C. globosum* KMITL-N0802 coded as CG/H gave the highest inhibition against spore production of *F. oxysporum* f. sp *lycopersici* which ED_{50} value was 157 µg/ml, followed with the crude extract coded as CL/H from *C. lucknowense* CLT which ED_{50} value was 188 µg/ml. Pure compounds, Chaetoglobosin C which extracted from *C. globosum* KMITL-N0802 and Chaetomanone A which extracted from *C. lucknowense* CLT were tested to inhibit the growth of *F. oxysporum* f.sp. *lycopersici* NKSC01. Chaetoglobosin C gave the highest result to inhibit spore production of *F. oxysporum* f.sp. *lycopersici* NKSC01 which ED_{50} value was 17 ppm followed with Chaetomanone A which ED_{50} value was 22 ppm, respectively. These two antagonistic fungi have a trend to use as biological control agent to control *F. oxysporum* f. sp *lycopersici* causing tomato *Fusarium* wilt.

Key words: Tomato wilt, biological control

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THE FOURTH ANNUAL MEETING OF THAI MYCOLOGICAL ASSOCIATION (TMA) AND MYCOLOGY CONFERENCE IN THAILAND

October 24, 2009

THEME: BUILDING UP MYCOLOGY IN THAILAND

Organized by

Thai Mycological Association (TMA)
Faculty of Agricultural Production,
Maejo University, Chiang Mai

The 4th Annual Meeting of Thai Mycological Association and
Mycology Conference in Thailand, 24 October 2009

Biological control of tomato Fusarium wilt using bioactive compounds from antagonistic fungi

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Abstract: The experiment was done to evaluate the efficacy of antagonistic fungi and its substance as biological control agent to control tomato Fusarium wilt. Chaetoglobosin C from *Chaetomium globosum* N0802, Chaetomanone A from *Chaetomium lucknowense* CLT and Trichotoxin A50 from *Trichoderma harzianum* PC01 were tested for their abilities to inhibit the growth of *Fusarium oxysporum* f. sp. *lycopersici*, caused Fusarium wilt of tomatoes. All tested bioactive compounds could inhibit mycelial growth over 80% and inhibit conidial production of the pathogen over 95%. The Effective Dose (ED_{50}) of Chaetoglobosin C, Chaetomanone A and Trochotoxin A50 were 17, 22 and 18 µg/ml, respectively. Each bioactive compound was applied by spraying onto inoculated tomato plants to control wilt disease in pot experiment. Result showed that those bioactive compounds could decrease disease incidence over 50% and promoted plant growth parameters such as plant height and plant canopy with significantly compared to chemical and inoculated control.

Key words: Bioactive compounds, *Fusarium oxysporum* f. sp. *lycopersici*, *Chaetomium globosum*, *Chaetomium lucknowense*, *Trichoderma harzianum*, biological control

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THE ANNUAL MEETING OF THAI MYCOLOGICAL ASSOCIATION (TMA) AND MYCOLOGY CONFERENCE IN THAILAND

OCTOBER 28-29, 2006

THEME: BUILDING UP MYCOLOGY IN THAILAND

Organized by

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King Mongkut's Institute of Technology Ladkrabang (KMITL), Bangkok 10520,
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TMA_P_11

Study on Fusarium Wilt of Tomato

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Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp *lycopersici* was studied. The isolation of pathogen was done from infected fruits, leaves, stems, roots and rhizosphere soil of tomato plants where collected samples from infested areas planted to tomato:- Amphur Pakchong, Nakhon Ratchasima Province, Roi-ed Province, Burirum Province, Pathumthani Province and Bangkok. It was found 31 isolates of *Fusarium* spp. that varies in characteristic of colony and conidia. It can be divided into 4 groups according to morphological studies such as colonies, (1) white and cottony (2) pinkish white and cottony (3) peach and cottony and (4) white with purple tint and cottony. Each isolate was identified under compound microscope Some isolates show sparse to abundant macroconidia, fusoid and 3-6 septate, hyaline. Microconidia are ellipsoid or oval ,1-2 septate, hyaline. These isolates will be done for pathogenicity, molecular phylogeny and for further studies.

APPENDIX II

Analysis of variance

Table 1 Disease severity index of tomato wilt at 21 days after inoculation.

Treatments	Disease severity index				Mean
	R1	R2	R3	R4	
BKFC01	1.00	1.00	1.00	1.00	1.00d
BKFC03	1.00	1.00	1.00	1.00	1.00d
BKFC04	1.00	1.00	1.00	1.00	1.00d
BKFC06	1.00	1.00	1.00	1.00	1.00d
BKFC07	1.00	1.00	1.00	1.00	1.00d
BKFC12	1.00	1.00	1.00	1.00	1.00d
BKRC02	1.00	1.00	1.00	1.00	1.00d
BKSC02	1.00	1.00	1.00	1.00	1.00d
BRC01	1.00	1.00	1.00	1.00	1.00d
BRC02	1.00	1.00	1.00	1.00	1.00d
BRC03	2.50	3.50	4.00	2.75	3.19c
KK2	5.00	6.00	6.00	6.00	5.75a
KSoC01	1.00	1.00	1.00	1.00	1.00d
KSoC02	3.00	3.50	3.00	4.00	3.38c
KSoC03	1.00	1.00	1.00	1.00	1.00d
KSoC04	1.00	1.00	1.00	1.00	1.00d
MRC02	1.00	1.00	1.00	1.00	1.00d
MSC02	1.00	1.00	1.00	1.00	1.00d
MSC04	1.00	1.00	1.00	1.00	1.00d
MSoC01	1.00	1.00	1.00	1.00	1.00d
MSoC02	1.00	1.00	1.00	1.00	1.00d
MSoC03	1.00	1.00	1.00	1.00	1.00d
NKRC02	3.50	4.00	5.00	4.00	4.13b
NKRC04	4.00	5.00	4.50	4.00	4.38b
NKRC09	3.00	4.00	3.75	2.75	3.38c
NKRC11	1.00	1.00	1.00	1.00	1.00d
NKSC01	6.00	6.00	6.00	6.00	6.00a
NKSC02	6.00	6.00	6.00	5.50	5.88a
NKSoC01	1.00	1.00	1.00	1.00	1.00d
NKSoC01	1.00	1.00	1.00	1.00	1.00d
NRC04	1.00	1.00	1.00	1.00	1.00d

Table 1 (continued) Disease severity index of tomato wilt at 21 days after inoculation.

Treatments	Disease severity index				Mean
	R1	R2	R3	R4	
NSC01	4.50	3.75	4.00	5.00	4.31b
NSC02	1.00	1.00	1.00	1.00	1.00d
NSC07	1.00	1.00	1.00	1.00	1.00d
NSC09	1.00	1.00	1.00	1.00	1.00d
NSoC01	1.00	1.00	1.00	1.00	1.00d
NSoC04	1.00	1.00	1.00	1.00	1.00d
PSC01	1.00	1.00	1.00	1.00	1.00d
PSC02	1.00	1.00	1.00	1.00	1.00d
PSC03	1.00	1.00	1.00	1.00	1.00d
PSC04	1.00	1.00	1.00	1.00	1.00d
PSC05	1.00	1.00	1.00	1.00	1.00d
SRC02	5.00	4.00	4.50	4.00	4.38b
SSoC02	1.00	1.00	1.00	1.00	1.00d
SSoC03	3.00	3.50	3.00	3.00	3.13c
SSoC04	2.75	3.50	3.00	3.00	3.06c
Control	1.00	1.00	1.00	1.00	1.00d

Table 2 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	45	426.90	9.49	162.06**	1.50	1.76
Ex.Error	38	8.08	0.06			
Total	83	434.98	2.38			

** significant difference at P = 0.01, GRAND MEAN = 1.85

CV = 13.10 %

LSD 0.05 = 0.34

LSD 0.01 = 0.45

Table 3 Colony diameter of dual-culture test at 30 days.

Treatments	Colony diameter (cm)				Mean
	R1	R2	R3	R4	
Control	9.00	9.00	9.00	9.00	9.00a
CG	2.40	2.40	2.80	2.80	2.60b
CL	0	0	1.90	2.10	1.00c
Thz	0.60	0.90	0.90	1.00	0.85c

Table 4 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	3	177.03	59.01	165.83**	3.49	5.95
Ex.Error	12	4.27	0.36			
Total	15	181.30	12.09			

** significant difference at P = 0.01, GRAND MEAN = 3.36

CV = 17.74 %

LSD 0.05 = 0.92

LSD 0.01 = 1.29

Table 5 Inhibition of mycelium growth of *Fusarium oxysporum* f.sp. *lycopersici* in dual-culture test at 30 days.

Treatments	Mycelial inhibition (%)				Mean
	R1	R2	R3	R4	
CG	73.33	73.33	68.89	68.89	71.11b
CL	100.00	100.00	78.89.00	76.67	88.89a
Thz	93.33	90.00	90.00	88.89	90.56a

Table 6 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	2	929.34	464.67	7.94**	4.26	8.02
Ex.Error	9	526.99	58.56			
Total	11	1456.34	132.39			

** significant difference at P = 0.01, GRAND MEAN = 83.52

CV = 9.16 %

LSD 0.05 = 12.24

LSD 0.01 = 17.59

Table 7 Number of conidia in dual-culture test at 30 days.

Treatments	Number of conidia (x 10^7 conidia)				Mean
	R1	R2	R3	R4	
Control	38.60	45.13	47.00	47.80	44.63a
CG	3.10	3.20	3.40	4.35	3.51b
CL	2.65	2.70	3.80	4.25	3.35b
Thz	0.002	0.002	0.002	0.003	0.002c

Table 8 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	3	5410.51	1803.51	392.18**	3.49	5.95
Ex.Error	12	55.18	4.60			
Total	15	5465.70	364.38			

** significant difference at P = 0.01, GRAND MEAN = 12.87

CV = 16.66 %

LSD 0.05 = 3.30

LSD 0.01 = 4.63

Table 9 Inhibition of sporulation in dual-culture test at 30 days.

Treatments	Conidial inhibition (%)				Mean
	R1	R2	R3	R4	
Chaetomium globosum KMITL-N0802	91.97	92.77	92.91	90.90	92.14b
Chaetomium lucknowense CLT	93.13	94.02	91.91	91.11	92.54b
Trichoderma harzianum PC01	99.99	99.99	99.99	99.99	99.99a

Table 10 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	2	156.39	78.19	93.38**	4.26	8.02
Ex.Error	9	7.54	0.84			
Total	11	163.92	14.90			

** significant difference at P = 0.01, GRAND MEAN = 94.89

CV = 0.96 %

LSD 0.05 = 1.46

LSD 0.01 = 2.10

Table 11 Colony diameter in crude extract of *Chaetomium globosum* KMITL-N0802 test.

Treatments	Colony diameter (cm)				Mean
	R1	R2	R3	R4	
CG/H 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	4.80	4.90	4.90	5.00	4.90ab
500	4.70	4.70	4.70	4.70	4.70cd
1000	4.60	4.70	4.70	4.70	4.68d
CG/E 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	4.80	4.90	4.90	4.90	4.88b
100	4.70	4.80	4.80	4.80	4.78c
500	4.50	4.50	4.60	4.60	4.55e
1000	4.00	4.20	4.30	4.30	4.20f
CG/M 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	4.90	4.90	4.90	4.90	4.90ab
500	4.70	4.70	4.70	4.80	4.73cd
1000	4.60	4.60	4.70	4.70	4.65d

Table 12 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	17	3.22	0.19	78.75**	1.84	2.34
A	5	2.26	0.45	187.43**	2.36	3.34
B	2	0.46	0.23	95.60**	3.15	4.98
AxB	10	0.51	0.05	21.03**	1.99	2.63
Error	54	0.13	0.002			
Total	71	3.35	0.05			

** significant difference at P = 0.01, GRAND MEAN = 4.83

CV = 1.02%

Table 13 Colony diameter in crude extract of *Chaetomium lucknowense* CLT test.

Treatments	Colony diameter (cm)				Mean
	R1	R2	R3	R4	
CL/H 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	5.00	5.00	5.00	5.00	5.00a
500	5.00	5.00	5.00	5.00	5.00a
1000	5.00	5.00	5.00	5.00	5.00a
CL/E 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	4.90	4.90	4.90	5.00	4.93ab
500	4.50	4.70	4.80	4.80	4.70c
1000	3.90	3.90	3.90	4.00	3.93d
CL/M 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	5.00	5.00	5.00	5.00	5.00a
500	5.00	5.00	5.00	5.00	5.00a
1000	4.80	4.90	4.90	5.00	4.90b

Table 14 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	17	4.51	0.27	150.84**	1.84	2.34
A	5	2.28	0.46	259.52**	2.36	3.34
B	2	0.45	0.23	127.97**	3.15	4.98
AxB	10	1.78	0.18	101.07**	1.99	2.63
Error	54	0.09	0.002			
Total	71	4.61	0.06			

** significant difference at P = 0.01, GRAND MEAN = 4.91

CV = 0.85%

Table 15 Colony diameter in crude extract of *Trichoderma lharzianum* PC01 test.

Treatments	Colony diameter (cm)				Mean
	R1	R2	R3	R4	
Thz/H 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	5.00	5.00	5.00	5.00	5.00a
500	5.00	5.00	5.00	5.00	5.00a
1000	5.00	5.00	5.00	5.00	5.00a
Thz/E 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	5.00	5.00	5.00	5.00	5.00a
500	5.00	5.00	5.00	5.00	5.00a
1000	4.30	4.40	4.40	4.50	4.40c
Thz/M 0	5.00	5.00	5.00	5.00	5.00a
10	5.00	5.00	5.00	5.00	5.00a
50	5.00	5.00	5.00	5.00	5.00a
100	4.80	4.80	4.90	4.90	4.85b
500	4.40	4.50	4.50	4.50	4.48c
1000	3.90	4.00	4.20	4.20	4.08d

Table 16 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	17	4.98	0.29	150.64**	1.84	2.34
A	5	2.82	0.56	289.83**	2.36	3.34
B	2	0.67	0.34	173.21**	3.15	4.98
AxB	10	1.49	0.15	76.53**	1.99	2.63
Error	54	0.11	0.002			
Total	71	5.08	0.07			

** significant difference at P = 0.01, GRAND MEAN = 4.88

CV = 0.90%

Table 17 Mycelial inhibition of crude extract of *Chaetomium globosum* KMITL-N0802 against *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Treatments	Mycelial inhibition (%)				Mean
	R1	R2	R3	R4	
CG/H 10	0	0	0	0	0f
50	0	0	0	0	0f
100	4.00	2.00	2.00	0	2.00e
500	6.00	6.00	6.00	6.00	6.00cd
1000	8.00	6.00	6.00	6.00	6.50cd
CG/E 10	0	0	0	0	0f
50	4.00	2.00	2.00	2.00	2.50e
100	6.00	4.00	4.00	4.00	4.50d
500	10.00	10.00	8.00	8.00	9.00b
1000	20.00	16.00	14.00	14.00	16.00a
CG/M 10	0	0	0	0	0f
50	0	0	0	0	0f
100	2.00	2.00	2.00	2.00	2.00e
500	6.00	6.00	6.00	4.00	5.50cd
1000	8.00	8.00	6.00	6.00	7.00c

Table 18 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	1123.73	80.27	69.46**	1.92	2.52
A	4	184.40	46.10	39.89**	2.61	3.83
B	2	46.53	23.27	20.13**	3.23	5.18
AxB	8	892.80	111.60	96.58**	2.18	2.99
Error	45	52.00	1.16			
Total	59	1175.73	19.93			

** significant difference at P = 0.01, GRAND MEAN = 4.07

CV = 26.43%

Table 19 Mycelial inhibition of crude extract of *Chaetomium lucknowense* CLT against *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Treatments	Mycelial inhibition (%)				Mean
	R1	R2	R3	R4	
CL/H 10	0	0	0	0	0d
50	0	0	0	0	0d
100	0	0	0	0	0d
500	0	0	0	0	0d
1000	0	0	0	0	0d
CL/E 10	0	0	0	0	0d
50	0	0	0	0	0d
100	2.00	2.00	2.00	0	1.50cd
500	10.00	6.00	4.00	4.00	6.00b
1000	22.00	22.00	22.00	20.00	21.50a
CL/M 10	0	0	0	0	0d
50	0	0	0	0	0d
100	0	0	0	0	0d
500	0	0	0	0	0d
1000	4.00	2.00	2.00	0	2.00c

Table 20 Analysis of Variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	1761.73	125.84	149.02**	1.92	2.52
A	4	440.40	110.10	130.38**	2.61	3.83
B	2	166.53	83.27	98.61**	3.23	5.18
AxB	8	1154.80	144.35	170.94**	2.18	2.99
Error	45	38.00	0.84			
Total	59	1799.73	30.50			

** significant difference at P = 0.01, GRAND MEAN = 2.07

CV = 44.46%

Table 21 Mycelial inhibition of crude extract of *Trichoderma harzianum* PC01 against *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Treatment	Mycelial inhibition (%)				Mean
	R1	R2	R3	R4	
Thz/H 10	0	0	0	0	0d
50	0	0	0	0	0d
100	0	0	0	0	0d
500	0	0	0	0	0d
1000	0	0	0	0	0d
Thz/E 10	0	0	0	0	0d
50	0	0	0	0	0d
100	0	0	0	0	0d
500	0	0	0	0	0d
1000	14.00	12.00	12.00	10.00	12.00b
Thz/M 10	0	0	0	0	0d
50	0	0	0	0	0d
100	4.00	4.00	2.00	2.00	3.00c
500	12.00	10.00	10.00	10.00	10.50b
1000	22.00	20.00	16.00	16.00	18.50a

Table 22 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	1905.73	136.12	145.85**	1.92	2.52
A	4	1041.07	260.27	278.86**	2.61	3.83
B	2	25.73	12.87	13.79**	2.99	2.99
AxB	8	838.93	104.87	112.36**	2.99	2.99
Error	45	42.00	0.93			
Total	59	1947.73	33.01			

** significant difference at P = 0.01, GRAND MEAN = 2.93

CV = 32.93%

Table 23 Number of conidia of *Fusarium oxysporum* f.sp. *lycopersici* NKSC01in crude extracts of *Chaetomium globosum* KMITL-N0802.

Treatments	Number of conidia (x 10^7 conidia)				Mean
	R1	R2	R3	R4	
CG/H 0	23.75	23.88	26.13	27.38	25.29a
10	15.53	16.35	17.40	18.90	17.05bc
50	13.10	13.28	15.23	15.98	14.4cde
100	12.45	13.65	14.30	14.65	13.76def
500	9.30	10.40	11.65	12.80	11.04fgh
1000	8.05	9.45	9.70	10.10	9.33h
CG/E 0	23.75	23.88	26.13	27.38	25.29a
10	17.40	18.10	18.40	19.80	18.43b
50	15.40	17.20	17.80	19.30	17.43b
100	15.10	16.00	17.00	17.60	16.43bcd
500	10.35	12.40	12.40	13.25	12.1efgh
1000	8.80	9.10	9.50	10.45	9.46gh
CG/M 0	23.75	23.88	26.13	27.38	25.29a
10	18.00	18.30	19.43	19.73	18.87b
50	17.55	18.9	19.05	19.20	18.68b
100	15.23	15.38	15.83	17.10	15.89bcd
500	10.65	10.75	13.40	14.90	12.43efg
1000	7.85	10.60	11.85	13.95	11.06fgh

Table 24 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	17	1816.37	106.85	51.49**	1.84	2.34
A	5	1110.37	222.07	107.03**	2.36	3.34
B	2	632.45	316.22	152.40**	3.15	4.98
AxB	10	73.55	7.36	3.54**	1.99	2.63
Error	54	112.05	2.07			
Total	71	1928.41	27.16			

** significant difference at P = 0.01, GRAND MEAN = 16.23

CV = 8.87%

Table 25 Number of conidia of *Fusarium oxysporum* f.sp. *lycopersici* NKSC01in crude extract of *Chaetomium lucknowense* CLT.

Treatments	Number of conidia (x 10^7 conidia)				Mean
	R1	R2	R3	R4	
CL/H 0	23.75	23.88	26.13	27.38	25.29a
10	16.00	16.40	17.20	18.80	17.10cdef
50	15.00	15.10	15.90	17.00	15.75defg
100	13.70	14.80	15.50	15.80	14.95efg
500	10.60	13.00	13.40	14.50	12.88gh
1000	5.70	5.80	6.70	7.30	6.38j
CL/E 0	23.75	23.88	26.13	27.38	25.29a
10	15.40	18.60	22.70	22.80	19.88bc
50	16.80	17.00	21.00	21.70	19.13bcd
100	11.20	13.80	16.50	19.30	15.20efg
500	10.80	12.00	12.70	13.30	24.40gh
1000	6.85	7.20	7.25	9.60	7.73ij
CL/M 0	23.75	23.88	26.13	27.38	25.29a
10	21.20	21.70	22.30	23.20	22.10ab
50	16.50	17.10	17.70	20.60	17.98cde
100	12.30	13.40	13.90	14.10	13.43fgh
500	9.60	10.00	11.50	11.50	10.65hi
1000	5.20	5.60	8.00	9.20	7.00j

Table 26 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	17	2516.45	148.03	44.66**	1.84	2.34
A	5	1749.26	349.85	105.56**	2.36	3.34
B	2	691.06	345.53	104.25**	3.15	4.98
AxB	10	76.13	7.61	2.30**	1.99	2.63
Error	54	178.98	3.31			
Total	71	2695.43	37.96			

** significant difference at P = 0.01, GRAND MEAN = 16.01

CV = 11.37%

Table 27 Number of conidia of *Fusarium oxysporum* f.sp. *lycopersici* NKSC01in crude extract of *Trichoderma harzianum* PC01.

Treatments	Number of conidia (x 10^7 conidia)				Mean
	R1	R2	R3	R4	
Thz/H 0	23.75	23.88	26.13	27.38	25.29a
10	19.00	20.30	23.10	24.20	21.65b
50	16.90	18.90	19.70	21.00	19.13bc
100	13.70	14.80	15.90	17.20	15.4defg
500	12.80	14.80	15.60	16.10	14.83fg
1000	11.20	12.30	12.40	12.50	12.1g
Thz/E 0	23.75	23.88	26.13	27.38	25.29a
10	19.20	20.33	22.20	25.10	21.71b
50	15.60	18.70	20.20	21.40	18.98bcd
100	14.10	16.70	18.90	19.58	17.32cdef
500	12.10	16.00	16.50	16.50	15.28efg
1000	0.60	0.71	0.88	0.94	0.78h
Thz/M 0	23.75	23.88	26.13	27.38	25.29a
10	19.05	18.90	19.50	22.80	20.06bc
50	18.45	18.50	18.60	18.90	18.61bcde
100	16.30	17.80	18.40	19.40	17.98cdef
500	11.80	12.00	14.80	15.20	13.45g
1000	0.55	0.55	0.64	0.80	0.64h

Table 28 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	17	3394.29	199.66	65.47**	1.84	2.34
A	5	1816.64	363.33	119.13**	2.36	3.34
B	2	1090.03	545.02	178.70**	3.15	4.98
AxB	10	487.62	48.76	15.99**	1.99	2.63
Error	54	164.69	3.05			
Total	71	3558.99	50.13			

** significant difference at P = 0.01, GRAND MEAN = 16.8750

CV = 10.3490

Table 29 Conidial inhibition of crude extract of *Chaetomium globosum* KMITL-N0802 againsted *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Treatments	Conidial inhibition (%)				Mean
	R1	R2	R3	R4	
CG/H 10	34.61	31.53	33.41	30.97	32.63ef
50	47.58	44.39	41.71	41.64	43.83d
100	44.84	42.84	45.27	46.49	44.86d
500	60.84	56.45	55.42	53.25	56.49bc
1000	66.11	60.43	62.88	63.11	63.13a
CG/E 10	26.74	24.20	29.58	27.68	27.05fg
50	35.16	27.97	31.88	29.51	31.13efg
100	36.42	33.00	34.94	35.72	35.02e
500	56.42	48.07	52.54	51.61	52.16c
1000	62.95	61.89	63.64	61.83	62.58ab
CG/M 10	22.94	20.85	27.09	27.94	24.71g
50	26.11	20.85	25.64	29.88	25.62g
100	35.87	35.59	39.42	37.55	37.11e
500	55.16	54.98	48.72	45.58	51.11c
1000	66.95	55.61	54.65	49.05	56.57bc

Table 30 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	10185.42	727.53	1.92**	66.05	2.52
A	4	1324.78	331.20	30.07**	2.61	3.83
B	2	86.65	43.33	3.93**	3.23	5.18
AxB	8	8773.98	1096.75	99.57**	2.18	2.99
Error	45	495.68	11.02			
Total	59	10681.09	181.04			

** significant difference at P = 0.01, GRAND MEAN = 42.93

CV = 7.73%

Table 31 Conidial inhibition of crude extract of *Chaetomium lucknowense* CLT againsted *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Treatments	Conidial inhibition (%)				Mean
	R1	R2	R3	R4	
CL/H 10	32.63	31.32	34.18	31.34	32.37ef
50	36.84	36.77	39.15	37.91	37.66e
100	42.32	38.02	40.68	42.29	40.83de
500	55.37	45.56	48.72	47.04	49.17cd
1000	76.00	75.71	74.36	73.33	74.85a
CL/E 10	29.26	22.11	13.13	16.72	20.31gh
50	35.15	28.81	19.63	20.75	26.09fg
100	52.84	42.21	36.85	29.51	40.35de
500	54.52	49.75	51.40	51.42	51.77bc
1000	71.16	69.85	72.25	64.94	69.55a
CL/M 10	10.74	9.13	14.66	15.27	12.45h
50	30.53	28.39	32.26	24.76	28.99f
100	48.21	43.89	46.80	48.50	46.85cd
500	59.58	58.12	55.99	58.00	57.92b
1000	78.11	76.55	69.38	66.44	72.62a

Table 32 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	20058.33	1432.74	72.08**	1.92	2.52
A	4	4387.20	1096.80	55.18**	2.61	3.83
B	2	44.64	22.32	1.12**	3.23	5.18
AxB	8	15626.48	1953.31	98.27**	2.18	2.99
Error	45	894.44	19.88			
Total	59	20952.77	355.13			

** significant difference at P = 0.01, GRAND MEAN = 44.12

CV = 10.11%

Table 33 Conidial inhibition of crude extract of *Trichoderma lharzianum* PC01 againsted *Fusarium oxysporum* f.sp. *lycopersici* NKSC01.

Treatments	Conidial inhibition (%)				Mean
	R1	R2	R3	R4	
Thz/H 10	20.00	14.99	11.60	11.61	14.55f
50	28.84	20.85	24.61	23.30	24.4de
100	42.32	38.02	39.15	37.18	39.17c
500	46.11	38.02	40.30	41.20	41.41c
1000	52.84	48.49	52.54	54.35	52.06b
Thz/E 10	19.16	14.87	15.04	8.33	14.35f
50	34.32	21.69	22.69	21.84	25.14de
100	40.63	30.07	27.67	28.49	31.72d
500	49.05	33.00	40.55	39.74	40.59c
1000	97.47	97.03	96.63	96.57	96.93a
Thz/M 10	19.79	20.85	25.37	16.73	20.69ef
50	22.32	22.53	28.82	30.97	26.16de
100	31.37	25.46	29.58	29.15	28.89d
500	50.32	49.75	43.36	44.49	46.98bc
1000	97.68	97.70	97.55	97.08	97.50a

Table 34 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	37082.71	2648.76	166.55**	1.92	2.52
A	4	7767.26	1941.81	122.10**	2.61	3.83
B	2	757.74	378.87	23.82**	3.23	5.18
AxB	8	28557.71	3569.71	224.46**	2.18	2.99
Error	45	715.66	15.90			
Total	59	37798.37	640.65			

** significant difference at P = 0.01, GRAND MEAN = 40.03

CV = 9.96%

Table 35 Colony diameter of *Fusarium oxysporum* f.sp. *lycopersici* NKSC01 in bioactive compound test.

Treatments	Concentrations (µg/ml)	Colony diameter (cm)				Mean
		R1	R2	R3	R4	
Chaetoglobosin C	0	5.00	5.00	5.00	5.00	5.00a
	10	4.80	4.90	5.00	5.00	4.93a
	25	4.70	4.70	4.80	4.80	4.75bc
	50	4.10	4.20	4.30	4.30	4.23d
	100	1.00	1.00	1.10	1.10	1.05f
Chaetomanone A	0	5.00	5.00	5.00	5.00	5.00a
	10	4.80	5.00	5.00	5.00	4.95a
	25	4.50	4.80	4.80	4.80	4.73bc
	50	3.70	3.80	3.80	3.90	3.8e
	100	0.80	0.80	0.90	0.90	0.85g
Trichotoxin A50	0	5.00	5.00	5.00	5.00	5.00a
	10	4.70	4.80	4.90	5.00	4.85ab
	25	4.60	4.70	4.70	4.70	4.68c
	50	3.70	3.80	3.80	3.90	3.8e
	100	0.90	1.00	1.00	1.00	0.98fg

Table 36 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	139.18	9.94	1556.06**	1.92	2.52
A	2	0.22	0.11	16.98**	3.23	5.18
B	4	138.58	34.65	5422.86**	2.61	3.83
AxB	8	0.38	0.05	7.43**	2.18	2.99
Error	45	0.29	0.006			
Total	59	139.47	2.36			

** significant difference at P = 0.01, GRAND MEAN = 3.91

CV = 2.05%

Table 37 Mycelial inhibition of chaetoglobosin C, chaetomanone A and trichotoxin A50 against *Fusarium oxysporum* f.sp. *lycopersici* NKSC01 in bioactive compound test.

Treatment	Concentrations (µg/ml)	Mycelial inhibition (%)				Mean
		R1	R2	R3	R4	
Chaetoglobosin C	10	0	4.00	0	2.00	1.50gh
	25	6.00	4.00	4.00	6.00	5.00ef
	50	14.00	16.00	18.00	14.00	15.50d
	100	78.00	78.00	80.00	80.00	79.00b
Chaetomanone A	10	0	0	4.00	0	1.00gh
	25	4.00	4.00	4.00	4.00	4.00efg
	50	24.00	22.00	24.00	26.00	24.00c
	100	82.00	82.00	84.00	84.00	83.00a
Trichotoxin A50	10	4.00	6.00	2.00	0	3.00fgh
	25	6.00	8.00	6.00	6.00	6.50e
	50	24.00	20.00	22.00	24.00	22.50c
	100	80.00	80.00	82.00	80.00	80.50ab

Table 38 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	11	48796.25	4436.02	1754.91**	2.08	2.80
A	2	84.50	42.25	16.71**	3.23	5.18
B	3	48577.58	16192.53	6405.84**	2.84	4.31
AxB	6	134.17	22.36	8.85**	2.33	3.29
Error	36	91.00	2.53			
Total	47	48887.25	1040.15			

** significant difference at P = 0.01, GRAND MEAN = 27.13

CV = 5.86%

Table 39 Number of conidia of *Fusarium oxysporum* f.sp. *lycopersici* NKSC01 in bioactive compound test.

Treatments	Concentrations	Number of conidia ($1x10^7$ conidia)				Mean
	(µg/ml)	R1	R2	R3	R4	
Chaetoglobosin C	0	13.50	13.68	14.25	15.00	14.11a
	10	9.50	10.38	10.75	11.63	10.57b
	25	5.40	5.60	8.20	8.40	6.9de
	50	3.20	3.90	4.00	4.70	3.95f
	100	0.08	0.08	0.10	0.13	0.10g
Chaetomanone A	0	13.50	13.68	14.25	15.00	14.11a
	10	8.25	8.38	8.88	10.50	9.00c
	25	5.20	5.60	6.00	7.30	6.03e
	50	3.40	3.80	3.90	4.10	3.80f
	100	0.15	0.18	0.18	0.2	0.18g
Trichotoxin A50	0	13.50	13.68	14.25	15.00	14.11a
	10	7.30	8.00	8.80	9.50	8.40cd
	25	6.00	6.90	7.70	7.80	7.10de
	50	3.80	4.00	4.70	5.30	4.45f
	100	0.20	0.20	0.23	0.25	0.22g

Table 40 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	14	1348.56	96.33	156.68**	1.92	2.52
A	2	2.52	1.26	2.05**	3.23	5.18
B	4	1335.00	333.76	542.85**	2.61	3.83
AxB	8	11.04	1.38	2.25**	2.18	2.99
Error	45	27.67	0.61			
Total	59	1376.23	23.33			

** significant difference at P = 0.01, GRAND MEAN = 6.87

CV = 11.42%

Table 41 Conidial inhibition of chaetoglobosin C, chaetomanone A and trichotoxin A50 against *Fusarium oxysporum* f.sp. *lycopersici* NKSC01 in bioactive compound test.

Treatments	Concentrations (µg/ml)	Conidial inhibition (%)				Mean
		R1	R2	R3	R4	
Chaetoglobosin C	10	38.52	37.68	30.00	38.89	36.27d
	25	58.91	61.48	57.89	51.33	57.40c
	50	72.63	74.81	72.12	72.67	73.06b
	100	98.72	98.89	98.77	86.67	95.76a
Chaetomanone A	10	23.84	22.47	29.63	24.56	25.13e
	25	44.00	42.46	58.91	60.00	51.34c
	50	71.93	76.29	68.67	71.39	72.07b
	100	91.67	92.98	99.44	99.45	95.89a
Trichotoxin A50	10	41.31	38.25	45.93	36.67	40.54d
	25	49.38	55.56	45.96	48.00	49.73c
	50	67.02	70.65	71.85	64.67	68.55b
	100	98.52	98.42	98.53	98.33	98.45a

Table 42 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	11	26467.62	2406.15	118.11**	2.08	2.80
A	2	172.95	86.48	4.24**	3.23	5.18
B	3	25766.39	8588.80	421.58**	2.84	4.31
AxB	6	528.28	88.05	4.32**	2.33	3.29
Error	36	733.42	20.37			
Total	47	27201.04	578.75			

** significant difference at P = 0.01, GRAND MEAN = 63.68

CV = 7.09%

Table 43 Disease severity index of tomato after inoculated with pathogen and sprayed with bioactive compounds 15 days.

Treatments	Disease severity index			Mean
	R1	R2	R3	
Chaetoglobosin C	2.30	1.70	3.30	2.43b
Chaetomanone A	3.30	3.30	2.00	2.87b
Trichotoxin A50	2.00	2.70	3.30	2.67b
prochloraz	3.30	3.30	3.70	3.43ab
Inoculated control	3.70	4.70	4.70	4.37a
Non-inoculated control	1.00	1.00	1.00	1.00c

Table 44 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	5	18.76	3.75	11.11**	3.11	5.06
Ex.Error	12	4.05	0.34			
Total	17	22.81	1.34			

** significant difference at P = 0.01, GRAND MEAN = 2.79

CV = 20.80 %

LSD 0.05 = 1.03

LSD 0.01 = 1.45

Table 45 Plant disease immunity of tomato after inoculated with pathogen and sprayed with bioactive compounds 15 days.

Treatments	Plant disease immunity (%)			Mean
	R1	R2	R3	
Chaetoglobosin C	54.05	51.06	29.79	44.97a
Chaetomanone A	45.95	29.79	29.79	35.18a
Trichotoxin A50	45.95	42.55	29.79	39.43a
prochloraz	29.79	29.79	21.28	26.95a
Inoculated control	-	-	-	-
Non-inoculated control	-	-	-	-

Table 46 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	3	519.27	173.09	1.93^{ns}	4.07	7.59
Ex.Error	8	717.52	89.69			
Total	11	1236.79	112.44			

^{ns} non-significant difference at P = 0.05, GRAND MEAN = 36.63

CV 5.85 %

LSD 0.05 = 17.83

LSD 0.01 = 25.94

Table 47 α-tomatine quantification of tomato after inoculated with pathogen and sprayed with bioactive compounds 5 days.

Treatments	α-tomatine (ug/g)			Mean
	R1	R2	R3	
Chaetoglobosin C	758.99	738.66	742.37	746.67a
Chaetomanone A	584.74	447.7	572.58	535.01b
Trichotoxin A50	659.99	641.53	497.59	599.7b
prochloraz	390.23	432.18	283.64	368.68c
Inoculated control	341.16	348.24	395.12	361.51c
Non-inoculated control	267.71	381.23	234.2	294.38c

Table 48 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	5	447731.62	89546.32	20.32**	3.11	5.06
Ex.Error	12	52877.28	4406.44			
Total	17	500608.90	29447.58			

** significant difference at P = 0.01, GRAND MEAN = 484.33

CV = 13.71 %

LSD 0.05 = 118.10

LSD 0.01 = 165.58

Table 49 α-tomatine quantification of tomato after inoculated with pathogen and sprayed with bioactive compounds 10 days.

Treatments	α-tomatine (ug/g)			Mean
	R1	R2	R3	
Chaetoglobosin C	369.25	308.34	368.57	348.72b
Chaetomanone A	377.05	239.45	352.45	322.98b
Trichotoxin A50	495.77	527.35	453.53	492.22a
prochloraz	177.31	185.52	209.64	190.82c
Inoculated control	204.9	198.68	136.04	179.87c
Non-inoculated control	353.03	286.04	324.49	321.19b

Table 50 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	5	198386.85	39677.37	21.96**	3.11	5.06
Ex.Error	12	21685.37	1807.11			
Total	17	220072.22	12945.42			

** significant difference at P = 0.01, GRAND MEAN = 309.30

CV = 13.74 %

LSD .05 = 75.63

LSD .01 = 106.04

Table 51 α-tomatine quantification of tomato after inoculated with pathogen and sprayed with bioactive compounds 15 days.

Treatments	α-tomatine (ug/g)			Mean
	R1	R2	R3	
Chaetoglobosin C	157.37	209.73	256.5	207.87b
Chaetomanone A	221.98	297.79	242.98	254.25b
Trichotoxin A50	205.59	172.84	236.69	205.04b
prochloraz	110.49	150.82	132.16	131.56c
Inoculated control	79.25	76.48	76.66	77.46c
Non-inoculated control	375.12	358.84	363.76	365.91a

Table 52 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Treatment	5	150061.28	30012.26	32.80**	3.11	5.06
Ex.Error	12	10980.55	915.05			
Total	17	161041.83	9473.05			

** significant difference at P = 0.01, GRAND MEAN = 206.95

CV = 14.62 %

LSD 0.05 = 53.82

LSD 0.01 = 75.45

Table 53 Disease severity index at 30 days after treatments of Bio-agent formulation.

Treatments	Disease severity index				Mean
	R1	R2	R3	R4	
T1	3.80	4.00	4.20	4.80	4.20a
T2	2.20	2.80	3.20	3.40	2.90bc
T3	2.20	2.40	3.60	3.80	3.00b
T4	2.40	2.40	3.00	3.40	2.80b
T5	2.80	3.20	3.60	4.00	3.40b
T6	1.00	1.00	1.00	1.00	1.00d

Table 54 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	3.66	1.22	14.52**	3.29	5.42
Treatment	5	22.27	4.45	53.03**	4.56	2.90
Ex.Error	15	1.26	0.08			
Total	23	27.19	1.18			

** significant difference at P = 0.01, GRAND MEAN = 2.88

CV = 10.05 %

LSD 0.05 = 0.44

LSD 0.01 = 0.60

Table 55 Disease reduction at 30 days after treatments of Bio-agent formulation.

Treatments	Disease reduction (%)				Mean
	R1	R2	R3	R4	
T1	-	-	-	-	-
T2	42.11	30.00	23.81	29.17	31.27ab
T3	42.11	40.00	14.29	20.83	29.31ab
T4	36.84	40.00	28.57	29.17	33.65a
T5	26.32	20.00	14.29	20.00	20.15b
T6	-	-	-	-	-

Table 56 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	670.31	223.44	7.94**	3.86	6.99
Treatment	5	417.82	139.27	4.95**	3.86	6.99
Ex.Error	15	253.33	28.15			
Total	23	1341.46	89.43			

** significant difference at P = 0.01, GRAND MEAN = 28.59

CV = 18.55 %

LSD 0.05 = 8.49

LSD 0.01 = 12.19

Table 57 Disease severity index at 60 days after treatments of Bio-agent formulation.

Treatments	Disease severity index				Mean
	R1	R2	R3	R4	
T1	4.20	4.80	4.80	4.80	4.70a
T2	2.60	2.60	2.60	2.60	2.60b
T3	2.00	2.80	3.00	3.20	2.75c
T4	2.40	2.60	2.80	3.00	2.70c
T5	3.40	3.60	3.60	4.20	3.70ab
T6	1.00	1.00	1.00	1.00	1.00c

Table 58 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	0.89	0.30	5.83**	3.29	5.42
Treatment	5	29.86	5.97	116.84**	2.90	4.56
Ex.Error	15	0.77	0.05			
Total	23	31.52	1.37			

** significant difference at P = 0.01, GRAND MEAN = 2.90

CV = 7.80 %

LSD 0.05 = 0.34

LSD 0.01 = 0.47

Table 59 Disease reduction at 60 days after treatments of Bio-agent formulation.

Treatments	Disease reduction (%)				Mean
	R1	R2	R3	R4	
Fol	-	-	-	-	
N0802	38.10	45.83	45.83	45.83	43.90a
CLT	52.38	41.67	37.50	33.33	41.22a
PC01	42.86	45.83	41.67	37.50	41.97a
prochloraz	19.05	25.00	25.00	12.50	20.39b
control	-	-	-	-	

Table 60 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	120.95	40.32	1.36**	3.86	6.99
Treatment	3	1463.76	487.92	16.45**	3.86	6.99
Ex.Error	9	267.02	29.67			
Total	15	1851.73	123.45			

** significant difference at P = 0.01, GRAND MEAN = 36.88

CV = 14.77 %

LSD .05 = 8.71

LSD .01 = 12.52

Table 61 Plant height of tomatoes at 30 days after treatment with Bio-agent formulation.

Treatments	Plant height (cm)				Mean
	R1	R2	R3	R4	
Fol	19.60	14.40	14.00	12.40	15.10d
N0802	19.20	18.80	18.80	19.00	18.95bcd
CLT	29.60	21.40	24.00	21.80	24.20ab
PC01	20.20	25.80	20.40	25.00	22.85bc
prochloraz	18.20	19.00	16.20	17.80	17.80cd
control	30.80	30.80	26.60	26.00	28.55a

Table 62 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	32.63	10.88	1.80**	3.29	5.42
Treatment	5	478.27	95.66	15.86**	2.90	4.56
Ex.Error	15	90.46	6.03			
Total	23	601.36	26.15			

** significant difference at P = 0.01, GRAND MEAN = 21.24

CV = 11.56 %

LSD .05 = 3.70

LSD .01 = 5.12

Table 63 Plant height of tomatoes at 60 days after treatment with Bio-agent formulation.

Treatments	Plant height (cm)				Mean
	R1	R2	R3	R4	
Fol	40.00	35.00	25.00	28.00	32.00b
N0802	49.30	46.00	46.80	45.00	46.78a
CLT	47.30	34.50	44.70	39.20	41.43ab
PC01	38.70	48.20	42.50	57.50	46.73a
prochloraz	32.30	33.60	49.00	2.00	35.48ab
control	47.60	52.60	50.00	47.80	49.50a

Table 64 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	17.81	5.94	0.12**	3.29	5.42
Treatment	5	977.12	195.42	4.11**	2.90	4.56
Ex.Error	15	713.70	47.58			
Total	23	1708.63	74.29			

** significant difference at P = 0.01, GRAND MEAN = 41.98

CV = 16.43 %

LSD 0.05 = 10.39

LSD 0.01 = 14.37

Table 65 Plant canopy of tomatoes at 30 days after treatment with Bio-agent formulation.

Treatments	Plant canopy (cm)				Mean
	R1	R2	R3	R4	
Fol	24.60	15.80	13.60	11.40	16.35d
N0802	26.40	28.00	21.60	26.60	25.65bc
CLT	39.00	25.60	28.60	29.20	30.60ab
PC01	22.40	36.80	29.60	27.80	29.15ab
prochloraz	19.80	21.80	17.00	18.00	19.15cd
control	35.20	40.20	30.40	30.80	34.15a

Table 66 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	109.18	36.39	1.83**	3.29	5.42
Treatment	5	950.09	190.02	9.54**	2.90	4.56
Ex.Error	15	298.65	19.91			
Total	23	1357.92	59.04			

** significant difference at P = 0.01, GRAND MEAN = 25.84

CV = 17.27 %

LSD 0.05 = 6.72

LSD 0.01 = 9.30

Table 67 Plant canopy of tomatoes at 60 days after treatment with Bio-agent formulation.

Treatments	Plant canopy (cm)				Mean
	R1	R2	R3	R4	
Fol	37.70	28.50	22.50	22.00	27.68b
N0802	40.80	37.80	42.50	43.30	41.10a
CLT	39.00	26.00	35.30	30.60	32.73ab
PC01	35.70	40.40	38.00	51.00	41.28a
prochloraz	24.70	27.00	35.00	29.00	28.93b
control	42.20	46.40	38.20	37.20	41.00a

Table 68 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	17.81	5.94	0.12**	3.29	5.42
Treatment	5	977.12	195.42	4.11**	2.90	4.56
Ex.Error	15	713.70	47.58			
Total	23	1708.63	74.29			

** significant difference at P = 0.01, GRAND MEAN = 41.98

CV = 16.43 %

LSD 0.05 = 10.39

LSD 0.01 = 14.37

Table 69 Fruit number of tomatoes after treated with Bio-agent formulation.

Treatments	Fruit number (fruit/plant)				Mean
	R1	R2	R3	R4	
Fol	2.40	1.80	0.80	1.00	1.50c
N0802	7.80	9.40	10.20	12.80	10.05a
CLT	12.60	4.00	4.80	8.60	7.50ab
PC01	9.60	9.20	7.00	11.40	9.30a
prochloraz	1.40	1.40	0.80	6.40	2.50bc
control	9.20	8.00	6.60	3.60	6.85ab

Table 70 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	22.79	7.60	1.32**	3.29	5.42
Treatment	5	249.13	49.83	8.64**	2.90	4.56
Ex.Error	15	86.47	5.76			
Total	23	358.39	15.58			

** significant difference at P = 0.01, GRAND MEAN = 6.28

CV = 38.21 %

LSD 0.05 = 3.62

LSD 0.01 = 5.00

Table 71 Fruit weight of tomatoes after treated with Bio-agent formulation.

Treatments	Fruit weight (g)				Mean
	R1	R2	R3	R4	
Fol	25.89	22.56	7.35	5.59	15.35d
N0802	158.61	149.27	116.29	111.07	133.81a
CLT	152.136	107.35	68.11	50.01	94.40bc
PC01	145.10	126.06	110.35	99.75	120.32ab
prochloraz	66.34	24.74	12.71	12.22	29.00d
control	100.09	95.14	83.39	51.27	82.47c

Table 72 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	9907.89	3302.63	17.51**	3.29	5.42
Treatment	5	9209.13	46045.67	48.82**	2.90	4.56
Ex.Error	15	2829.49	188.63			
Total	23	58783.05	2555.79			

** significant difference at P = 0.01, GRAND MEAN = 79.22

CV = 17.34 %

LSD 0.05 = 20.70

LSD 0.01 = 28.62

Table 73 Fruit width of tomatoes after treated with Bio-agent formulation.

Treatments	fruit width (cm)				Mean
	R1	R2	R3	R4	
Fol	2.19	2.36	1.98	1.70	1.65b
N0802	2.47	2.43	2.48	2.32	2.43a
CLT	2.20	2.21	2.47	2.40	2.32ab
PC01	2.29	2.42	2.42	2.38	2.38a
prochloraz	2.13	2.08	2.60	2.12	2.23ab
control	2.24	2.29	2.33	2.15	2.25ab

Table 74 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	0.13	0.04	1.61	3.29	5.42
Treatment	5	0.34	0.07	2.56*	2.90	4.56
Ex.Error	15	0.40	0.03			
Total	23	0.86	0.04			

* significant difference at P = 0.05, GRAND MEAN = 2.28

CV = 7.14 %

LSD 0.05 = 0.25

LSD 0.01 = 0.34

Table 75 Fruit length of tomatoes after treated with Bio-agent formulation.

Treatments	Fruit length (cm)				Mean
	R1	R2	R3	R4	
Fol	2.70	3.01	2.83	2.28	2.71ab
N0802	2.96	2.98	2.98	2.84	2.94a
CLT	2.58	2.86	3.18	2.97	2.90a
PC01	2.67	2.51	3.21	2.8	2.80ab
prochloraz	2.57	2.65	2.83	2.83	2.72ab
control	2.53	2.64	2.60	2.21	2.50b

Table 76 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	0.31	0.10	2.50*	3.29	5.42
Treatment	5	0.51	0.10	2.48*	2.90	4.56
Ex.Error	15	0.62	0.04			
Total	23	1.44	0.06			

* significant difference at P = 0.05, GRAND MEAN = 2.76

CV = 7.35 %

LSD 0.05 = 0.31

LSD 0.01 = 0.42

Table 77 Stem fresh weight of tomatoes after treated with Bio-agent formulation.

Treatments	Stem fresh weight (g)				Mean
	R1	R2	R3	R4	
Fol	21.60	25.00	17.60	17.60	20.45c
N0802	83.00	62.60	53.40	48.80	61.95a
CLT	49.00	36.60	48.00	58.00	47.90abc
PC01	32.60	51.60	64.20	53.00	50.35ab
prochloraz	20.60	43.80	10.00	46.40	30.20bc
control	43.60	47.40	51.40	35.60	44.50abc

Table 78 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	48.70	16.23	0.10**	3.29	5.42
Treatment	5	4442.23	888.45	5.31**	2.90	4.56
Ex.Error	15	2509.15	167.28			
Total	23	7000.08	304.35			

** significant difference at P = 0.01, GRAND MEAN = 42.56

CV = 30.39 %

LSD 0.05 = 19.49

LSD 0.01 = 26.959

Table 79 Stem dry weight of tomatoes after treated with Bio-agent formulation.

Treatments	Stem dry weight (cm)				Mean
	R1	R2	R3	R4	
Fol	4.20	3.40	3.60	3.00	3.55c
N0802	14.20	10.40	9.40	8.20	10.55a
CLT	9.40	5.20	7.80	9.40	7.95abc
PC01	8.60	12.20	12.40	11.60	11.20a
prochloraz	4.40	5.00	1.40	7.60	4.60bc
control	8.20	10.20	10.00	7.20	8.90ab

Table 80 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	1.65	0.55	0.12**	3.29	5.42
Treatment	5	194.63	38.93	8.82**	2.90	4.56
Ex.Error	15	66.17	4.41			
Total	23	262.44	11.41			

** significant difference at P = 0.01, GRAND MEAN = 7.79

CV = 26.95 %

LSD 0.05 = 3.16

LSD 0.01 = 4.38

Table 81 Root fresh weight of tomatoes after treated with Bio-agent formulation.

Treatments	Root fresh weight (cm)				Mean
	R1	R2	R3	R4	
Fol	2.20	1.60	1.80	1.40	1.75c
N0802	6.20	6.20	4.20	5.80	5.60a
CLT	6.00	1.80	3.40	5.20	4.10abc
PC01	4.80	3.80	5.20	4.40	4.55ab
prochloraz	1.40	4.60	0.40	2.80	2.30bc
control	9.40	5.00	6.00	3.00	5.85a

Table 82 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	7.98	2.66	1.04**	3.29	5.42
Treatment	5	56.98	11.40	4.48**	2.90	4.56
Ex.Error	15	38.19	2.55			
Total	23	103.15	4.48			

** significant difference at P = 0.01, GRAND MEAN = 4.02

CV = 39.64 %

LSD 0.05 = 2.40

LSD 0.01 = 3.33

Table 83 Root dry weight of tomatoes after treated with Bio-agent formulation.

Treatments	Root dry weight (cm)				Mean
	R1	R2	R3	R4	
Fol	0.26	0.26	0.23	0.26	0.25b
N0802	1.00	1.18	0.84	0.85	0.98a
CLT	1.02	0.35	0.76	0.79	0.73a
PC01	1.03	0.78	0.88	0.93	0.91a
prochloraz	0.16	0.59	0.07	0.38	0.30b
control	1.02	0.98	1.21	0.75	0.99a

Table 84 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	0.03	0.01	0.25**	3.29	5.42
Treatment	5	2.23	0.45	11.49**	2.90	4.56
Ex.Error	15	0.58	0.04			
Total	23	2.85	0.12			

** significant difference at P = 0.01, GRAND MEAN = 0.69

CV = 28.54 %

LSD 0.05 = 0.30

LSD 0.01 = 0.41

Table 85 Increase in yield of tomatoes after treated with Bio-agent formulation.

Treatments	Increase in yield (%)				Mean
	R1	R2	R3	R4	
Fol	-	-	-	-	-
N0802	83.68	84.89	93.68	94.97	89.31a
CLT	82.98	78.98	89.21	88.82	85.00a
PC01	82.16	82.1	93.34	94.4	88.00a
prochloraz	60.97	8.81	42.17	54.26	41.55b
control	74.13	76.29	91.19	89.10	82.68a

Table 86 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	968.16	322.72	3.27**	3.49	5.95
Treatment	4	6498.65	1624.66	16.47**	3.26	5.41
Ex.Error	12	1183.78	98.65			
Total	19	8650.59	455.29			

** significant difference at P = 0.01, GRAND MEAN = 77.31

CV = 12.85 %

LSD 0.05 = 15.30

LSD 0.01 = 21.46

Table 87 Disease severity index of tomatoes at 30 days after treated with bioactive compounds *in vivo*.

Treatments	Disease severity index				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	2.00	1.50	3.50	2.00	2.25bc
Chaetoglobosin C 50 µg/ml	2.5	2.00	2.00	1.50	2.00bcd
Chaetoglobosin C 100 µg/ml	1.50	2.50	2.00	2.50	2.13bcd
Chaetomanone A 10 µg/ml	2.00	2.50	1.50	1.50	1.88bcd
Chaetomanone A 50 µg/ml	2.50	2.00	1.50	2.00	2.00bcd
Chaetomanone A 100 µg/ml	2.00	1.50	2.00	2.00	1.88bcd
Trichotoxin A50 10 µg/ml	2.00	2.00	2.50	2.00	2.13bcd
Trichotoxin A50 50 µg/ml	1.00	2.00	1.50	1.50	1.50cd
Trichotoxin A50 100 µg/ml	2.00	1.00	2.00	2.50	1.88bcd
prochloraz	3.50	2.50	3.50	2.50	3.00ab
Inoculated control	5.00	3.00	4.00	3.50	3.88a
Non-inoculated control	1.00	1.00	1.00	1.00	1.00d

Table 88 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	0.79	0.26	0.96**	2.92	4.51
Treatment	11	22.88	2.08	7.56**	2.16	2.98
Ex.Error	33	9.08	0.28			
Total	47	32.75	0.70			

** significant difference at P = 0.01, GRAND MEAN = 2.13

CV = 24.69 %

LSD 0.05 = 0.73

LSD 0.01 = 0.97

Table 89 Disease severity index of tomatoes at 45 and 60 days after treated with bioactive compounds *in vivo*.

Treatments	Disease severity index				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	1.50	1.50	3.00	2.00	2.00c
Chaetoglobosin C 50 µg/ml	2.00	1.50	2.00	1.50	1.75c
Chaetoglobosin C 100 µg/ml	1.50	2.00	1.50	2.00	1.75c
Chaetomanone A 10 µg/ml	1.50	2.00	1.50	1.50	1.63cd
Chaetomanone A 50 µg/ml	2.00	2.00	1.50	2.00	1.88c
Chaetomanone A 100 µg/ml	2.00	1.50	2.00	2.00	1.88c
Trichotoxin A50 10 µg/ml	2.00	1.50	2.00	2.00	1.88c
Trichotoxin A50 50 µg/ml	1.00	2.00	1.5	1.5	1.5cd
Trichotoxin A50 100 µg/ml	2.00	1.00	2.00	2.5	1.88c
prochloraz	4.00	2.50	3.0	3.o	3.13b
Inoculated control	5.00	4.50	4.00	3.50	4.25a
Non-inoculated control	1.00	1.00	1.00	1.00	1.00d

Table 90 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	0.29	0.10	0.49**	2.92	4.51
Treatment	11	31.54	2.87	14.37**	2.16	2.98
Ex.Error	33	6.58	0.20			
Total	47	38.42	0.82			

** significant difference at P = 0.01, GRAND MEAN = 2.04

CV = 21.88 %

LSD 0.05 = 0.63

LSD 0.01 = 0.83

Table 91 Plant height of tomatoes at 30 days after treated with bioactive compounds *in vivo*.

Treatments	Plant height (cm)				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	36.00	34.50	34.00	37.50	35.50a
Chaetoglobosin C 50 µg/ml	31.50	47.00	43.00	22.50	36.00a
Chaetoglobosin C 100 µg/ml	40.00	43.00	39.50	38.50	40.25a
Chaetomanone A 10 µg/ml	36.50	30.50	40.50	42.50	37.50a
Chaetomanone A 50 µg/ml	43.00	34.00	41.50	42.50	40.25a
Chaetomanone A 100 µg/ml	29.00	28.50	34.00	35.00	31.63ab
Trichotoxin A50 10 µg/ml	40.00	32.50	38.50	44.00	38.75a
Trichotoxin A50 50 µg/ml	28.00	32.50	31.50	35.50	31.88ab
Trichotoxin A50 100 µg/ml	34.00	34.50	23.00	32.50	31.00ab
prochloraz	22.00	37.50	18.00	15.0	23.13b
Inoculated control	23.50	20.50	27.00	20.00	22.75b
Non-inoculated control	40.00	48.00	28.00	44.00	40.00a

Table 92 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	28.02	9.34	0.24**	2.92	4.51
Treatment	11	1676.56	152.41	3.95**	2.16	2.98
Ex.Error	33	1274.05	38.61			
Total	47	2978.62	63.38			

** significant difference at P = 0.01, GRAND MEAN = 34.05

CV = 18.25 %

LSD 0.05 = 8.70

LSD 0.01 = 11.50

Table 93 Plant height of tomatoes at 45 days after treated with bioactive compounds *in vivo*.

Treatments	Plant height (cm)				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	48.50	45.00	48.00	50.00	47.88a
Chaetoglobosin C 50 µg/ml	45.50	59.00	55.50	32.00	48.00a
Chaetoglobosin C 100 µg/ml	54.00	49.50	51.00	48.50	50.75a
Chaetomanone A 10 µg/ml	45.00	35.50	47.50	50.50	44.63a
Chaetomanone A 50 µg/ml	54.00	46.00	53.5.00	53.00	51.63a
Chaetomanone A 100 µg/ml	46.00	40.00	43.50	50.00	44.88a
Trichotoxin A50 10 µg/ml	43.50	42.50	46.50	49.50	45.50a
Trichotoxin A50 50 µg/ml	45.00	45.50	47.50	53.50	47.88a
Trichotoxin A50 100 µg/ml	49.00	46.00	28.00	47.00	42.5a
prochloraz	30.00	43.00	26.00	21.00	30.00b
Inoculated control	30.00	25.00	33.00	24.00	28.00b
Non-inoculated control	51.00	53.50	44.00	53.00	50.38a

Table 94 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	13.04	4.35	0.10**	2.92	4.51
Treatment	11	2586.79	235.16	5.48**	2.16	2.98
Ex.Error	33	1417.33	42.95			
Total	47	4017.17	85.47			

** significant difference at P = 0.01, GRAND MEAN = 44.33

CV = 14.78 %

LSD 0.05 = 9.18

LSD 0.01 = 12.13

Table 95 Plant height of tomatoes at 60 days after treated with bioactive compounds *in vivo*.

Treatments	Plant height (cm)				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	64.50	62.50	60.00	63.50	62.63a
Chaetoglobosin C 50 µg/ml	63.50	72.50	68.00	50.00	63.50a
Chaetoglobosin C 100 µg/ml	64.00	60.00	64.50	58.50	61.75a
Chaetomanone A 10 µg/ml	60.50	52.00	63.00	65.50	60.25a
Chaetomanone A 50 µg/ml	63.00	53.50	61.50	60.50	59.63a
Chaetomanone A 100 µg/ml	56.00	53.00	58.00	63.00	57.5a
Trichotoxin A50 10 µg/ml	52.00	53.50	52.50	62.50	55.13a
Trichotoxin A50 50 µg/ml	59.00	56.50	61.00	65.00	60.38a
Trichotoxin A50 100 µg/ml	65.50	63.00	58.50	63.00	62.50a
prochloraz	40.00	53.00	38.50	34.00	41.38b
Inoculated control	39.00	38.00	42.00	35.00	38.50b
Non-inoculated control	62.00	65.50	55.00	62.00	61.13a

Table 96 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	2.52	0.84	0.03**	2.92	4.51
Treatment	11	3050.85	277.35	9.65**	2.16	2.98
Ex.Error	33	948.60	28.75			
Total	47	4001.98	85.15			

** significant difference at P = 0.01, GRAND MEAN = 57.02

CV = 9.40 %

LSD 0.05 = 7.51

LSD 0.01 = 9.92

Table 97 Plant canopy of tomatoes at 30 days after treated with bioactive compounds *in vivo*.

Treatments	Plant canopy (cm)				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	17.50	25.00	25.00	31.00	24.63ab
Chaetoglobosin C 50 µg/ml	22.50	35.00	32.00	20.00	27.38ab
Chaetoglobosin C 100 µg/ml	32.00	30.50	30.00	32.00	31.13a
Chaetomanone A 10 µg/ml	28.00	25.00	29.00	28.50	27.63ab
Chaetomanone A 50 µg/ml	32.00	29.50	29.00	35.50	31.50a
Chaetomanone A 100 µg/ml	23.50	20.50	27.50	25.00	24.13ab
Trichotoxin A50 10 µg/ml	25.50	24.50	30.50	29.50	27.50ab
Trichotoxin A50 50 µg/ml	21.00	22.00	26.00	30.50	24.88ab
Trichotoxin A50 100 µg/ml	26.00	24.50	18.00	20.50	22.25bc
prochloraz	18.00	27.00	12.5.00	10.00	16.88cd
Inoculated control	14.50	16.50	15.00	14.00	15.00d
Non-inoculated control	30.00	34.00	21.5.00	23.00	27.13ab

Table 98 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	25.21	8.40	0.40**	2.92	4.51
Treatment	11	1110.25	100.93	4.83**	2.16	2.98
Ex.Error	33	689.54	20.90			
Total	47	1825.00	38.83			

** significant difference at P = 0.01, GRAND MEAN = 25

CV = 18.28 %

LSD 0.05 = 6.40

LSD 0.01 = 8.46

Table 99 Plant canopy of tomatoes at 45 days after treated with bioactive compounds *in vivo*.

Treatments	Plant canopy (cm)				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	26.00	22.00	27.00	24.00	24.75abc
Chaetoglobosin C 50 µg/ml	23.50	31.50	25.00	21.00	25.25abc
Chaetoglobosin C 100 µg/ml	30.00	31.50	28.50	27.50	29.38a
Chaetomanone A 10 µg/ml	22.50	23.50	23.50	26.50	24.00abc
Chaetomanone A 50 µg/ml	31.00	25.50	31.50	28.00	29.00a
Chaetomanone A 100 µg/ml	26.00	22.50	29.00	23.00	25.13abc
Trichotoxin A50 10 µg/ml	28.50	24.00	30.50	28.50	27.88ab
Trichotoxin A50 50 µg/ml	27.50	24.00	25.50	32.00	27.25ab
Trichotoxin A50 100 µg/ml	26.50	26.00	12.00	24.00	22.13bc
prochloraz	21.00	25.50	17.00	15.00	19.63cd
Inoculated control	17.00	18.50	18.00	16.00	17.38d
Non-inoculated control	27.00	31.50	26.00	30.00	28.63a

Table 100 Analysis of variance

Source of variation	df	SS	MS	F	Ft	
					0.05	0.01
Block	3	11.6823	3.8941	0.31**	2.92	4.51
Treatment	11	632.1406	57.4673	4.52**	2.16	2.98
Ex.Error	33	419.3802	12.7085			
Total	47	1063.2031	22.6213			

** significant difference at P = 0.01, GRAND MEAN = 25.03

CV = 14.24 %

LSD 0.05 = 4.99

LSD 0.01 = 6.60

Table 101 Plant canopy of tomatoes at 60 days after treated with bioactive compounds *in vivo*.

Treatments	Plant canopy (cm)				Mean
	R1	R2	R3	R4	
Chaetoglobosin C 10 µg/ml	34.00	27.50	35.00	32.00	32.13b
Chaetoglobosin C 50 µg/ml	30.00	38.00	34.00	31.50	33.38ab
Chaetoglobosin C 100 µg/ml	37.00	38.50	36.50	35.00	36.75ab
Chaetomanone A 10 µg/ml	31.00	33.00	33.50	39.00	34.13ab
Chaetomanone A 50 µg/ml	39.00	33.50	40.50	39.50	38.13a
Chaetomanone A 100 µg/ml	34.50	31.50	38.50	30.00	33.63ab
Trichotoxin A50 10 µg/ml	39.00	33.50	42.50	38.00	38.25a
Trichotoxin A50 50 µg/ml	37.00	30.50	33.50	38.00	34.75ab
Trichotoxin A50 100 µg/ml	35.50	35.00	35.50	37.00	35.75ab
prochloraz	28.00	32.00	24.00	19.00	25.75c
Inoculated control	25.00	29.00	25.00	22.00	25.25c
Non-inoculated control	35.00	41.50	34.00	40.00	37.63ab

Table 102 Analysis of variance

Source of variation	df	SS	MS	F	Ft.	
					0.05	0.01
Block	3	6.13	2.04	0.16**	2.92	4.51
Treatment	11	830.29	75.48	6.02**	2.16	2.98
Ex.Error	33	414.00	12.55			
Total	47	1250.42	26.60			

** significant difference at P = 0.01, GRAND MEAN = 33.79

CV = 10.48 %

LSD 0.05 = 4.96

LSD 0.01 = 6.55

AUTHOR BIOGRAPHY

The author, Miss Chamaiporn Charoenporn, was born on June 1, 1968 in Rayong Province, Thailand. She graduated a Bachelor degree in Agriculture (B.Sc.) from Department of Plant production, Program in Plant Pest Management, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang in 1990. She obtained Master degree in Agriculture (M.Sc.) from Department of Plant Pathology, Faculty of Agriculture, Kasetsart University in 1994. Since August 1994, she worked as a lecturer in Department of Agriculture, Faculty of Science and Technology, Nakhon Ratchasima Rajabhat University, Nakhon Ratchasima province, Thailand. A present academic position is Assistance Professor. In the year 2006, she decided to leave for study in the Doctor of Philosophy Program in Biotechnology in Plant Pathology, International College, King Mongkut's Institute of Technology Ladkrabang. She received some financial support by Nakhon Ratchasima Rajabhat University during her study.