AGRICULTURAL INPUTS USED FOR GOOD AGRICULTURAL PRACTICE (GAP), CHEMICAL AND ORGANIC METHODS ON DIFFERENT VARIETIES OF RICE CULTIVATION IN CAMBODIA.

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Thesis:	Agricultural inputs used for good agricultural practice (GAP), chemical and organic methods on different varieties of rice cultivation in Cambodia.
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ABSTRACT

Curvularia lunata found to be seriously caused leaf spot of rice var. IR66 and Sen Pidoa planted in Cambodia. It is a first reported of leaf spot of rice var. IR66 in Cambodia. All isolates were proved for pathogenicity. Bi-culture antagonistic test showed that *Chaetomium cupreum* significantly inhibited sporulation of *C. lunata* when compared to the control plate. The antagonistic fungus *C. cupreum* CC3003 express antifungal activity against *C. lunata* causing leaf spot of rice var. Sen Pidoa in bi-culture test. Hexane-crude extract, ethyl acetate-crude extract and methanol-crude extract from *C. cupreum* CC3003 inhibit spore production of 95.13%, 94.92% and 87.90%, respectively which the ED_{50} values were 6.41µg/ml, 0.83µg/ml and 7.81µg/ml, respectively. Nano-CGH, nano-CGE and nano-CGM from *Chaetomium globosum* KMITL-N0805 expressed antifungal activity against *C. lunata* causing leaf spot of rice var. Sen Pidoa at ED_{50}values of 1.21ppm/ml, 1.19ppm/ml and 1.93 ppm/ml, respectively. It is demonstrated that nano particles caused the disruption and distortion of pathogen cells leading to loss of pathogenicity.

In pot experiments, testing fungal metabolites to inhibit *C. lunata* causing leaf spot of rice var. Sen Pidoa trended to increase in plant height, tiller number that treated with spore suspension of *Chaetomium,* bioproduct of *Chaetomium*, nano product from *C. cupreum* and chemical Butochlonazole when compared to the non-inoculated and inoculated controls. Rice seedlings treated with spore suspension of *Chaetomium*, bioproduct of *Chaetomium*, nano product from *C. cupreum* and chemical Tebuconasole showed significantly lower disease index than the

inoculated control. The efficacy of *Chaetomium* sp to control leaf spot of rice var. IR66 caused by *C. lunata* in pot experiment was further resulted that *C. cupreum* significantly reduced disease incidence of leaf spot caused by *C. lunata* . With this spraying spore suspension of *C. cupreum*, *Chaetomium*-biofungicide and chemical fungicide (Tebuconasole) to inoculated rice seedlings with *C. lunata* that significantly reduced disease incidence. Moreover, testing nano-products against *C. lunata* to control leaf spot of rice var. Sen Pidoa in a pot experiment showed that nano-CGH, nano-CGE and nano-CGM from *C. globosum* KMITL-N0805 significantly controlled leaf spot of rice var. Sen Pidoa. The disease severity index revealed that nano-CGM gave a significantly lower disease severity index than nano-CGH and nano-CGE when compared to non-treated control. It revealed that nano-CGH, nano-CGE and nano-CGM significantly increased in plant height and number of tillers when compared to the non-treated control.

In Field application of *Chaetomium* sp to control leaf spot of rice var. IR66 showed that chemical method gave the better result in plant height, number of tillers and all parameters including yield than GAP and organic methods which significantly differed when compared to non-treated control. Further field experiment for rice var. Sen Pidoa showed that the organic method significantly decreased leaf spot infection caused by *C. lunata*, followed by chemical method and GAP method. The chemical method gave the best results in all plant parameters, followed by the GAP and organic methods. The chemical method gave the highest panicle/plant, panicle length, panicle weight, grain weight/plant which significantly differed from the GAP and organic methods. The chemical method also gave the best results in filled grain/panicle, unfilled grain/panicle, grain weight/plot, dry hay weight/plot, biomass weight/plot and harvest index, and was significantly better than the GAP and organic methods. This is the first report using *C. cupreum* CC3003 to control leaf spot of rice var. Sen Pidoa caused by *C. lunata* in Cambodia.

หัวข้อวิทยานิพนธ์:	**การใช้ปัจจัยการผลิตการเกษตรในการผลิตข้าวโดยวิธีเกษตรดีที่เหมาะสม วิธีการใช้สารเคมี และวิธีเกษตรอินทรีย์**
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บทคัดย่อ

Curvularia lunata เป็นเชื้อราสาเหตุทำให้เกิดโรคใบจุดในข้าว พันธุ์ IR66 และ Sen Pidoa ในประเทศกัมพูชา ซึ่งเป็นรายงานครั้งแรกที่พบโรคใบจุดในข้าวพันธุ์ IR66 ในกัมพูชา ทุก isolates ที่แยกได้ นำมาพิสูจน์ความสามารถในการเกิดโรค จากการทดสอบการเลี้ยงเชื้อในอาหารร่วมพบว่า *Chaetomium cupreum* สามารถยับยั้งการสร้างสปอร์ *C. lunata* ได้อย่างมีนัยสำคัญยิ่งทางสถิติเมื่อเปรียบเทียบกับไม่ใช้วิธีการใด *Ch. cupreum* CC3003 มีคุณสมบัติเป็นจุลินทรีย์ต่อต้านเชื้อรา *C. lunata* สาเหตุโรคใบจุดของข้าวได้ สารสกัดรวม Crude hexane extract, Crude ethyl acetate extract and Crude methanol extract จากเชื้อรา *Ch. cupreum* CC3003 สามารถยับยั้งการสร้างสปอร์ของเชื้อสาเหตุโรคได้ 95.13%, 94.92% และ 87.90% ตามลำดับ ซึ่งมีค่า ED_{50} เท่ากับ 6.41 µg/ml, 0.83

μg/ml and 7.81μg/ml ตามลำดับนอกจากนี้. Nano-CGH, nano-CGE และ nano-CGM ที่พัฒนาจาก *Chaetomium globosum* KMITL-N0805 มีคุณสมบัติในการยับยั้งเชื้อรา *C. lunata* สาเหตุโรคใบจุดของข้าว พันธุ์ Sen Pidoaที่ ED_{50} เท่ากับ 1.21ppm/ml, 1.19ppm/ml และ 1.93 ppm/ml ตามลำดับ และ nano particles สามารถทำลายเซลล์ของเชื้อสาเหตุโรคและสูญเสียความสามารในการก่อ โรค

การทดสอบในกระถางทดลอง พบว่าสารออกฤทธิ์จากเชื้อรา *Chaetomium* สามารถยับยั้งโรคใบจุดของข้าว พันธุ์ Sen Pidoa ที่เกิดจากเชื้อ *C. lunata* และการใช้สปอร์แขวนลอยของ *Chaetomium* ชีวภัณฑ์ *Chaetomium* สารนาโนจาก *Chaetomium cupreum* และ สารเคมีป้องกันกำจัดเชื้อรา Butochlonazole สามารถเพิ่มความสูงของต้น การแตกกอ ได้อย่างมีนัยสำคัญยิ่งทางสถิติเมื่อเปรียบเทียบกับไม่ใช้วิธีการใด ต้นกล้าข้าวที่ฉีดพ่นด้วย สปอร์แขวนลอยของ *Chaetomium*, ชีวภัณฑ์ *Chaetomium* สารนาโนจาก *Chaetomium cupreum* และ สารเคมีป้องกันกำจัดเชื้อรา Butochlonazole มีดัชนีการเกิดโรคใบจุดต่ำกว่า ไม่ใช้วิธีการใด และจากการทดลองในข้าวพันธุ์ IR66 พบว่า *Ch. cupreum* มีประสิทธุภาพในการควบคุมโรคและลดการเกิดโรคใบจุดที่เกิดจากเชื้อรา การฉีดพ่นต้นข้าวที่ปลูกเชื้อสาเหตุโรค *C. lunata* ด้วยสปอร์แขวนลอย *Ch. cupreum*, ชีวภัณฑ์ *Chaetomium* และ สารเคมีป้องกันกำจัดเชื้อรา สามารถลดการเกิดโรคได้เมื่อเปรียบเทียบกับไม่ใช้วิธิการใด นอกจากนี้ การทดสอบสาร nano-products จาก *Chaetomium* ในการควบคุมเชื้อรา *C. lunata* สาเหตุโรคใบจุดของข้าว พันธุ์ Sen Pidoa ในกระถางทดลอง พบว่า สาร nano-CGH, nano-CGE และ nano-CGM จาก *Ch. globosum* KMITL-N0805 สามารถควบคุมโรคใบจุดของข้าวพันธุ์ SenPidoa การใช้สาร nano-CGM มีดัชนีการเกิดโรคต่ำกว่าสาร nano-CGH และ nano-CGE เมื่อเปรียบเทียบกับไม่ใช้วิธีการใด นอกจากนี้ สาร nano-CGH, nano-CGE และ nano-CGM สามารถเพิ่มความสูงและการแตกกอ ได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับไม่ใช้วิธีการใด

การทดสอบในแปลงทดลอง ในข้าวพันธุ์ IR66 พบว่าวิธีการใช้สารเคมี มีความสูง การแตกกอ และผลผลิต ดีกว่า วิธีการเกษตรดีที่เหมาะสม และวิธีการเกษตรอินทรีย์ เมื่อเปรียบเทียบกับไม่ใช้วิธีการใดส่วนการทดสอบในแปลงทดลองกับข้าวพันธุ์ Sen Pidoa พบว่าวิธีการเกษตรอินทรีย์สามารถลดการเกดโรคใบจุดที่เกิดจากเชื้อรา *C. lunata* รองลงมาคือ วิธีการใช้สารเคมี และวิธีการเกษตรดีที่เหมาะสม อย่างไรก็ตาม วิธีการใช้สารเคมี ได้ผลดีกว่า วิธีการเกษตรดีที่เหมาะสม และวิธีเกษตรอินทรีย์ ซึ่งวิธีการใช้สารเคมีมีปริมาณรวงข้าว ความยาวรวงข้าว น้ำหนักรวงข้าว ผลผลิตเมล็ดข้าวและฟางข้าว ดีกว่าวิธีการเกษตรดีที่เหมาะสม และวิธีเกษตรอินทรีย์ อย่างไรก็ตาม พบว่าเป็นรายงานครั้งแรกในการใช้ *Ch. cupreum* CC3003 ควบคุมโรคใบจุดที่เกิดจากเชื้อรา *C.lunata* ของข้าวพันธุ์ Sen Pidoaในประเทศกัมพูชา

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ABBREVIATION

Abbreviation	Meaning
CG-KMITL-N0805	*Chaetomium globosum*
CC3003	*Chaetomium cupreum*
No. Conc	Number of Concentration
Col-dia	Colony diameter
No-con	Number of conidia
PDA	Potato dextrose agar
Vs	Versus
WA	Water agar
KMITL	King Mongkut'sInsitute of Technology Ladkrabang
SRI	System of Rice Intensification
GAP	Good Agriculture Practice
PDB	Potato dextrose Broth
var.	Variety
EtoAC	Ethyl acetate
H	Hexane
MeOH	Methanol
C.cupreum	*Cheatomium cupreum*
C.lunata	*Curvularia lunata*
nano-CGH	Crude Hexane extract from *Cheatomium globosum*
nano-CGE	Crude Ethyl accetate extract from *Cheatomium globosum*
nano-CGM	Crude Methanol extract from *Cheatomium globosum*

CHAPTER 1

INTRODUCTION

1.1 Statement and Significant of Problem

Rice (*Oryza sativa* L.) belongs to Gramineae and it is the most economically important food crop in many developing countries (Matsuo *et al.* 1995). Rice is central to the lives of billions of people around the world. It is the staple food for 2.5 billion people and growing rice is the largest single use of land for producing food, covering 9 % of the earth's arable land. Rice provides 21% of global human per capita energy and 15% of per capital protein. Only 6-7% of the world's rice crop that is traded to the world markets. Thailand, Vietnam, China and the United States are the world's largest exporters. The United States produces 1.5% of the world's rice crop with Arkansas, California and Louisiana producing 80% of the U.S. rice crop, 85% of the rice that is produced in the world is used for direct human consumption (IRRI. 2001). About 57% of rice is grown on irrigated land, 25% on rainfed lowland, 10% on the uplands, 6% in deepwater, and 2% in tidal wetlands (Chopra and Prakash. 2002). Rice can be grown in many different environments especially in tropical climates like in Southeast Asia (Alford and Duguid. 1998 and Chaudhary *et al.* 2001). In Cambodia, rice cultivation is planted in rainfed lowland areas without irrigation. Total cultivated area for rice production in 24 provinces in 2009 was 2,719,080 ha and in 2010 was 2,795,892 ha, but the harvested area in 2009 was 2,674,603 ha and in 2010 was 2,777,323 ha only. The total yield production of rice in 2009 was 7,175,473 tons, and average yield was 2.84 t/ha and the total yield production of rice in 2010 was 8,249,452 tons, and average yield was 2.97 t/ha (Nesbitt. 1995; Pracilio *et.al,* 1997; Maeder. 2002; MAFF. 2010). It is needed to improve rice productivity by either using GAP or organic methods and to study on improving rice yield by insects and disease control, improve soil fertility by adding organic fertlizer and other environmental factors.

Brown leaf spot is one of the problem associated with rice disease of many varieties in Cambodia which caused by *Curvularia lunata* especially in the last few year. The pathogen is not only infected leaves but also infected in rice seeds caused by *C. lunata* leading to low yield and poor quailty (Simon and Lal. 2013). The other encountered problem of rice production that has been faced on increasingly application of chemical fungicides and pathogen become resistant to chemical fungicides and toxic residue polluted in surrounding environment and risk for human

being. Biological control of plant pathogens has successful provided a relatively recent strategy for integrating with other control measures. It could reduce the over use of chemical fungicides, improving agro-ecosystem and maintain natural balance. There are several reports on the potential use of biological control agents against plant pathogens (Kaewchai *et al.* 2009). *Chaetomium* spp. is one of the strictly saprophytic antagonists against several plant pathogens (Soytong and Quimio. 1989) e.g. *Phytophthora palmivora* (Pehprome and Soytong. 1996) and *Colletotrichum gloeosporioides* (Noiaium and Soytong. 1999) and *Pyricularia oryzae* (Soytong and Quimio. 1989; Soytong. 1992ab). The alternative method is challenging to find the safety agricultural inputs like bio-fertilizer and bio-pesticides to be used instead of those toxic chemicals for the use in rice production in term of good agricultural practice (GAP) and organic agriculture (Soytong *et al.* 2001).

Observation and preliminary disease diagnosis found that leaf spot of rice caused by *Curvularia lunata* become one of serious disease in Cambodia especially in rice var. Sen Pidoa (Tann and Soytong. 2016). With this, Kamaluddeen *et al.* (2013) reported that *Curvularia lunata* causing leaf spot for the first time in India that symptom expressed brown leaf spot and finally become blight. Moreover, there are reported that *Curvularia lunata* caused many symptoms in rice eg. grain discoloration (Groves and Skolko. 1945), leaf spot (Patwick. 1950), black kernel and seedling blight (Martin. 1939) and sheath rot of rice is reported for the first time in Tamil Nadu, India (Lakshmanan. 1992). *Curvularia lunata* causing leaf spots on *Sorghum bicolor* was reported for the first time in Pakistan (Akram *et al.* 2014). Biological control of plant diseases is widely distributed to farmers to decrease the use of toxic chemical fungicides that polluted to the environment and harmful to living things.. *Chaetomium* spp. belong to the Ascomycota is reported as biocontrol agent against several plant pathogens (Soytong *et al.* 2001; Alford and Duguid. 1998). *Chaetomium globosum* and *Ch. cupreum* are successfully applied to control rice blast caused by *Pyricularia oryzae* (Soytong and Quimio. 1989).

The search for effective alternative methods of plant disease control is mandated by the need to reduce or eliminate to non target effects on humans and environment. Recently, nanotechnology becomes to be a new technology for building, re-structuring, controlling and devising materials at the molecular level. Molecular nanotechnology involves building organic materials into defined structures, atom by atom or molecule by molecule, often by self-assembly or self-organization. Agricultural applications of nanotechnology have greatly advanced in recent years (Li *et al.* 2011). Scientists are actively engaged in the synthesis and investigation of

organic nano materials including different kinds of nano particles having unusual optical, physical, and biological properties (Elibol *et al.* 2003; Salata. 2004). The uses of nanotechnology in agriculture are being explored and progressed. Precision farming, for example, along with nano-delivery systems are becoming the new "industrial revolution" in agriculture (Soutter. 2012). As such, there is great potential for nano science and technology in providing state-of-the-art solutions for various challenges faced by agriculture and society today (Ditta. 2012). Nano particles can serve as 'magic bullets', containing bioactive substances from antagonistic fungi which can enable effective penetration through cuticles and tissues, allowing slow and constant release of the active substances. The most popular shapes of nano materials being used for biocides delivery are nano spheres, nano capsules, and nano gels (Perlatti *et al.* 2013). Nanotechnology can provide green and efficient alternatives for disease and insect pest management in agriculture without compromising nature (Rai and Ingle. 2012). Moreover, nanotechnology has great potential in agriculture to enhance life quality through application for crop production (Ditta. 2012). Some nano particles have been formulated containing pesticides in colloidal suspensions or as powders, at the nano or micro scale. These preparations have advantages to increase the stability of active organic compounds, systemic activity, synergism, and specificity, and reducing foliar settling and leaching (Perlatti *et al.* 2013). Nano-formulations have been done not only for synthetic insecticides but also in alternative products such as natural products (herbal extracts) and microorganisms to control insects and diseases. The climate changes that woud concern in sustainable use of natural resources, get rid of toxic pesticides and fertilizers need to be addressed immediately (Ditta. 2012). However, there are very few reports for the use of nano carrier systems in agriculture (Nguyen *et al.* 2012). Recently, the application of bio-active compounds from different *Chaetomium* species has been actively proven to be an effective antifungal properties against several plant pathogens (Soytong *et al.* 2001). It is to safe and protect the environment with appropriate method for controlling plant disease. The construction and characterization of copolymer nano particle loaded with bio-active compounds rather than toxic pesticides is needed to investgate in this research findinds. The aim of this research was partly designed nano particles from active compounds of *Chaetomium globosum* KMITL-N0805 and tested to control *Curvularia lunata* causing leaf spot of rice.

The research findings are included testing and developing new biological products as agricyultural inputs for rice cultivation with good agricultural practice (GAP) and organic methods.

1.2 Objectives of the Study

1. To isolate, identification and pathogenicity test of leaf spot rice varieties IR66 and Sen Pidoa.
2. To conduct biculure antagonist test againt *Curvularia lunata* isolate frome Rice varieties Sen Pidoa and IR66.
3. To conduct the *Chaetomium cupreum* extraction and testing the antifungal metabolites from *Chaetomium cupreum* against *Curvularia lunata* in PDA plate
4. Testing nano-material from *Chaetomium globosum* KMITL-N08505 against *Curvularia lunata* in PDA plate.
5. To evaluate the fungal metabolites from *Chaetomium cupreum* to inhibit *Curvularia lunata* causing leaf spot of rice var. Sen Pidoa in pot experiment,
6. To test the efficacy of *Chaetomium cupreum* to control leaf spot of rice variety IR66 caused by *Curvularia lunata* in pot experiment,
7. To test nano-CGH, nano CGE and nano CGM from *Cheatomium globosum* KMITL-N0805 against *Curvularia lunata* in pot experiment.
8. Application of agricultural inputs for cultivation of rice variety IR66 and Sen Pidoa in the field.

1.3 Scopes of the Study

The research fiddling was to investigate seriously disease of rice var. IR66 and Sen Pidoa planted in Cambodia. All isolates of pathogen were proved for the pathogenicity test. The antagonistic fungi eg. *Chaetomium cupreum* CC3003 and *Chaetomium globosum* KMITL – N0805 were tested with leaf spot pathogen by bi-culture test. Control mechanism of *Chaetomium* spp. were also proved by fungal metabolites testing against rice pathogen. Fungal metabolites were performed to nano-particle to increase effective control. Formulation of those antagonists and their metabolites including nano-products were also evaluated in laboratory, pot experiments and field trials.

1.4 Location and Time of the Study

The studies were conducted at:

1. Faculty of Agricultural Technology and Faculty of Science, King Mongkut's Institute of Technology Ladkrabang (KMITL) - Bangkok 10520, Thailand.

2. The field site experiment were located at Ou rung Village, Pongro kroum Commune, Chi kreng District, Siem Reap Province, Kingdom of Cambodia.
3. The research was conducted from the years of 2012 to 2016

CHAPTER 2

LITERATURE REVIEW

2.1 Background

Rice (*Oryza sativa* L) is the staple food of Southeast Asian, especially in Cambodia. It is trended to produce for food security. Rice growing fields are also considered as a fundamental sector for Cambodian economy and has become a top priority for developmental programs because over 80% of people in Cambodia are living in rural areas and the majority of them are farmers (Nesbitt. 1995). The rice production is particularly important among the poor, where it accounts for 50-80% of daily caloric intake (IRRI. 2001).

Rice is a major commodity in world trade and has become the second most important cereal in the world after wheat in terms of production, due to a recent decline in maize production (Jones. 1995). It is widely cultivated throughout the tropical climates. In South-East Asia, where flood controls are effective, production is high yield. As noted in the introduction rice provides 21% of the per capita energy and 15% of the per capita protein (IRRI. 2002). In Cambodia, the flooded rice paddies provide a field of aquatic biodiversity, including fish, plants, amphibians, reptiles, mollusks, and crustaceans. Many of which the poors use as a meal to incorporate protein into their diets (International Year of Rice. 2004).

Cambodia is primarily a rural society with 85% of the population living in rural areas and agriculture is a vital part of the rural economy. Agriculture accounts for 30% of the gross domestic product (GDP) and provides 70% of the employment. An estimated 4.7 million Cambodian people (34.7%) live below the poverty line, and 21% of people in Cambodia live below the extreme poverty line. Approximately 90% of those poors live in rural areas. Their lilfe depend on agricultural production for their livelihood. Therefore, improved performance of the agricultural sector is essential to decrease poverty. Crops are produced on about 7.37 million ha (24% of the land) while forests cover about 56%. Rice is the dominant crop which covers about 3.57 million ha (80% of the agricultural land) including areas of receding and floating rice and rice paddies interspersed by villages. Field crops comprise of 6%, rubber 2%, garden crops 7%, orchards 1%, and others being slash and burn 8%. The cultivated areas for rice is about 2.44 million ha (MAFF. 2010-2011).

Rice can symbolize the state of food security in Cambodia. It is the quintessential food which contributes to 54% of the country's total crop production in 2007 and 68% of calories intake per capita. Additionally it is of interest that Cambodia has regained self-sufficiency in rice cultivation and exporting more than a decade. Cambodia has achieved a rice surplus of an estimated 2.67 million tons in 2007. This surplus due to both the steady increase of rice cultivated areas (from 1.8 million ha in 1993 to over 2.5 million ha in 2007) and productivity with an average yield per ha increased from 2.1 t/ha in 2003 to 2.81 t/ha in 2010 (MAFF annual report. 2010-2011).

2.2 Statement of problems

The majority of farmers are poor and still faced with many constraints in their production activities. However, they also faced about water control, soil fertility, varietal seed quality, pests and diseases leading to high production costs (Nesbitt. 1997). Cambodia is a subsistence economy reliant nearly entirely on agriculture. It is dependent on the production of rice as its primary food for employment and income. The vast majority of the population lives in rural areas (84%) and is dedicated to agricultural activities (82%). It is estimated about 20 % of all families that do not their own land and that 25% of owners have only 0.5 hectares. In recent year, jobs arising from agricultural activities has decreased. It is due to factors such as the introduction of machinery for agricultural work, competition with foreign exports and market constraint (NPRS and MDG. 2008).

About 21% of cultivated areas could provide an irrigation scheme that could cultivate rice crops during the dry season. It was low yield of 2.62 to 3.1 tons per hectare in dry season, but rice is still a major concern for cultivation in Cambodia regardless of season. Farmers are still implemented a traditional rice cultivation method using local rice seeds. The lack of purity seeds of rice usually creates a low yield too (MAFF Annual report. 2010-2011).

In the wet season, rice cultivation is reliant on rainfall, which in return can be a high risk from flooding as in 2011 and greatly affected to yield. In 2011, there were 18 provinces in Cambodia affected by floods. Around 431476 hectares of cultivated rice fields were affected by the floods and among these, there were 267184 hectares of damaged rice (Fao/wfp .2012).

Recently, the agricultural production and land cultivation areas have increased and many farmers have moved towards an increase in application of chemical fertilizers and chemical pesticides. In 2002, Cambodians imported 45,334 tons of agrochemicals, and altogether 936,753.882 tons of chemicals between the years 2003 to 2008. The imported chemical fertilizers

are sure to be more than listed due to lack of detailed nomenclature on chemical fertilizer recording procedures, based on (national profile on chemicals management in cambodia, in Harmonized System, H.S. Code 31.02.29.00. 2004). The labels of chemical fertilizers and pesticides are primarily (95%) labeled in foreign languages. This leads to inappropriate pesticide use, including the timing, frequency, concentration and type of products used. This misuse of product is widespread in Cambodia. Safety measures are often ignored or misunderstood by the people using them. This situation is only made the worse by peoples who lack of knowledge about harzadous risks associated with pesticides and due to inadequate labeling that is incomprehensible to the rural users (Preap and Kang. 2012).

2.3 Rice Cultivation in Cambodia

Rice is the primary stable food in Cambodia. The total cultivated area for rice production in 24 provinces in 2010 -2011 was 2,795,892 ha, but the harvested area was only 2,777,323 ha. The average yield was 2.970 t/ha and total production was 8,249,452t (MAFF Cambodia. 2010-2011). It seems to be low yield as the farmers faced the said problem eg insect pests, diseases and other factors.

Rice ecosystems in Cambodia are primarily divided into four types: rain fed lowlands (including areas with supplementary irrigation), rain fed uplands, areas of deepwater/floating cultivation, and dry season irrigated land. About 58% of the harvested rice comes from rain fed lowland ecologies, and 32 percent from deepwater ecologies as reported in developing sustainable rice production systems in Cambodia (Harry and Nesbitt. 2003).

Rice crops are primarily grown in the wet season under rain fed lowland conditions. The current total cultivated area for rice is about 2.44 million hectares suggesting that about 1.13 million hectares of rice land is not being utilized for rice cultivation (MAFF. 2009). Wet season rain fed lowland rice crops occupies about 84% of the total cultivated areas. In the dry season, rice crops with full and/or supplementary irrigation occupy only about 11%, and the remainders are deepwater and upland rice crops (Bell and Seng. 2008). Cultivated rice areas have recently increased dramatically by approximately 14% from 2000 to 2005. Total irrigated rice areas including supplementary irrigated areas increased by about 67% from 284,172 ha in 2001 (FAO. 2010) to 475,000 ha (RGC. 2009). In 2010 the Royal Government of Cambodia has planned to increase irrigated rice areas to 594,000 ha, thus also increasing the total rice production to 7.0 million metric tons with an average yield of 2.81t/ha (RGC. 2010).

2.4 Rice varieties in Cambodia

Rice varieties in Cambodia are released by the Cambodian Agricultural Research and Development Institute (CARDI) from 1990 up until 2011. They have covered 38 rice varieties. There are early duration varieties that are photoperiod insensitive and mature in less than 120 days which include 9 varieties (IR66, IR72, Kru, IR Kesar, Chul'sa, Baray, Rumpe, Rohat and Sen Pidoa) and 2 upland rice varieties (Sita and Rimke). Medium duration insensitive photoperiod varieties to weakly sensitive varieties which mature in 120 to 150 days that include 5 varieties (Sentepheap 1, Sentepheap 2, Sentepheap 3, Sarika and Popoul). Medium duration sensitive photoperiod varieties that flower from mid-October to mid-November including 6 varieties, CAR 1, CAR 2, CAR 3, CAR 11, Riang Chey and Phka Chan Sen Sar. Medium duration aromatic sensitive photoperiod sensitive varieties that flower from mid-October to mid-November which include 5 varieties Phka Rumchek, Phka Rumchang, Phka Rumduol, Phka Romdeng and Phka Romeat. Long duration photoperiod sensitive varieties that flower after mid-November include 8 varieties CAR 4, CAR 5, CAR 6, CAR 7, CAR 8, CAR 9, CAR 12 and CAR 13. The final 3 deepwater rice varieties are Don, Khao Tah Pech and Tewada (CARDI. 1990).

There has been a strategic look on agricultural investment and rice exportation of the RGC in 2010. MAFF has approved and released 10 rice varieties for promoting rice production in Cambodia and exportation. Those ten rice varieties are permitted for utilization in Cambodia. Those rice varieties included Phka Rumchek, Phka Rumchang, Phka Rumduol, Phka Romdeng and Phka Romeat, IR66, Sen Pidou, Chul'sa, CAR 4, CAR 6, and Riang Chey (Men *et al.* 2001). However, there was a constraint, as the rice seeds available in stock which were produced by CARDI which limited in number and could not supply the Cambodian farmers nationwide. It would require 5 to 10 years in order to produce the high quality seeds that requires to fill to the huge cultivated area in Cambodia.

2.5 Policy for System of Rice Intensification Technique

The System of Rice Intensification (SRI) has been sucessfully practiced for many years since it was originally developed it (Henri de Laulanie, 2011). The benefits of SRI, which have been demonstrated in over 40 countries, are an increase in yield of 50-100% or more, a reduction in seed requirements (up to 90%) and water savings (50% or more) Miguel A Altieri. (2012). Many SRI users have additionally reported a reduction in pests, diseases, grain shattering, unfilled grains and lodging. As a climate-smart agricultural methodology, additional environmental

benefits have increased from the reduction of agricultural chemicals, water use and methane emissions, such as a reduction in emissions that contribute to global warming.

Some techniques and principles of SRI have already known by some Cambodian farmers through their observations and experiences. There were not known experiences of growing rice by combining or integrating all of these techniques. Additionally, CEDAC organization has gained positive experience in soil and nutrient management in rice production, such as field levelling by dividing the rice field into smaller plots, establishing small canals in the field for the purpose of better field drainage, and the application of organic matter (green leaves and decomposed organic matter during the vegetative stage of rice). This experience is integrated into the practice of SRI. In 2000, SRI was launched and the first field trials were introduced by combining all good practices in rice production in Cambodia. Many farmers in Kampong Thom province are supported in collaboration with PDP-GTZ in order to find out how these technologies work under different agro-climatic conditions, as well as to further develop SRI in the Cambodian context. Until now more than one millions farmers have applied for the SRI technique to be applied on their land and SRI has already become integrated into the agriculture sector plan, as presently there is a SRI secretary based in MAFF (Summary of SRI in Cambodia. 2000).

2.6 World organic rice production

Organic farming is a form of ecological and sustainable agriculture that relies on techniques such as improving and maintaining soil fertility by adding organic compost, green manure, crop rotation and biological pesticides in the farm. Organic farming uses bio-fertilizers and bio-pesticides and do not allow any use of chemical fertilizers, chemical pesticides, synthetic plant growth regulators like hormones, antibiotics, genetically modified organisms (GMO) and nano materials (Paull. 2011).

Organic agricultural methods have become internationally regulation and legally enforced by several nations. These standards are largely based on the standards set by the International Federation of Organic Agriculture Movements (IFOAM), established in 1972 (Paull. 2010).

IFOAM defines the goal of organic farming as an organic agricultural producing system that sustains the health of soils, ecosystems and people. It is reliant on ecological processes, biodiversity and an adaptation to local conditions, rather than the use of inputs with adverse effects. Organic farms becomes the original type of agriculture, and have been practiced for thousands of years. Agrohemical methods are introduced during the industrial revolution. Some

methods were not well developed and had serious side effects from toxic chemicals and harzadius to environment snd human beings as well. It has started to do an organic agriculture movement since in the mid-1920s in Central Europe through the work of Rodolf Steiner. The increase in environmental awareness in the general population has transformed the original supply-driven movement to a demand-driven one. Premium prices and some government subsidies have attracted farmers to turning organic. In the developing world, many producers use traditional methods which are comparable to organic farming but are not certified. In other cases, farmers in the developing world have converted for economic reasons (Paull. 2011). With this, it is needed to promote organic agriculture to other part of the world including Cambodia.

Yong-Hwan Lee *et al.* (2003) reported that organic farming is responsible for agricultural ecosystems and plays a key role in ecological protection and agricultural production. There are no chemical fertilizers and pesticides in organic farming, so nutrients must come from plant growth supplied by organic fertilizers such as compost containing various organic materials. The management of soil fertility in organic farming is differed from other more conventional types of farming. For this reason, soil fertility such as soil chemistry, physical and biological properties must be concerned for crop productivity to determine on organically managed rice paddy soil. This was to manage soil fertility in a proper way for long-term rice cultivation. Yong-Hwan Lee e*t al.* (2003) and Otto. (2003) stated that organic farming become a key for material circulation in agricultural ecosystems and enhanced crop production with a minimal environmental factors. Organic farming encourages to maintain ecological balance, and maintains the general meaning of holistic production and management system, for enhancing the health of an agricultural ecosystem.

The CODEX are guidelines on an international standard for food production, specific agricultural techniques for organic production have been developed and applied in Korea based on CODEX guidelines (CODEX Alimentarius Commission. 1999). In the Philippines, the Department of Agriculture set in motion a one-year program to promote the shift from conventional rice production methods to organic rice production models in four pilot areas in the province of Pangasinan. The project was implemented by the La Liga Policy Institute in partnership with the local government units of Alaminos City and the municipalities of Burgos, Bani and Dasol. Local chief executives of the four LGUs – Mayor Hernani A. Braganza, Mayor Marcelo Navarro (Bani), Mayor Alberto Guiang (Burgos) and Mayor Noel Nacar (Dasol) – agreed to promote sustainable, organic and ecological agriculture in their respective localities as a

strategy to pursue local economic development. A member of Go Organic, Philippines, La Liga, promotes organic farming in the Philippines. The program was a part of the Philippines Department of Agricultures strategy to attain staple food self sufficiency within the term of President Aquino. Despite rice's significance, the Philippines is still a rice-importing country. "A concerted effort to increase riceproductivity is imperative". Organic matter is very important to improving soil productivity and has been used since agriculture began. Various organic matters such as oil cakes, fish meals, green manures, and compost, were all used in the early days of farming. Oil cakes and fish meals have become obsolete in modern rice production. Green manures and compost have become the important sources of organic matter. Recently, fresh rice straw has become a popular source of organic matter, especially in wetland rice production (Oh. 1978).

Compost production is being applied to increase rice yield (Park and Lee. 1969). The increase of soil fertility through organic matter application is essential in maintaining a stable high crop yield (Cooke. 1977). The market research company Organic Monitor estimated the global market for organic products in 2009 at 55 billion US dollars (€40 billion), roughly 5 percent more than in 2008. The largest amount of organic products are sold in Europe and North America. In Europe, over €18 billion was spent, with Germany leading at €5.8 billion, followed by France at €3 billion and the United Kingdom at €2.1 billion. The countries with the highest per capita spending are Denmark and Switzerland with more than €130 spent annually (Cambodia Organic Agriculture Association. 2011).

2.7 Organic rice production in Cambodia

Cambodia is a latecomer on the international organic agriculture scene. Cambodia's neighbour Thailand, for example, already has a long record of recognized organic agriculture. In 2006, several NGOs and entrepreneurs began development programs to conduct by international donors and individuals in government ministries. They established the Cambodian Organic Agriculture Association (COrAA). COrAA is established as a domestic organization where Cambodian stakeholders, especially those from the private-sectors, which would have ownership and take a leading role in promoting organic agriculture within Cambodia. Unfortunately, partially due to Cambodia being late in building a domestic organic sector, Cambodia has a limited capacity to advise interested farmers and entrepreneurs, as well as to certify their produce organic. Although a considerable number of farmers in Cambodia are still cultivating their crops,

especially rice, with little or no use of synthetic fertilizers and almost no pesticides; they do not necessarily to build up organic farms and do not know how to improve the fertility of the soil.

Cambodia Organic Agriculture Association (COrAA), (2011). Organic rice production is considered and certified by CorAA in 2008 after the CEDAC organization. It is formed by the farmers producing organic rice. There are 7,800 farmers involved in organic rice cultivation over 8,000ha of land. There are 790 farmer associations. At least 4,000 tonnes of paddy produced by farmers. In 2009-2010 there were 1500 tonnes of paddy sold to CEDAC by farmers associations. In 2007-2008, according to the data collected by 7,800 farmers that involved in associations, the average organic rice yield was 2,3 tonnes per hectare. In some areas, organic rice farms obtained an average yield slightly below two tons per hectare becose they are still lack of knowledge. Some farmers are able to plant green manure crops, such as beans, obtained an average of 3.5 to 5.4 metric tons per hectare (Cambodia Organic Agriculture Association. 2011).

2.8 Good Agriculture Practice (GAP) for Rice production

GAP is not new method in rice production. In Southeast Asia, farmers already used this method for planting rice and research is being done to facilitate diffusion and adoption of these practices (IRRI. 2009). Agriculture imput use for this GAP method which were bio-chemecal fertilizer, bio insecticide (*Metarhizium* and *Beauveria*), bio fungicide (CM product as Ketomium, nano CC3003), insecticide (abamectin), fungicide(benomyl, Tebuconazole). For disease and insect control: alternative spraying a mixture of every 15-20 days until harvest by (Tann and Kasem, 2016). GAP method were incresed and better of the plant height, number tiller, panicle lenght, number full grain seed, biomass yield and grain yield when compare with non treatment (Tann *et al.* 2011). The Good Agricultural Practices method is very similar to the System of Rice Intensification (SRI) which was reseach in Toek Vil agriculture research station in Cambodia (Tann *et al.* 2012). Noltze *et al.* (2012) stated that the Good Agricultural Practices and System of Rice Intensification can be understood assystem technologies defined as an integrated innovation to improve agriculturalproductivity and agro ecosystem resilience, involving different agronomic and management components with synergistic relationships.

2.9 Agriculture inputs for rice production

Tann *et al.* (2011) stated that biological products have been developed as agricultural inputs for safety during crop production. These biological products have been successfully applied to promote organic farms on a commercial scale. The organic farms are able to be

certified by BioAgriCert and IFOAM (International Federation for Organic Agriculture Movement). The microbial products are in turn used to reduce damage and decrease toxic chemicals in agricultural products and the surrounding environment and used to encourage sustainable development and production. These are microbial fertilizer, and biological humus to improve soil fertility and promote plant growth, and *Chaetomium* biological fungicide for disease control. The interest in agricultural inputs for organic crop production has increased in several countries. There needs to be further research for new agricultural inputs for application in organic farms. For example, a new biological fungicide could be released for controlling diseases such as *Emericella* sp. to control tomato wilt.

Biological fertilizers consists of effective isolates *Arthrobotrys oligospora* AO, *Aspergillus oryzae* AsO, *Aspergillus terreus* Ast, *Chaetomium lucknowens* CL, *Emericella nivea* EN, *Emericella rogulosa* ER, *Pseudoeurotium zonatum* EC, *Mucor plumbeus* MC, *Penicillium variabile* PV, *Pseudoeurotium ovale* EH, *Trichoderma hamatum* Thm-Bio1 and *Trichoderma harzianum* Thz-Bio2. The fertilizers come in either powder or pellet forms to apply at different stages of growth at the rate of 1,250 – 2,500 kg/hectare (Soytong *et al.* 2011).

Biofertilizers with high phosphorous consist primarily of the selected microorganisms *Aspergillus niger*, *Pennicillium* sp., *Chaetomium lucknowense*, Actinomycetes C4-8, Actinomycetes T1-V, Actinomycetes T2-4, Actinomycetes T2-10 and Actinomycetes T2-Y. These microorganisms were used to sterilize ground rock phosphate and/or potassium feldspar. It was found that sterilized rock phosphate incubated with *Penicillium* sp. could release a significant amount of the available phosphorus at a rate of 166.75 ppm, higher than the non-treated control that released available phosphorus at the rate of 8.00 ppm. Biofertilizers with high potassium contained sterilized potassium feldspar and Actinomycetes T2-4 which can significantly increase the release of available potassium at the rate of 20.00 ppm compared to the non-treated control that releases available potassium at the rate of 9.50 ppm. The other tested microorganism in this study also released some amount of available phosphorous and potassium. High phosphorus-biofertilizer and high potassium-biofertilizer could be developed to be applied on soil for organic crop production (Nguyen Huu *et al.* 2011).

Chaetomium is commercialized as a new broad spectrum biological fungicide in powder or suspension concentration forms mixing 22-strains of *Chaetomium cupreum* and *C. globosum*. The mechanism of disease control is competition, antibiosis/lysis, antagonism, induced immunity in plants and hyphal interference. *Ch. cupreum* found to produce rotiorinol and *Ch. globosum*

produces chaetoglobosin-c. Those antibiotic substances can inhibit several plant pathogens. It has been registered as patent rights namely: *Chaetomium* as a new broad spectrum mycofungicide: Int. cl.[5] AO 1 N 25/12. The main purpose is to prevent soil-borne plant pathogens, such as *Phytophthora* spp., *Pythium* spp, and *Fusarium* spp. It is additionally compatible for mixing with selected chemical pesticides. These can alternatively be sprayed with many pesticides at the rate of 3-5 kg or L per hectare. Applications in the fields have been successful in several countries, including Thailand, China, Costa Rica, Vietnam, Laos, Philippines, Bangladesh, Cambodia, Georgia and Russia (Soytong *et al.* 2011).

Chaetomium-biological fungicide can be used for perennial crops such as fruit trees (e.g. apple, peach, citrus, black pepper, coffee, guava, durian, and mango etc). *Chaetomium*-biological fungicide controls several plant pathogens on the fruit trees, such as *Pyricularia oryzae, Phytophthora parasitica, Phytophthora palmivora, Phytophthora infestans, Fusarium oxysporum, Sclerotium rolfsii, Rhizoctonia solani, Thielaviopsia paradoxa, Rigidophorus microporus, Colletotrichum gloeosporioides. Chaetomium*-biological fungicide may also be used for annual crops (e.g. kale, chinese cabbage, radish, cucumber, chilli, potato, rice, corn, soybean, watermelon, grape and tobacco etc). It requires being applied at the rate of 3-5 kg per hectare by mixing biological hums at the rate of 10g per 20 litres of water, and adding organic compost into the soils every 2-4 months for plant protection (Soytong *et al.* 2011). There are many benefits to using mycofungicides and biofertilizers which include decreasing the occurrence of plant disease by inhibiting the growth of pathogens, suppressing the amount of inocula of pathogens, improving the uptake of nutrients from the soil or atmosphere, and producing bioactive compounds, hormones and enzymes which encourage plant growth. These benefits protect and increase the crop production. There are several commercial mycofungicides and fungal biofertilizers currently available worldwide. Using mycofungicides and fungal biofertilizers offer a more environmentally friendly alternative than chemical fungicides and chemical fertilizers. Although there are some limitations in using these products. Their success is affected by environmental conditions, there are difficulties during application, they have a limited shelf life, and they are slow acting compared to their chemical product equivalents, which may discourage farmers from using them. Research on the development of mycofungicides and fungal biofertilizers needs to be carried out so more effective products can be produced (Kaewchai *et al.* 2009).

Soytong. (2004) stated that application of chemical fungicides has been recognized as a cause of environmental pollution and it leaves chemical residues in the soil, water and agricultural products. Continuous use of chemical fungicides leads to the development of resistance in pathogens (Prechaprome and Soytong. 1996) biological control of plant pathogens has provided a successful strategy when integrated with other control measures. It reduces the heavy use of chemical fungicides, which in turn improves agro-ecosystems and helps maintain the natural balance. There are several reports on the potential use of biological control agents against plant pathogens. *Chaetomium* spp. is one of the strictly saprophytic antagonists against several plant pathogens, such as *Phytophthora palmivora* (Prechaprome and Soytong. 1996; Sodsa-art and Soytong. 1999) and *Colletotrichum gloeosporioides* (Noiaium and Soytong. 1999).

Agriculture safety in crop production would be divided into 3 types as follows: good agricultural practices (GAP) of crops, pesticide-free production and organic crop production. The primary purpose is to lessen the use of toxic chemical pesticides. This research finding aims to prove microbial products used for organic crop production in the field are beneficial in several aspects. The bioproducts are afterwards able to be evaluated and certified as agricultural inputs of the International Federation of Organic Agriculture Movement (IFOAM) for organic vegetable production to meet organic standards of IFOAM (Soytong. 2004).

CHAPTER 3

MATERIALS AND METHODS

3.1 Laboratory tests

3.1.1 Isolation, identification and pathogenicity test of rice pathogen

Leaf spots of rice variety IR66 and Sen Pidoa were isolated from leaf symptoms by tissue transplanting method (Soytong and Quimio. 1989). The mycelia on water agar (WA) were cultured onto potato dextrose agar (PDA) until get pure culture. All isolates were identified by morphologically observation under compound microscope. All isolates were tested for pathogenicity test followed the method of Koch's Postulate. The pathogen inoculum was prepared as spore suspension of $1x10^6$ spore/ml. The inoculum was inoculated to 20 day rice seedlings planted in pots of 30cm diameter by spraying to seedlings, then covered with plastic bags to maintain moisture content. The appeared symptom was re-isolated to be pure culture and identified to confirm species.

3.1.2 Bi-culture antagonistic test against *Curvularia lunata* isolated from rice variety IR66 and Sen Pidoa

Chaetomium cupreum CC3003 was offered by Assoc. Prof. Dr. Kasem Soytong. *Ch. cupreum* was tested against *Curvularia lunata* causing brown leaf spot in bi-culture plates. The test was performed by using the method of Soytong, (1992). The fungal antagonists and the virulent isolate of *C. lunata* were made bi-culture on potato dextrose agar (PDA) and incubated at the room temperature (28-30°C). The edge of actively growing colony of *C. lunata* and *Chaetomium cupreum* was cut with 0.5mm diameter by the sterilized cork borer and one agar plug of each fungus was cultured to the opposite sides on the PDA plates of 9cm diameter and separately culture of *Chaetomium cupreum* and *C. lunata* served as a controls, then incubated at the room temperature (28-30°C) for four weeks. Data were collected as colony diameter (cm) and sporulation which counted on Haemacytometer under compound microscope. The experiment was done using a completely randomized design (CRD) with four replications. Data collection were recorded as colony diameter (cm), spore number of tested pathogen, and computed the analysis of variance (ANOV), then compared treatment means using Duncan's Multiple Range Test (DMRT) at P = 0.05 and 0.01.

3.1.3 Antifungal metabolites from *Chaetomium cupreum* CC3003 against *Curvularia lunata* in vitro

The crude hexane-extract, ethyl acetate and methanol from *Chaetomium cupreum* CC3003 were made. The method of *Chaetomium cupreun* extraction were explained as follows: Fungal growth and extraction of crude extracts- *Chaetomium cupreum* was cultured in potato dextrose broth (PDB) and incubated at room temperature (28-30°C) for 4 weeks. Fungal biomass were removed the liquid by cheesecloth filtration and dried over night at 28-30°C for 3 days. The extraction was performed by the method described by Kanokmedhakul *et al.* (2006). The air-dried fungal biomass of *C. cupreum* was ground and extracted with hexane (1:1 vol/vol) and incubated by shaking for 3 days at room temperature. The solvent was separately taken out the marc by filtration through filter paper (Whatman No.4). The marc from hexane extraction was extracted with ethyl acetate (EtOAc) and followed with methanol (MeOH) using the same method as hexane. The solvents were separately evaporated to yield crude hexane, ethy acetate and methanol extracts, respectively (Fig. 1).

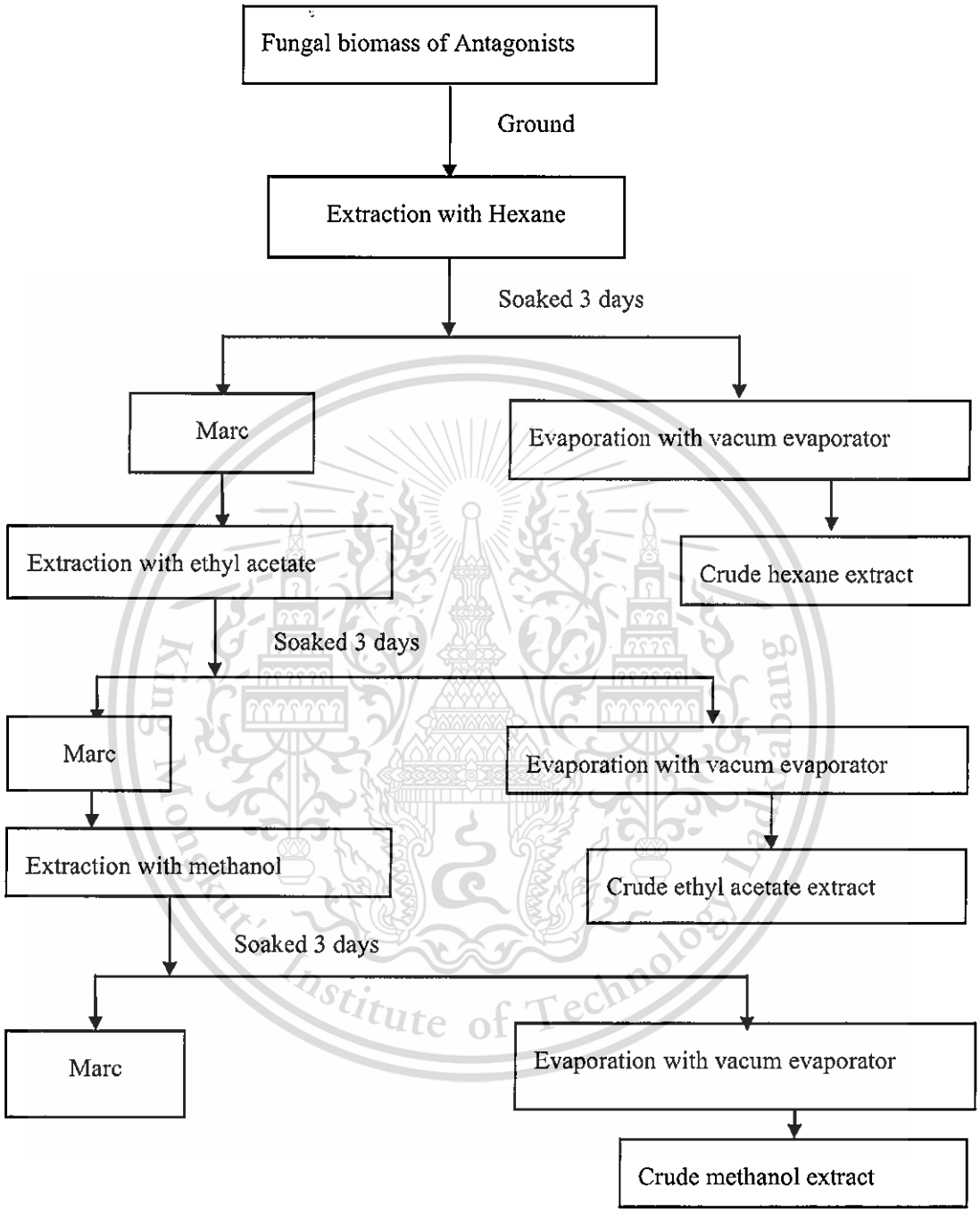


Fig. 1 Diagram of *Chaetomium cupreum* extraction.

The antifungal metabolites from *Chaetomium cupreum* against *Curvularia lunata* was done by using poisonous method Soytong *et al.* (2001) in PDA plate of 5cm diameter. The experiment was done by using two factorial experiments in Completely Randomized Design (CRD) with four replications. Factor A represented crude hexane, ethyl acetate and methanol extracts. Each crude extract was dissolved with 2% dimethyl sulfoxide (DMSO), mixed into PDA before autoclaving at 121°C for 30 min and factor B represented the concentrations of 0, 10, 50, 100, 500 and 1000 µg/ml of each crude extract. The 7 days old of colony advance margin of *Curvularia lunata* was cut with 3mm and sub-cultured to the middle of PDA plate in each concentration of crude extract, and incubated at room temperature (28-30°C) for 4 days. Data were collected as colony diameter (cm) and spore production. Data were computed analysis of variance (ANOVA) and treatment means were compared using the Duncan's multiple range test (DMRT) at P=0.01. The effective dose of ED_{50} values was computed using probit analysis.

3.1.4 Testing nano-materials derived from *Chaetomium globosum* KMITL–N0805 against *Curvularia lunata* in laboratory

Nano-particles used in this experiment that made from metabolites of *Chaetomium globosum* KMITL-N 0805 are offered by Joselito Dar and Kasem Soytong (KMITL, Bangkok, Thailand) who firstly investigated this new nano-particles which developed and characacterized nanomaterial loaded with active compounds from *Chaetomium* sp. The crude hexane, crude ethyl acetate and crude methanol extracts from *C. globosum* KMITL-N 0805 were separately formed into nano-particles by electro spinning. Exactly 2g of polylactic acid was dissolved in 10ml tetrahydrofuran. The mixture was heated until the polylactic acid was totally melted. The extracts was dissolved in a few drops of dimethyl sulfoxide and heated to dissolve completely and then the two mixtures were added together. The resulting mixture was loaded into a syringe and placed into the electro-spinning set-up. The tip of the syringe was clipped to the positive pole while aluminum foil was clipped to the negative pole and served as the collector. The voltage used was 25 to 30 kilovolts. Nano-particles containing no active compound were also made and served as the control. The product was carefully scraped from the aluminum foil and stored in tightly capped bottles. The characteristics of the products namely nano-CGH , nano-CGE and nano-CGM were noted, viewed under the scanning electron microscope and the properties were analyzed using Fourier Transform Infrared spectroscopy (FTIS) (Dar and Soytong. 2014).

The experiment was performed by using a Completely Randomized Design (CRD) with four replications. Treatments were inoculated control, nano-CGH , nano-CGE and nano-CGM

which derived from crude hexane, crude ethyl acetate and crude methanol extracts of *C. globosum*. The nano particles were tested for inhibition *Curvularia lunata* causing leaf spot of rice variety Sen pidoa. The experiment was performed by using two factorial experiments in completely randomized design (RCD) with four replications. Factor A represented nano particles, which consisted of nano-CGH, nano-CGE and nano-CGM, and factor B represented concentrations of 0, 1, 5 and 10 ppm/ml of the nano particles. Each crude extract was dissolved in 2% dimethyl sulfoxide (DMSO), mixed into PDA before autoclaving at 121°C, for 30 min. The tested pathogen, *Curvularia lunata* was cultured on PDA and incubated at room temperature for 5 days. The colony margin was cut with 3mm by using the sterilized cork borer. The agar plug of the pathogen was transferred to the middle of the PDA plate of 5cm diameter containing each concentration and incubated at room temperature (28-30°C) for 4 days. The number of spores was recorded and percentage of inhibition was computed as described above. Data was statistically computed analysis of variance. Treatment means were compared by DMRT at P=0.05 and P=0.01. The effective dose (ED_{50}) was computed by using probit analysis.

3.2 Pot experiments

3.2.1 Testing fungal metabolites from *Chaetomium cupreum* CC3003 to inhibit *Curvularia lunata* causing leaf spot of rice variety Sen Pidoa in pot experiment

The experiment was performed by using Completely Randomized Design (CRD) with four replications. Treatments were set up as follows: Inoculated with *Curvularia lunata* (T1), spore suspension of *C. cupreum* CC3003 1 x 10^6spore/ml (T2), Bio fungicide (*C. cupreum*) at 20g/20 L of water (T3), nano-particle of *C. cupreum* (T4) at 10ppm/ml and and chemical fungicide (Tebuconasole) 0.1ml/L of water (T5). All treatments were inoculated with *C. lunata* and followed the treatments above. Rice seeds variety Sen Pidoa were soaked in clean water for 24 hours in moisten paper until germination, then planted into pot (3 seedlings per pot). The 15 days rice seedlings were inoculated to wounded leaves with 1 x 10^6spore/ml, three wounded leaves/seedlings were done. Each treatment was applied as mentioned above at every 15 days until harvest.

Data were collected as plant heights (cm), number of tillers at 65 days. Disease index of leaf spot at 65 days was recorded as follows: level 1= no symptoms 0%, level 2= small blighted spot and still healthy tissue 1-25%, level 3= dead cells in the area of blighted spot 1-2mm and turn brown color 26-50%, level 4= expanded lesion in oval shape 1-2cm and cell death in the center of lesion 51-75% and level 5= diseased area over 76% which modified from (Soytong and

Quimio. 1989). Data were computed analysis of variance (ANOVA) and treatment means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and P=0.01.

3.2.2 Efficacy of *Chaetomium Cupreum* to control leaf spot of rice variety IR66 caused by *Curvularia lunata* in pot experiment

The experiment was done by using a Completely Randomized Design (CRD) with four replications. Treatments were performed as follows: Inoculated with *Curvularia lunata* (T1), spore suspension of *C. cupreum* 1 x 10^6spore/ml (T2), biofungicide (*C. cupreum*) 20g/20 L of water (T3), and chemical fungicide (Tebuconasole) 0.1ml/L of water (T4). All treatments were inoculated with *C. lunata* and followed the treatment above. Rice seeds variety IR 66 were soaked in clean water for 24 hours on put in moisten paper until germination, then planted into pot (3 seedlings per pot). The 15 days old rice seedlings were inoculated to wounded leaves with 1 x 10^6spore/ml, three wounded leaves/seedlings. Each treatment was applied as mentioned above at every 15 days until harvest. Data were collected as plant heights (cm), number of tillers at 35 days . Disease index at 95 days was modified from (Soytong and Quimio.1989) and recorded as follow: [1] Disease index was modified from Soytong. (2014) which followed the level as follow: level 1 = leaf spot 0%, level 2 = leaf spots 1-10%, level 3 = leaf spots 11-20%, level 4 = leaf spots 21-30%, level 5 = leaf spots 31-40%, level 6 = leaf spots 41-50%, level 7 = 51-60 %, 8 = 61-70%, level 8 = 71-80%, level 9 = 81-90% and level 10= 91-100%. All data were statistical computed analysis of variance and treatment means were compared by Duncan Multiple's Range Test (DMRT) at P=0.05 and 0.01.

3.2.3 Testing nano-products derived from *Chaetomium globosum* KMITL-N0805 against *Curvularia lunata* caused leaf spot of rice variety Sen Pidoa in pot experiment

The experiment was done by using Complete Randomized Design (CRD) with four replications. Treatments were as follows: inoculated (Control), nano-CGH, nano-CGE and nano-CGM at concentration of 10ppm/ml. The leaves of 20-day-old seedlings of rice variety Sen Pidoa planted in pots were inoculated with 1 x 10^6spore/ml of *C. lunata* to the all treatments and followed the treatment above to control the disease that inoculated. The disease index (DI) at 30 days, 45 days and 60 days was scored as follows: level 0 = no symptom, 1 = 1-25% infection on leaves, 2 = 26-50% infection on leaves, 3 = 51-75% infection on leaves and 4 = 76-100% infection on leaves. Disease reduction (DR) was defined as ((Disease index in the inoculated control) – (Disease index in treatment)) / Disease index in inoculated control x 100. Additional data included plant heights (cm) and number of tillers at 30 days, 45 days and 60 days. Data were

statistically computed by ANOVA and treatment means were compared with DMRT at P= 0.05 and P=0.01. The comparison of treated and non-treated spores was carried out under a compound microscope.

3.3 Field experiments

3.3.1 Application of agricultural inputs for cultivation of rice variety IR66 in the field.

The field experiment was conducted at Ourong village, Pongro khroum Commune, Chi kreng District, Siem Reap province Cambodia which in the area of disease epidemic or infestation to the rice. The rice in experiment was natturally infected by *Curvularia lunata* causing leaf spot symptoms and observed.

The experiment was performed using a Completely Randomized Design (CRD) with four replications and treatments were as follows: the non-treated control (T1), organic method (T2) which applied organic fertilizer at 4.5kg/plot during transplanting, liquid biofertilizer 40cc/20L applied every 20 days untill harvesting, bioinsecticide (*Metarhizium* and *Beauveria*) at the rate of 40cc/20L of water, biofungicid (*Chaetomium cupreum*) at the rate of 10g/20L of water every 20 days until harvest. Good agriculture practice (T3) was applied the chemical-organic biofertilizer (12-3-3) at 1.5kg/plot. Disease and insect control were controlled by alternative spraying with bioinsecticide plus biofungicide and chemical insecticide (Buprofezin 25%WP 30g/20L) plus chemical fungicide, (Tebuconasole) 20cc/20L) every 20 days until harvest. The chemical method (T4) was applied urea 46-0-0 at the rate of 0.75kg/plot in early stage and 15-15-15 before flowering stage at the rate 0.75kg/plot and spraying with chemical insecticide (Buprofezin 25%WP 30g/20L) plus chemical fungicide, (Tebuconasole) 20cc/20L) every 20 days until harvest. The plot size was 6m x 5m ($30m^2$). Each replication was separated by 0.5m bund. Twenty-day-old seedlings of rice variety IR66 were transplanted in the spacing of 25cm × 25cm. Fertilizer application was done according to the above-mentioned treatments for individual experimental plot. The weed control method for all treatments were done in the same way by manual. The water management was necessary maintained for rice cultivation. At the panicle initiation phase to ripening stage which maintained the water level of 5 -10cm and drained off the water from the field 10 days before harvesting. Harvested plants were left in the field for 4-5 days for sun drying. Threshing was done by manually, and grains were obtained and weighed at 14% moisture contain. The data were collected as follows: plant heights (cm), number of tillers

per plant at 80 days. The length and weight of panicles, number panicle per plant and weight of grains per panicle, numbers of filled and unfilled grains per panicle and grain ,bio mass and dry straw yields per plot, and harvest Index(5%) at harvesting time. The disease index (DI) on leaves at 80 days was scored as follows: 1= no symptoms 0%, 2= small blighted spots 1-25%, 3= dead cells in the area of blighted spots 1-2 mm and turning brown 26-50%, 4= expanded oval-shaped lesions 1-2cm and cell death in the center of lesion 51-75%, and 5= diseased area over 76% . and disease reduction were calculated by followed the formular (% disease reduction = (disease index in control - disease index in treatment) / disease index incontro x 100%. [modified from Soytong and Quimio. (1989)]. The data was statistically computed by ANOVA.

3.3.2 Application of agricultural inputs for cultivation of rice variety Sen Pidoa in the field.

The field experiment was conducted at Ourung Village, Pongro khroum Commune, Chi kreng District, Siem Reap province Cambodia which in the area of disease epidemic or infestation to the rice. The rice in experiment was natturally infected by *Curvularia lunata* causing leaf spot symptoms and observed.

The experiment was conducted by using a Completely Randomized Design (CRD) with 4 replications and 4 treatments were done as follows: the non-treated control (T1), organic method (T2), GAP method (T3) and chemical method (T4). The non-treated control was not used any bio-products and chemicals. Organic method was used *Chaetomium cupreum* (10cc/20L of water), applied organic fertilizer 4.5kg/plot, liquid biofertilizer 40cc/20L, bioinsecticide (*Metarhizium* and *Beauveria*) (50cc/20L of water) every 20 days until harvest. GAP method (good agricultural practice) was applied the chemical-organic biofertilizer (12-3-3) 1.5kg/plot, alternative spraying between bio-insecticide together with *Chaetomium cupreum* at the rate of 10cc/20L and chemical insecticide (Buprofezin 25%WP 30g/20L) together with chemical fungicide (Tebuconasole 20cc/20L) every 20 days until harvest. Chemical method was applied urea 46-0-0 (0.75kg/plot) in early stage and 15-15-15 before flowering stage (0.75kg/plot) and spraying with Buprofezin 25%WP (30g/20L) together with Tebuconasole (20cc/20L) every 20 days until harvest. Data were collected as plant heights (cm), number of tillers per plant at 50 days and 80 days. The length and weight of panicles, number of panicle per plant and weight of grains per panicle at 80 days. The grain and dry hay yields per plot at harvesting time. The Disease Index on leaves at 80 days. The disease index on leaves at 80days and on grain at harvesting time were scored as follows: 1= no symptoms 0%, 2= small blighted spots 1-25%, 3= dead cells in the area of blighted

spots 1-2mm and turning brown 26-50%, 4= expanded oval-shaped lesions 1-2cm and cell death in the center of lesion 51-75%, and 5= diseased area over 76%. and disease reduction were calculated by followed the formular (% disease reduction = (disease index in control - disease index in treatment) / disease index incontro x 100%. [modified from Soytong and Quimio. (1989)]. The data were statistically computed by ANOVA .Data were computed analysis of variance (ANOVA) and treatment means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and P=0.01.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Laboratory tests

4.1.1 Isolation, identification and pathogenicity test of rice pathogen

Curvularia lunata was found to be the causing agent to infect rice variety IR66 and Sen Pidoa (Fig. 2). All isolates were tested for pathogenicity to 20 days old seedlings by inoculating spore suspension at concentration of 1 x 10^6spores/ml. The rice seedlings of both varieties showed clearly symptom of leaf spot and re-isolation to confirm species (Fig.3). The most virulent isolate was used for further experiment. The symptoms were observed from leaves, spots were brown in color, the maximum infection was recorded as leaf sheath. This is a first reported of rice leaf spot caused by *C. lunata* in Cambodia. Result showed that *C. lunata* causing leaf spots of rice var. IR66 and Sen Pidoa which were isolated and proved for pathogenicity. However, Ou. (1985) stated that *C. lunata* is one of the most commonly encountered fungal genera which may infect rice varieties upto 80%. Simon and Lal. (2013) also reported a new blight disease of rice caused by *C. lunata* from Uttar Pradesh in India. The symptoms were observed from leaves, spots were brown in color, the maximum infection was recorded as leaf sheath. It used to report that *C. lunata* is one of the most commonly found to infect rice seeds over 80% leading to grain discoloration (Ou. 1985) and also causing leaf spot or leaf blight of rice and other hosts (Kamaluddeen *et al.* 2013; Alcorn. 1991). Moreover, Salleh *et al.* (1996) also reported in other host as brown spot of Asparagus caused by *Curvularia* spp.

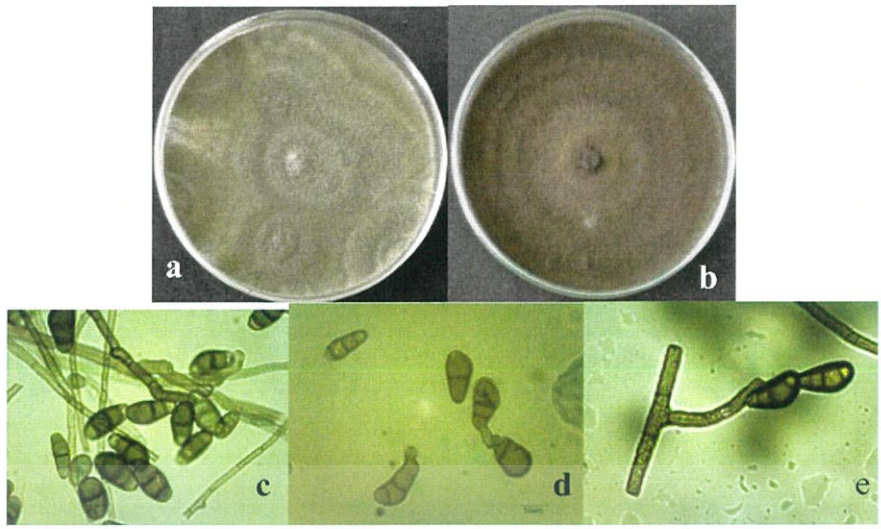


Fig. 2 *Curvularia lunata* causing leaf spot of rice, a and b = culture on PDA, c= conidiophores and conidia, d= conidia and e = conidiophores and conidia

Fig. 3 Pathogenecity tests for rice variety IR66 and Sen Pidoa

4.1.2 Bi-culture antagonistic test against *Curvularia lunata* isolated from rice variety IR66 and Sen Pidoa

Chaetomium cupreum CC3003 (Fig. 4) actively expressed antifungal activity against *Curvularia lunata* isolated from rice variety IR66 in bi-culture after 28-days incubation. In bi-culture the *C. cupreum* significantly inhibited spore production of *C. lunata* at 28.55% when compared to the control plate. *C. lunata* in bi-culture with *C. cupreum* plate produced 183.44 x 10^6 spores/ml compared to control plate at 256.72 x 10^6spores/ml. *C. cupreum* significantly inhibited colony growth of *C. lunata* in bi-culture plate by 21.78% at 28 days. The colony diameter of *C. lunata* in bi-culture plate was 7.04 cm compared to the control plate at 9.00 cm in Table 1. *Chaetomium cupreum* also resulted to inhibit *Curvularia lunata* causing leaf spot of rice var Sen Pidoa in bi-culture test, which significantly suppressed spore reduction of *C. lunata* of 41.03% when compared to the control plate (Table 2). As result, antagonistic activity tests between *C. cupreum* and *C. lunata* done in bi-culture test. *C. cupreum* is reported to control rice blast pathogen caused by *Pyricularia oryzae* in the Philippines (Soytong. 1992b). Moreover, the *C. cupreum* isolate used in this study is reported by (Kanokmedhakul *et al.* 2006) who found three new azaphilones named rotiorinols A-C (1-3), two new stereoisomers, (-)-rotiorin (4) and epi-isochromophilone II (5), and a known compound, rubrorotiorin (6), isolated from *C. cupreum*. Compounds 1, 3, 4, and 6 exhibited antifungal activity against Candida albicans with IC50 values of 10.5μg/ml,16.7μg/ml, 24.3μg/ml, and 0.6μg/ml, respectively. It is indicated that the control mechanism of *C. cupreum* implies antibiosis. It is similar expression as report of Soytong *et al.* (1992) who stated that *C. cupreum* gave a good result to antagonize the rice blast pathogen caused by P. oryzae in the Philippines. It is interesting that *C. cupreum* used in this study can be produced antibiotic substances which was preliminary studied by (Kanokmedhakul *et al.* 2006). It explained that *C. cupreum* could produce and release these bioactive compounds againist *C. lunata* causing leaf spot of rice var. IR66 and Sen Pidoa (Fig. 5)

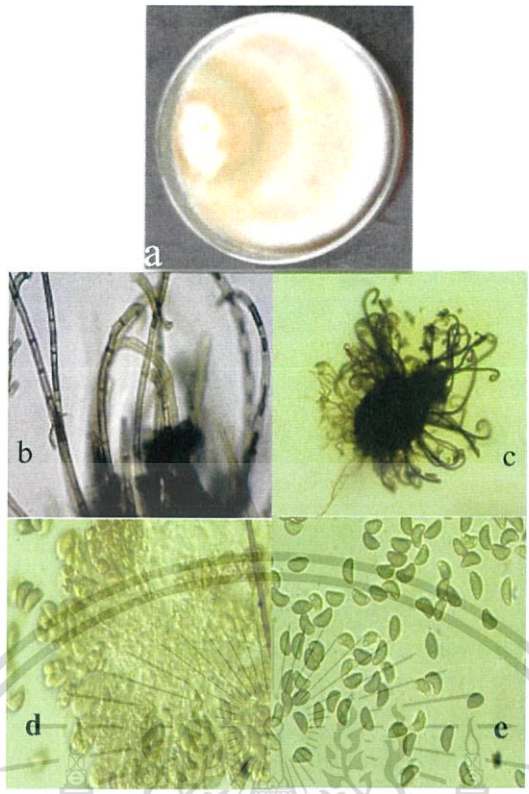


Fig. 4 *Chaetomium cupreum*, a = colony on PDA, b = terminal hairs, c = fruiting body, d = asci and e = ascospores

Table 1. *Chaetomium cupreum* to inhibit colony growth and spore production of *Curvularia lunata* isolated from rice variety IR66 in bi culture PDA plate at 28 days

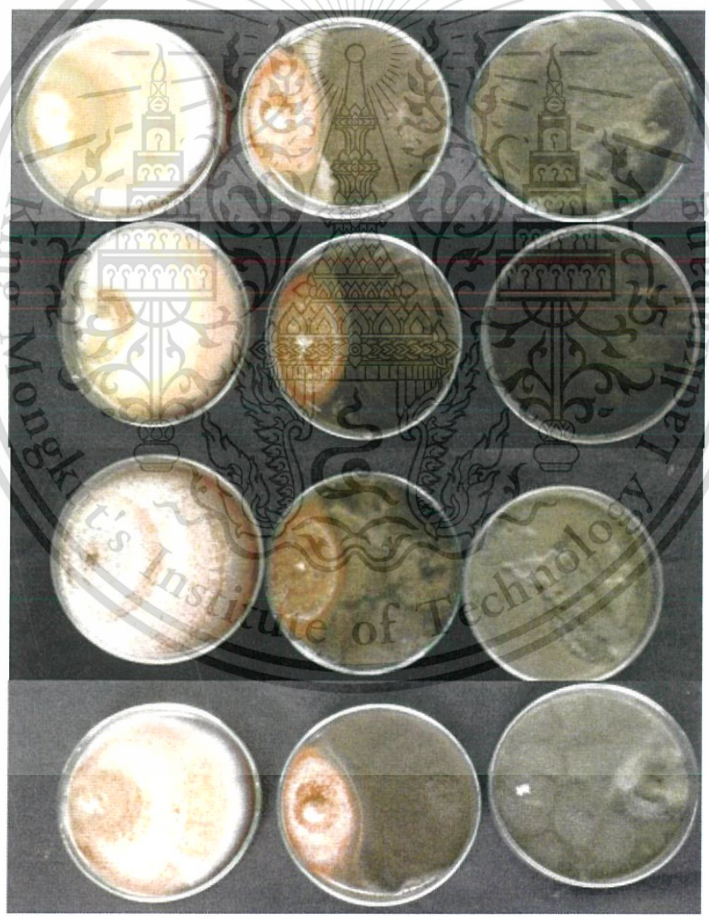
Treatments	Colony diameter (cm) of *C.lunata*	[2/] % Inhibition of colony diameter (cm)	Number spore production (x 10^6 spore/ml)	% Inhibition number of spore of *C.lunata*
Control (*C. lunata)*	9.00 a[1]	-	256.72 a	-
Bi-culture	7.04 b	21.77	183.44 b	28.54
CV (%)	2.03	-	18.50	-

[1/] Mean of four replications. Means followed by a common letter are not significantly different by DMRT at P = 0.01 . [2/] Inhibition(%) = (R1 - R2) / R1 x 100 ; (R1) = colony diameter or sporulation of pathogen in control plate and R2= colony diameter or sporulation of pathogen in bi-culture PDA plate.

Table 2. Efficacy of *Chaetomium cupreum* to inhibit spore production of *Curvularia lunata* isolated from rice variety Sen Pidoa

Treatments	Spore production of *C. lunata* (x 10^6 spore/ml)	[2/] % Inhibition spore reduction of *C. lunata*
Control (*C. lunata)*	256.72 a[1]	-
Bi-culture	151.38 b	41.03
CV (%)	18.50	-

[1/] Mean of four replication, means followed by a common letter were not significantly different by DMRT at P = 0.05. [2/] Inhibition (%) = (R1 - R2 / R1) x 100 ; R1 = number of spore in control plate , R2 = number of spore in bi-culture plate



Antagonist **Bi-culture** **Pathogen**

Fig. 5 Bi-culture antagonistic test between *C. cupreum* and *Curvularia lunata*

4.1.3 Antifungal metabolites from *Chaetomium cupreum* CC3003 against *Curvularia lunata* in vitro

Antifungal metabolites from *C. cupreum* CC3003 inhibited the growth and spore production of *C. lunata* causing leaf spot of rice as shown in Table 2. The extraction of antifungal metabolites from *C. cupreum* using Hexane, Ethyl acetate (EtoAC) and Methanol at 1,000µg/ml inhibited spore production of *C.lunata* by 95.13%, 94.92% and 87.90%, respectively. The ED_{50} values of Hexane, EtoAC and Methanol extracts from *C. cupreum* CC3003 were 6.417.81µg/ml, 0.837.81µg/ml and 7.81µg/ml, respectively (Table 3) and figure 6. The application method of these antibiotic substances was sprayed onto the plant surface or directly to the pathogen cells which it is high molecular weight substances. With this, Tathan *et al.* (2012a) reported that crude extracts of *C. cupreum* expressed antifungal activity against *Dreschera oryzae* causing leaf blight of rice. Moreover, it is clearly shown under compound microscope that the pathogen spores were abnormal due to antagonistic substances extracted with hexane, EtoAC and methanol extracts from *C. cupreum* released into pathogen cells and resulted to broken the pathohen cells (Fig. 6). Soytong and Quimio. (1989) also reported this phenomenon namely antibiosis and lysis that antagonistic substances could destroy the pathogen cells leading to the pathogen loss of pathogenicity. It was interested that *C. cupreum* was reported to antagonize *P. oryzae* causing rice blast in the Philippines (Soytong and Quimio. 1992).

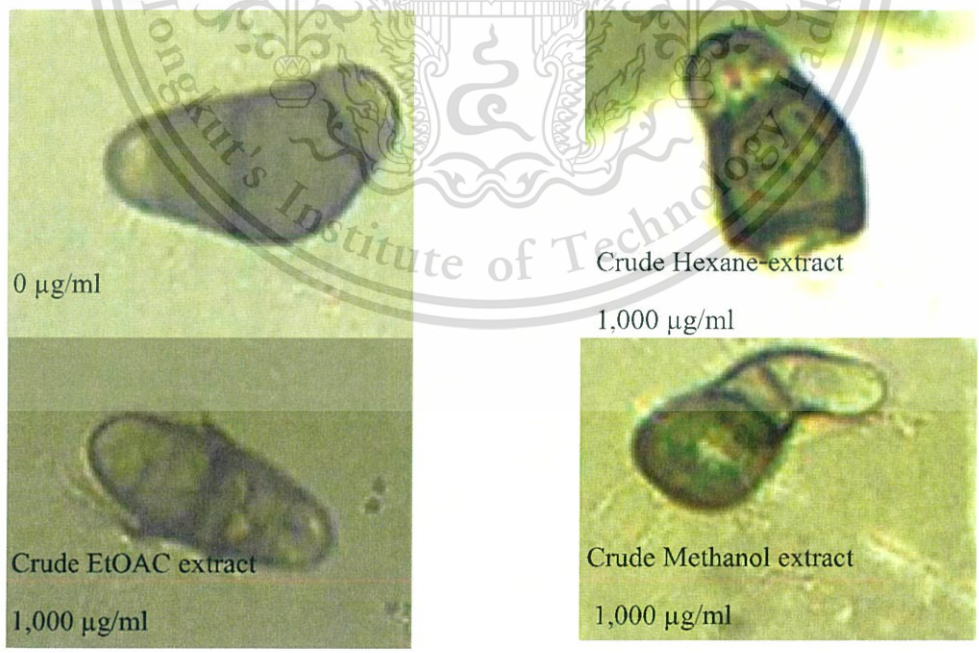


Fig. 6 Effects of hexane, ethyl acetate and methanol extracts from *Chaetomium cupreum* were destroy the *Curvularia lunata*.

Table 3. Antifungal metabolites of hexane, ethyl acetate and methanol extracts from *Chaetomium cupreum* against *Curvularia lunata*

Crude extracts	Conc. (µg/ml)	Colony diameter (cm)	Colony inhibition (%)	Spore number (x 10^6 spore /ml)	Spore inhibition (%)	ED_{50} (µg/ml)
	0	5.00 a[1]	-	77.00 a	-	
	10	4.38 bc	12.40 hi	64.25 c	16.56 k	
Crude	50	4.23 def	15.40 efg	46.88 d	39.12 j	6.41
hexane	100	4.14 fg	17.20 de	30.00 f	61.04 h	
	500	4.05 gh	19.00 cd	9.88 j	87.17 c	
	1000	3.84 i	23.20 b	3.75 l	95.13 a	
	0	5.00 a	-	71.50 b	-	
Crude	10	4.48 b	10.40 i	21.50 g	69.93 f	
ethyl	50	4.34 cd	13.20 gh	12.63 i	82.34d	0.83
acetate	100	4.28 cde	14.40 fgh	9.63 j	86.53 c	
	500	4.01 h	19.80 c	6.88 k	90.38 b	
	1000	3.60 j	28.00 a	3.63 l	94.92 a	
	0	5.00 a	-	78.5 a	-	
	10	4.46 b	10.80 i	37.38 e	52.38 i	
Crude	50	4.30 cde	14.00 fgh	28.50 f	63.69 g	7.81
methanol	100	4.21 ef	15.80 ef	18.63 h	76.27 e	
	500	4.13 fg	17.40 de	14.00 i	82.17 d	
	1000	4.01 h	19.80 c	9.50 j	87.90 c	
C.V (%)		1.74	10.74	4.47	2.23	

[1/] Means of four replications, means followed by a common letter were not significantly different by DMRT at P = 0.01

4.1.4 Testing nano-materials derived from *Chaetomium globosum* KMITL-N0805 against *Curvularia lunata* in laboratory

The results showed that nano-CGH, nano-CGE and nano-CGM from *Chaetomium globosum* KMITL-N0805 at a concentration of 10 ppm/ml showed significantly inhibited spore production of *C.lunata* by 92.70%, 93.44% and 84.17%, respectively. These nano-products,

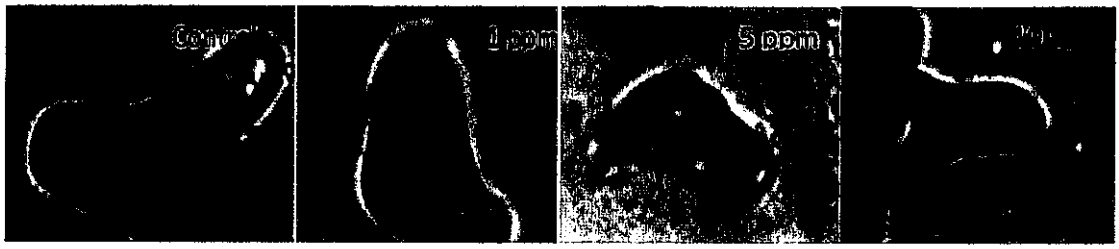
nano-CGH, nano-CGE and nano-CGM showed antifungal activity against *Curvularia lunata* at ED_{50} values of 1.21ppm/ml, 1.19ppm/ml and 1.93 ppm/ml, respectively (Fig. 7, 8 and Table 4). It was shown that all tested nano-products caused disruption and distortion of spores leading to loss of pathogenicity (Fig. 8). This research represents the first report that nano-particles derived from *Chaetomium globosum* KMITL-N0805 expressed antifungal activity against *Curvularia lunata* causing leaf spot of rice. Further research finding was made the chemical substances smaller for easy to enter the destroy the pathogen cells or easy to entered into plant cells for protection or induce immunity in plants. As a result, the particle size in *C. globosum* KMITL-N0805 was 241 nm. while particle size in the control (PLA alone) ranged from 185-218 nm. (Dar and Soytong. 2014). As comparison, the nano-products, nano-CGH, nano-CGE and nano-CGM were more effectively expressed antifungal activity against *C. lunata* ; which the ED_{50} values of 1.21ppm/ml, 1.19ppm/ml and 1.93 ppm, respectively; than crude hexane, ethyl acetate and methanol extracts were shown higher ED_{50} of 6.41µg/ml, 0.83µg/ml and 7.81µg/ml, respectively. In a previous research finding, Kanokmedhakul *et al.* (2006) reported that *C. globosum* KMITL-N0805 produces a novel anthraquinone-chromanone namely chaetomanone as well such known compounds as *chaetoglobosin C* and echinulin. *Chaetomanone* and echinulin were active against to the bacterium Mycobacterium *tuberculosis*. Soytong *et al.* (2001) stated that *chaetoglobosin C* from *C. globosum* KMITL-N0805 can actively inhibit several plant pathogens e.g. *Curvularia lunata* (leaf spot of corn), *Colletotrichum* sp. (citrus anthracnose), *Fusarium oxysporum* f.sp. *lycopersici* (tomato wilt) etc. Moreover, there are reports that the enzyme CHI46 which produced by *C. globosum* can efficiently degrade cell walls of the phytopathogenic fungi: *Rhizoctonia* solani, Fusarium oxysporum, Sclerotinia sclerotiorum, Sclerotinia tritici, and *Phytophthora* sojae indicating that CHI46 may be involved in the biocontrol mechanism of *C. globosum* (Liu *et al.* 2008).

Fig. 7 Samples of nano-particles from *Chaetomium globosum* KMITL-N0805 and *Chaetomium cupreum* CC3003.

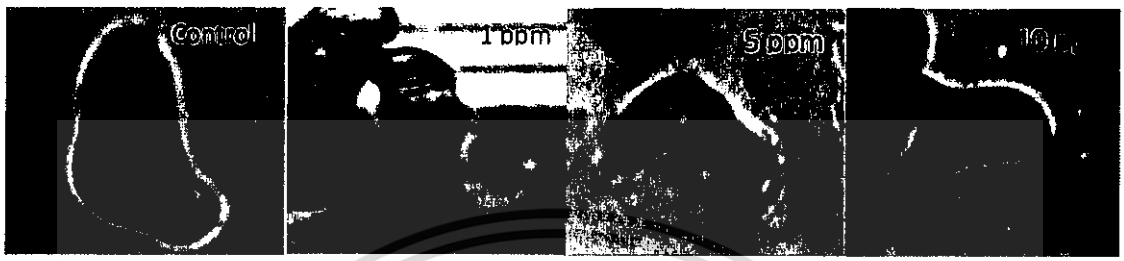
Table 4. Efficacy of nano-CGH, nano-CGE and nano-CGM from *Chaetomium globosum* KMITL-N0805 against *Curvularia lunata*

Nano-materials	Conc. ppm/ml	Colony diameter (cm)	Colony diameter (%)	Spore number (x 10^6spore /ml)	Spore inhibition (%)	ED_{50} (ppm/ml)
Nano-CGH	0	5.00 a[1]	-	6.85 a	-	
	1	4.90 b	2.00	4.25 d	37.96	1.21
	5	4.78 e	4.40	1.80 f	73.72	
	10	4.65 h	7.00	0.50 g	92.70	
Nano-CGE	0	5.00 a	-	6.10 b	-	
	1	4.87 bc	2.60	4.55 cd	25.41	1.19
	5	4.77 de	4.60	2.50 ef	59.02	
	10	4.69 f	6.20	0.40 g	93.44	
Nano-CGM	0	5.00 a	-	5.15 c	-	
	1	4.88 bc	2.40	2.89 e	43.88	1.93
	5	4.77 de	4.60	2.30 ef	55.34	
	10	4.69 f	6.20	0.30 g	84.17	
C.V (%)		3.56	-	8.44	-	

[1]Average of four replications, Means followed by a common letter in each column are not significantly different by DMRT at P = 0.01



Nano- CGH



Nano- CGE

Nano- CGM

Fig. 8 Efficacy of nano products were destroy the spores of *Curvularia lunata*

4.2 Pot experiments

4.2.1 Testing fungal metabolites from *Chaetomium cupreum* CC3003 to inhibit *Curvularia lunata* causing leaf spot of rice variety Sen Pidoa in pot experiment

The results showed that the heights of plants treated with a spore suspension of *Chaetomium cupreum* (28.49cm), biofungicide- *Chaetomium spp.* (28.92cm), nano product from *C. cupreum* (28.04cm) and Chemical fungicide -tebuconazole (27.16 cm) were not significantly differed from the inoculated control (26.91cm) at 65 days after planting (Table5). The Plant height increased after treated with spore suspension of *C. cupreum,* biofungicide (*C. cupreum),* nano-particles of *C. cupreum* and Chemical fungicide (Teboconazole) which were 5.55%, 6.95%, 4.03% and 0.92%, respectively, when compared to the inoculated controls (Table 5.).

Soytong *et al.* (2001) stated that metabolites produced by *Chaetomium* spp. inhibited several plant pathogens including *C. lunata.* It was clearly demonstrated that treatment with spore a suspension of *Chaetomium cruprem*, biofungicide (*C. cupreum),* nano product from *C. cupreum* and tebuconazole gave higher number of tillers which were 9.25, 9.63, 9.88 and 9.94, respectively, than the inoculated control 6 at 65 days after planting. All treatments increased the number of tillers from 35.14% to 39.64% when compared to the inoculated control (Table 6). Similarly Tathan *et al.* (2012) found that the metabolites from *Chaetomium* spp. could inhibit *Drechslera oryzae* which causes leaf spot of rice. Rice seedlings treated with a spore a suspension of *Chaetomium cruprem*, biofungicide (*Chaetomium cruprem*), nano product from *C. cupreum* and Chemical fungicide(tebuconazole) showed significantly lower disease indices (DI) of 1.75, 1.50, 1.50 and 1.25, respectively, than the inoculated control of 3.00. In the treatment of spore a suspension of *Chaetomium cruprem*, biofungicide (*Chaetomium cruprem*), nano-product from *C. cupreum* and Chemical fungicide(tebuconazole) showed significantly disease reduction which were 41.67%, 50%, 50%, and 58.33%, respectively, when compared with inoculated control. (Table 7 and similarly figure 9).

Testing fungal metabolites from *Chaetomium cruprem* to inhibit *C. lunata* causing leaf spot of rice var. Sen Pidoa in pot experiment was demonstrated. It is clearly noted that treated with spore a suspension of *Chaetomium cruprem* CC3003, biofungicide of *Chaetomium cruprem,* nano-product from *C. cupreum* and chemical fungicide (tebuconazole) gave higher number of tillers which were 9.25cm, 9.63cm, 9.88cm, and 9.94cm than the inoculated controls 6cm in 65 days after planting. Soytong *et al.* (2001) stated that metabolites produced from *Chaetomium* spp could inhibited several plant pathogens including *C. lunata*. The number of tiller increased in treatment of spore a suspension of *Chaetomium cruprem* CC3003, biofungicide of *Chaetomium cruprem,* nano-product from *C. cupreum* and chemical fungicide (tebuconazole) which were 35.14%, 37.69%, 39.27% and 39.64%, respectively, when compared to the inoculated control in Table 6. Similar report was found from the work of Tathan *et al.* (2012a) who stated that the metabolites from *Chaetomium* spp could inhibit *Drechslera oryzae* which causing leaf spot of rice and enhanced plant growth parameters.

Table 5. Efficacy of fungal metabolites from *Chaetomium cupreum* and a fungicide on plant heights of rice variety Sen Pidoa inoculated with *Curvularia lunata* in a pot experiment at 65 days

Treatments	Plant heights (cm)	% Plant heights increase
Inoculated control	26.91a[1]	-
Spore suspension of *C. cupreum*	28.49a	5.55
Biofungicide (*C. cupreum*)	28.92a	6.95
Nano-particles of *C. cupreum*	28.04a	4.03
Chemical fungicide (Tebuconazole)	27.16a	0.92
C.V (%)	7.35 %	-

[1]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01.

Table 6. Efficacy of fungal metabolites from *Chaetomium cupreum* and a fungicide on number of tillers per plant of rice variety Sen Pidoa inoculated with *Curvularia lunata* in a pot experiment at 65 days.

Treatments	Number of tillers(cm)	% Number tiller increase
Inoculated control	6.00 c[1]	-
Spore suspension of *C. cupreum*	9.25 b	35.14
Biofungicide (*C. cupreum*)	9.63 ab	37.69
Nano-particles of *C. cupreum*	9.88 a	39.27
Chemical fungicide (Tebuconazole)	9.94 a	39.64
C.V (%)	14.36 %	-

[1]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01.

Table 7. Efficacy of fungal metabolites of *Chaetomium cupreum* on *Curvularia lunata* leaf spot index and disease reduction in rice variety Sen Pidoa in a pot experiment at 65 days.

Treatments	Disease Index	[2/] Disease reduction (%)
Inoculated control	3.00 a[1]	-
Spore suspension of *C. cupreum*	1.75 ab	41.67
Bio fungicide (*C. cupreum*)	1.5 b	50.00
Nano particles of *C. cupreum*	1.5 b	50.00
Chemical fungicide (Tebuconazole)	1.25 b	58.33
C.V (%)	3.11	-

[1]Means of four replications. Means followed by a common letters were not significantly different by DMRT at P=0.01.

[2/] Disease Index (DI) was rated using the following scheme:

1= no symptoms 0%,

2= small blighted spots 1-25%,

3= dead cells in the area of blighted spots 1-2 mm and turning brown 26-50%,

4= expanded oval-shaped lesions 1-2 cm and cell death in the center of lesion 51-75%, and

5= diseased area over 76 % [modified from Soytong and Quimio. (1989)]

Inoculated control with *Curvularia lunata*

Spore suspension of *C. cupreum*

Biofungicide (*C. cupreum*)

Nano-particles of *C. cupreum*

Chemical fungicide (Tebuconazole)

Fig. 9 Effects of fungal metabolites of *Chaetomium cupreum* agaist *C. lunata* causing leaf spot disease index and disease reduction in rice variety Sen Pidoa in a pot experiment at 35 days.

4.2.2 Efficacy of *Chaetomium cupreum* to control leaf spot of rice variety IR66 caused by *Curvularia lunata* in pot experiment

Result showed that *Chaetomium cupreum* significantly reduced disease incidence of leaf spot caused by *Curvularia lunata*. Rice seedlings treated with biofungicide (*Chaetomium cruprem*), chemical fungicide(tebuconazole) and spore suspension of *Chaetomium cruprem*, showed significantly lower disease index (DI) of 1.75 DI, 2.00 DI, and 2.25 DI, respectively than the inoculated control with *Curvularia lunata* of 7.25 DI. With this spraying spore suspension of *C. cupreum*, biofungicide (*C. cupreum*) and chemical fungicide (tebuconasole) to inoculated rice seedlings with *C. lunata* that reduced disease which were 68.97%, 75.86% and 72.41%, respectively (Table 8 and similarly figure 10). The plant heights in treatment of spore suspension of *C. cupreum*, biofungicide and chemical fungicide (tebuconasole) to inoculated rice seedlings with *C. lunata at* 35 days which were 18.77cm, 18.33cm, and 18.94cm were not significantly differed with control at 14.16cm. Plant height significantly increased in treatments of spore suspension of *C. cupreum,* biofungicide (*C. cupreum*) and chemical fungicide (tebuconasole) 24.56%, 22.79% and 25.24%, respectively when compared to the inoculated control. The number of tillers also were not significantly differed in treatments of spore suspension of *C. cupreum*, biofungicide (*C. cupreum*) and chemical fungicide (tebuconasole) which were 4.94tiller, 5.50tiller, and 4.94tiller, respectively when compared with control at 3.25tiller. The number of tillers also significantly increased in treatments of spore suspension of *C. cupreum*, biofungicide (*C. cupreum*) and chemical fungicide (tebuconasole) 34.21%, 40.91% and 34.21%, respectively (Table 9). The efficacy of *Chaetomium cupreum* to control leaf spot of rice var. IR66 caused by *C. lunata* in pot experiment was also clearly shown. Similar reports stated by Soytong. (2014) that *Chaetomium cochliodes* proved to be a new antagonistic fungus against brown leaf spot of rice var. Pittsanulok2 caused by *Drechslera oryzae* in Thailand. It showed good inhibition of mycelia growth of 38.18% and inhibited inoculum production of 71.55%. *C. cochliodes* was formulated in different forms for applying to control brown leaf spot of rice. Biological products formulated from *C. cochliodes* were tested to control brown leaf spot of rice caused by *D. oryzae*. Result showed that biopowder formulation gave significantly highest to control leaf spot and highest plant growth when compared to the non-treat control, followed by applying crude extract of *C. cochliodes*, benlate and spore suspension of *C. cochliodes*. Moreover, bio-powder formulation gave significantly increased in plant growth over 44% and followed by crude extract of *C. cochliodes*, spore suspension of *C. cochliodes* and benlate.

Table 8 . Efficacy of treatments on disease index, and disease reduction in rice variety IR 66 at 95 days

Treatments	Disease index	[1]Disease reduction (%)
Inoculated control	7.25 a[2]	-
Spore suspension of *C. cupreum*	2.25 b	68.97
Biofungicide (*C. cupreum*)	1.75 c	75.86
Chemical fungicide (Tebuconazole)	2.00 bc	72.41
C.V (%)	25.00	-

[1] Disease index was modified from Soytong. (2014) which followed the level as follow:

level 1 = leaf spot 0%,

level 2 = leaf spots 1-10%,

level 3 = leaf spots 11-20%,

level 4 = leaf spots 21-30%,

level 5 = leaf spots 31-40%,

level 6 = leaf spots 41-50%,

level 7 = 51-60 %, 8 = 61-70%,

level 8 = 71-80%,

level 9 = 81-90% and

level 10= 91-100%.

[2/]Average of four replications. Means followed by a common letter in each column are not significantly different by DMRT at P=0.01.

Table 9. Efficacy of treatment on rice plant height of rice variety IR66 at 35 days in a pot experiment

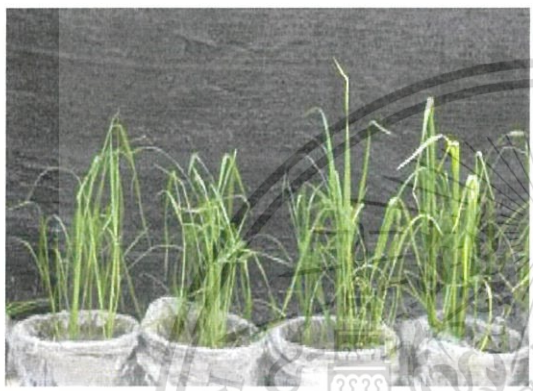
Treatments	Plant heights (cm)	[2/] Increase in plant heights (%)	Number of tillers	Increase in number of tillers (%)
Inoculated control	14.16 c[1]	-	3.25 c	-
Spore suspension of *C. cupreum*	18.77 b	24.56	4.94 b	34.21
Biofungicide (*C.cupreum*)	18.34 ab	22.79	5.50 a	40.91
Chemical fungicide (Tebuconazole)	18.94 a	25.24	4.94 b	34.21
C.V (%)	15.40 %		24.49 %	

[1]Average of four replications. Means followed by a common letter in each column are not significantly different by DMRT at P=0.01.

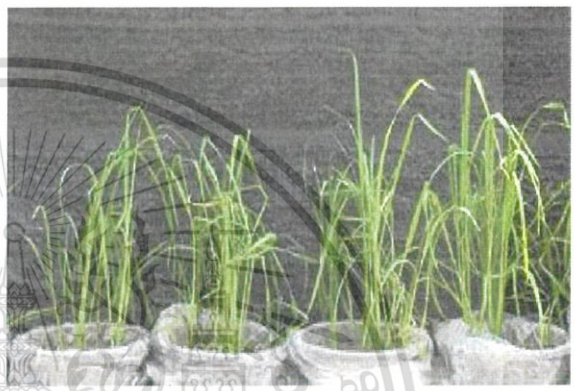
[2/]Increased in number of tillers (%) = (R1 - R2 / R1) x 100 ; R1 = number of tillers in each treatment and R2 = number of tillers in each control.

Inoculated control with *C. lunata*

Spore suspension of *C. cupreum*



Biofungicide (*C. cupreum*)



Chemical fungicide (Tebuconazole)

Fig.10 Testing *Chaetomium cupreum* to control leaf spot of rice variety IR66 caused by *Curvularia lunata* in pot experiment at 35 days.

4.2.3 Testing nano-products from *Curvularia globosum* KMITL-N0805 against *Curvularia lunata* caused leaf spot of rice variety Sen Pidoa in pot experiment

Testing nano-products to control leaf spot of rice var. Sen pidoa caused by *Curvularia lunata* was investigated in a pot experiment. The results showed that nano-CGH, nano-CGE and nano-CGM from *Chaetomium globosum* KMITL-N0805 significantly controlled leaf spot of rice var. Sen pidoa at 30, 45 and 60 days after treatment. The disease index (DI) at 30 days revealed that nano-CGM gave a significantly lower disease index(DI) which was 2.25DI and followed by nano-CGH and nano-CGE both of which 2.75DI when compared to non-treated control at 3.62DI shown in table 10. After treatment for 45 days, the nano-products were not significantly different from each other in disease index which were 2.50DI, 2.68DI and 2.75DI respectively, but were significantly different when compared to the non-treated control 4.12DI. After treatment for 60

days, nano-CGH and nano-CGM were not significantly differed in disease index 2.25DI, followed by nano-CGE at 2.50DI when compared to non-treated control at 4.25DI. Disease reduction after treatment for 30 days, nano-CGM gave the best disease reduction at 37.85% which was significantly higher than nano-CGH at 24.03% and nano-CGE at 24.03%. At 45 days after treatment, nano-CGH gave the best disease reduction at 39.32% which was higher than nano-CGE and nano-CGM which were 34.95% and 33.25%, respectively. Moreover, 60 days after treatment, nano-CGH and nano-CGM gave a higher disease reduction at 47.06% than nono-CGE at 41.18% as shown in Table 10. It was concluded that nano-CGH, nano-CGE and nano-CGM from *Chaetomium globosum* KMITL-N0805 could decrease leaf spot of rice var. Sen pidoa caused by *Curvularia lunata*. The plant increasing after treatment for 30 days , nano-CGH gave the best plant height increasing at 1.79% which was higher than nano-CGM 1% and nano CGE at 0.81%. At 45 days after treatment , nano-CGM gave the best plant height increasing at 12.92% which was higher than nano-CGH at 11.32% and nano-CGE at 7.99%, respectively. And at the 60 days after treatment, nano-CGH have the best incresing plant height at 2.80% which higher than the nono-CGE and nano-CGM at 1.83%, 1.20% as shown in Table 11. The number tiller after treatment for 30 days, nano-CGE gave the best number tiller increasing at 44.03% and higher than nano-CGH at 31.19% and nano-CGM at 27.18%. At 45 days after treatment, nano-CGH gave the best number tiller increasing at 33.71% follow by nano-CGE at 10.86% and nano-CGM at 9.65%. At 60 days after treatment, nona-CGH gave the best in number tillers increasing at 22.43% and follow by nano-CGE and nano-CGM at 13.97% and 10.15%, respectively. as shown in Table 12. It was revealed that nano-CGH, nano-CGE and nano-CGM significantly increased in plant height and number of tillers at 30, 45 and 60 days when compared to the non-treated control as shown in Tables 11 and 12. Testing nano-products to control leaf spot of rice var. Sen pidoa caused by *C. lunata* in a pot experiment revealed that nano-CGH, nano-CGE and nano-CGM from *C. globosum* KMITL-N0805 significantly reduced leaf spot of rice var. Sen pidoa at 30, 45 and 60 days after treatments. Previous reports from *C. globosum*, an important biocontrol fungus, can inhibit spot blotch of wheat (Aggarwal *et al.* 2004), suppressed the development of rice blast and wheat leaf rust (Park *et al.* 2005) and reduced the primary inoculum of *Diaporthe phaseolorum* f. sp. *meridionalis* in soil-surface soybean stubble under field conditions (Dhingra *et al.* 1987). Moreover, *Chaetomium* spp. have been reported to degrade cellulolytic plant debris to increase organic matter in the soil and a specific isolate of *C. globosum* can inhibit *Pyricularia oryzae* causing rice blast disease (Soytong and Quimio. 1989). The nano-CGH, nano-CGE and

nano-CGM from *Ch. globosum* KMITL-N0805 decreased leaf spot of rice var. Sen pidoa caused by *C. lunata* showing possibility to develop systemic biofungicide for disease control. It was concluded that nano-CGH, nano-CGE and nano-CGM from *C. globosum* KMITL-N0805 decreased leaf spot of rice var. Sen Pidoa caused by *C. lunata* and significantly increased in plant height and number of tillers when compared to the non-treated control. This research finding represents the first report that nano-particles derived from *C. globosum* KMITL-N0805 can be controlled *C. lunata* causing leaf spot of rice.

Table 10. Disease index and disease reduction of rice variety Sen Pidoa after treatment with nano-materials made from *Chaetomium globosum* KMITL-N0805

Treatments	Disease Index (DI)			Disease Reduction (DR) (%)		
	30 days	45 days	60 days	30 days	45days	60 days
Inoculated control	3.62 a[1]	4.12 a	4.25 a	-	-	-
Nano-CGH	2.75 b	2.5 b	2.25 c	24.03b	39.32 a	47.06a
Nano-CGE	2.75 b	2.68 b	2.50 b	24.03b	34.95ab	41.18b
Nano-CGM	2.25 c	2.75 b	2.25 c	37.85a	33.25b	47.06a
C.V (%)	2.48	4.83	2.21	6.47	9.16	4.43

[1/]Average of four replications. Means followed by a common letter in each column are not significantly different by DMRT at P=0.01.

[2] Disease index was modified from Soytong. (2014) which followed the level as follow:

level 1 = no symtops 0%,

level 2 = 1-25% infected on leaves,

level 3 = 26-50% infected on leaves,

level 4 = 51-75% infected on leaves,

level 5 = 76-100% infected on leaves.

Table 11. Efficacy of nano materials derived from extracts of *Chaetomium globosum* on plant height of rice variety Sen Pidoa after transplanting.

Treaments	Plant heights			Increased plant heights (%)		
	30 days	45 days	60 days	30 days	45days	60 days
Inoculated control	14.78a[1]	15.90c	24.63b	-	-	-
Nano-CGH	15.05a	17.93ab	25.34a	1.79 a	11.32b	2.80 a
Nano-CGE	14.90a	17.28bc	25.09a	0.81 c	7.99 c	1.83 b
Nano-CGM	14.93a	18.26a	24.93ab	1.00b	12.92a	1.20 b
C.V.(%)	1.12	3.60	0.71	5.49	8.61	19.69

[1]Average of four replications. Means followed by a common letter are not significantly different by DMRT at P = 0.01

Table 12. Efficacy of nano materials derived from extracts of *Chaetomium globosum* on tiller of rice variety Sen pidoa after transplanting.

Treaments	Number tillers			Increased tillers (%)		
	30 days	45 days	60 days	30 days	45days	60 days
Inoculated control	1.50 c[1]	4.68 c	12.31d	-	-	-
Nano-CGH	2.18 a	7.06 a	15.87 a	31.19 b	33.71 a	22.43 a
Nano-CGE	2.68 b	5.25 b	14.31 b	44.03 a	10.86 b	13.97 b
Nano-CGM	2.06 b	5.18 b	13.56 c	27.18 b	9.65 b	10.15 c
C.V (%)	14.59	3.54	1.83	5.51	6.56	5.04

[1]Average of four replications. Means followed by the same letter are not significantly different by DMRT at P = 0.01

4.3 Field experiments

4.3.1 Application of agricultural inputs for cultivation of rice variety IR66 in the field.

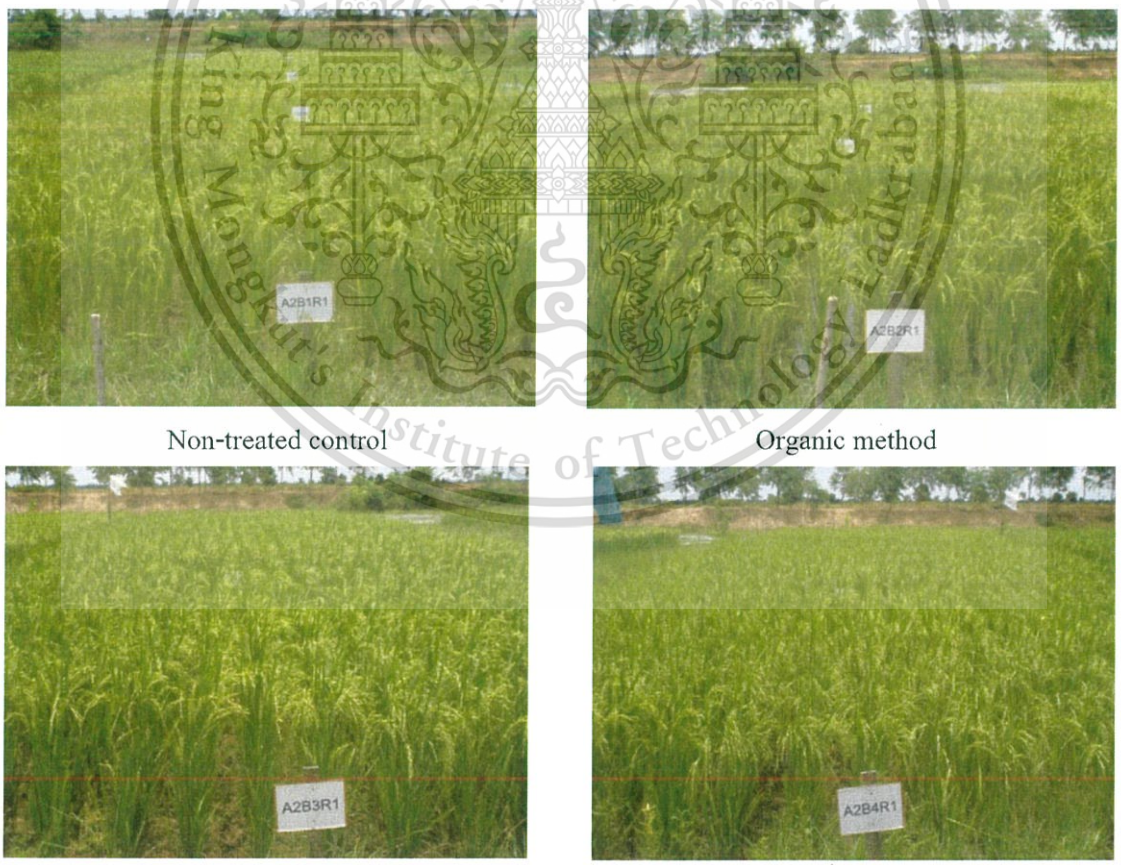
The organic, good agricultural practice (GAP) and chemical methods were tested for rice cultivation of var. IR 66 in a field trial in Cambodia. Result showed that the chemical method gave the highest plant height at 80 days which was 72.55cm, followed by the GAP method at

67.2cm and organic method at 62.35cm which were significantly different from the non-treated control at 53.19cm. The chemical and GAP methods gave the best results in number of tillers at 80 days which were 14 and 15tillers, respectively, followed by organic method at 12 which was significantly different from the non-treated control at 6 as seen in Table 13 and fig. 11.

Table 13. Efficacy of treatments on plant heights and number of tillers /plant of rice variety IR66 at 80 days in the field trial.

Treatments	Plant heights (cm)	Number of tillers/plant
Non- treated control	53.19 d[1]	6 c
Organic method	62.35 c	12 b
GAP method	67.2 b	15 a
Chemical method	72.55 a	14 a
C.V (%)	1.71	9.58

[1]Average of four replications Means followed by a common letter in each column are not significantly different by DMRT at P=0.01.



Non-treated control Organic method

GAP method Chemical method

Fig.11 Plant heights and number tillers comparison between each treatment at 80 days

The chemical method also gave the best results in panicle/plant, panicle lengths(cm), panicle weight(g), grain weight(g)/plant which were 13panicles/plant, 26.09cm, 4.70g and 4.05g, respectively, which significantly differed when compared to GAP which were 18 panicles/plant, 25.38cm, 4.24g and 3.60g, respectively, and the organic method which were 11 panicles/plant, 24.83cm, 3.36g and 2.90g as seen in Table 14.

Table 14. Efficacy of treatments on panicles and grains for rice variety IR66 in the field trial

Treatments	# Panicle/ plant	Length of panicle (cm)	Panicle weight(g)/p-	Grains weight(g)/panicle
Non-treated control	6 c[1]	23.25 bc	2.56 c	2.25 c
Organic method	11 b	24.83 ab	3.36 b	2.90 b
GAP method	18 a	25.38 a	4.24 a	3.60 ab
Chemical method	13 b	26.09 a	4.70 a	4.05 a
C.V (%)	9.81	3.30	10.31	11.41

[1]Average of four replications. Means followed by a common letter in each column are not significantly different by DMRT at P=0.01.

Number of filled grain and unfilled grain / panicle, grain and dry hay weight (kg) per plot ($20m^2$ planted area) at 14%MC, bio mass weight ang harvest index were gathered. Chemical method gave the best results in filled grain/panicle, unfilled grain/panicle, grain weight(kg)/plot, dry hay weight(kg)/plot, biomass weight(kg)/plot and Harvest Index (5%) which were 111 full grain per panicles, 15 unfull grain per panicles, 10.55kg/plot, 25.97kg/plot, 41.04kg/plot and 0.31 HI, respectively which significantly differed from GAP method which were 106 full grain per panicles, 12 unfill grain per panicles, 9.65kg/plot, 28.49kg/plot, 35.62kg/plot and 0.27HI, respectively, and the organic method which 104 fill grain per panicles, 7 unfill grain per panicles, 6.34kg/plot, 16.52 kg/plot, 22.61 kg/plot and 0.27 HI, respectively (Table 15 and figure 12-23).

Table 15. Efficacy of treatments on grains, dry hay, biomass and harvest index of rice variety IR66 at 14% of miosture contain (MC) .

Treatments	Filled grain/ panicle	Unfilled grain/ panicle	Grains weight (kg)/plot	Dry hay weight (kg)/plot	Bio mass weight (kg)/plot	Harvest Index (5%)
Non-treated control	79 c[1]	16 a	4.35 c	8.89 d	13.24 d	0.33 a
Organic method	104 b	7 c	6.34 b	16.52 c	22.61 c	0.27 b
GAP method	106 b	12 b	9.65 a	28.49 a	35.62 b	0.27 b
Chemical method	111 a	15 a	10.55 a	25.97 b	41.04 a	0.31 ab
C.V (%)	9.57	22.73	11.41	13.40	11.86	7.10

[1]Average of four replications. Means followed by a common letter in each column are not significantly different by DMRT at P=0.01.

This experiment revealed that chemical and GAP application gave better result than organic method. This contradicts the previous experiment of Tann *et al.* (2012b) in which the organic method gave better rice straw weight than non-treated control, GAP and chemicals at 115 days of harvesting. The organic method increased plant heights and tiller number per plant by 3.06% and 57.69%, respectively at 60 days. The GAP method increased in plant heights and number tillers by 11.23% and 69.44%, respectively while the chemical method increased plant height and tillers number by 6.73% and 62.71%, respectively. The grain weight (yield) increased by the GAP, chemical and organic methods by 59.15%, 55.38% and 44.23%, respectively. This may due to different location of experimental sites, soil fertility, disease and different tested variety (Stanhill. 1990; Maeder. 2002). The organic method requires evaluation of many factors for completely successful cultivation (Paull. 2011).

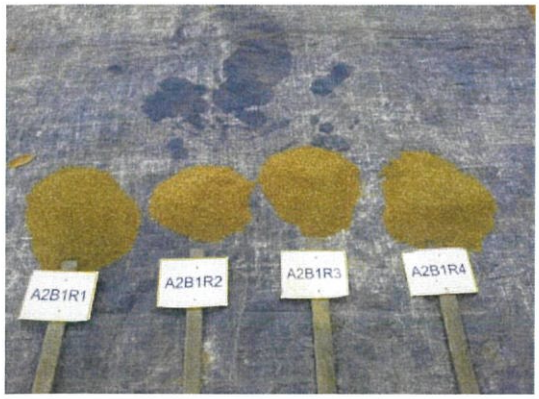


Fig. 12 Grains yield/plot in Non-treated control

Fig. 13 Grains yield/plot in Organic method

Fig. 14 Grains yield/plot in GAP method

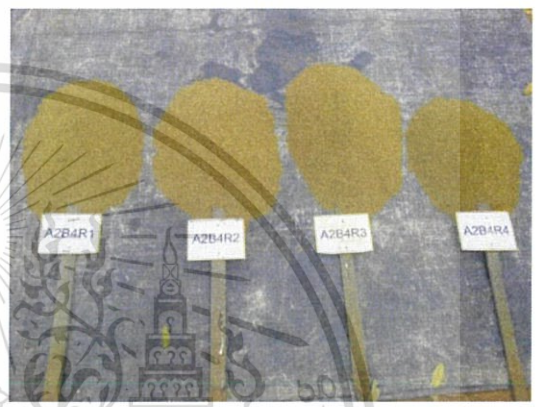


Fig. 15 Grains yield/plot in Chemical method

Fig. 16 Dry hays/plot in Non-treated control

Fig. 17 Dry hays/plot in Organic method

Fig. 18 Dry hays/plot in GAP method

Fig. 19 Dry hays/plot in Chemical method

Fig. 20 Bio mass/plot in Non-treated control

Fig. 21 Bio mass/plot in Organic method

Fig. 22 Bio mass/plot in GAP method

Fig. 23 Bio mass/plot in Chemical method

Result showed that organic and GAP methods were not significantly differed and low disease index of leaf spot caused by *C. lunata* as 1.75DI and 3DI, respectively, and followedby chemical method at 4DI, when compared the natural infection control that disease index was 4.50DI. Organic method showed the highest leaf spot disease reduction of 61.11%, and followed by GAP method , Chemical methods which leaf spot disease reduction of 33.33% and 11.11%, respectively(Table 16). Reviewd literature, Tann *et al.* (2012) reported similar results in rice var. Senpidoa causing rice blast disease (*Pyricularia oryzae*), which stated that the naturally disease incidence of rice blast was increased in non-treated control both in pot experiment. The disease index of rice blast in pot experiment of organic, GAP and chemical methods were 2.00DI, 2.50DI and 3.25DI, respectively. The organic method was the highest decreased rice blast disease of 80%, followed by GAP and chemical methods which were 50% and 40%, respectively.

Table 16. Disease index of leaf spot on leaves caused by *Curvularia lunata* on rice var. IR66 at 80 days in field experiment

Treatments	Disease index on leaves	[1]Disease reduction on leaves (%)
Non-treated control	4.50 a[2]	-
Organic method	1.75 b	61.11
GAP method	3.00 ab	33.33
Chemical method	4.00 a	11.11
CV (%)	24.77	-

[1]Disease index(DI) was rated using the following scheme: 1= no symptoms 0%, 2= small blighted spots 1-25%, 3= dead cells in the area of blighted spots 1-2 mm and turning brown 26-50%, 4= expanded oval-shaped lesions 1-2 cm and cell death in the center of lesion 51-75%, and 5= diseased area over 76% [modified from Soytong and Quimio. (1989)]. [2]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01

4.3.2 Application of agricultural inputs for cultivation of rice variety Sen Pidoa in the field.

The results showed that the chemical method showed significantly reduced leaf spot disease by 60%, followed by organic method and GAP method which reduced leaf spot disease caused by *Curvularia lunata* by 40% of both, respectively (Table 17). Soytong. (2014) also found that a bio-formulation of *Chaetomium cochliodes* gave good control of brown leaf spot of rice caused by *Curvularia lunata*. Moreover, blight symptom caused by *Curvularia lunata* in grains were significantly reduced by the chemical method at 66.80% compared to organic and GAP methods which reduced disease by 40% and 33.40%, respectively when compared to the non-treated control as seen in Table 17. This research found that plant heights and at 50days and were not significantly different among the organic method at 44.75cm, GAP method at 41.29cm and chemical methods at 44.25cm when compared to the non-treated control at 39.50cm. And the chemical method at 80 days gave the best plant heights at 69.20cm and was significantly difference when compared with control at 61.40cm (Table 18 and figure 24, 25). The organic method, GAP method and chemical method at 50 days old showed significanty in number tillers at 11 tillers/plant, 12 tillers/plant, 13 tillers/plant respectively, all treatments were significantly

difference with control at 6. The chemical method at 80 days also showed significanty in tiller at 22 tillers/plant, followed by GAP and organic method at 21tillers/plant and 16 tillers/plant respectively, all treatments were significanty difference compared with control at 9 as seen in Table 18 and figure 24, 25. The GAP method at 80 days showed the best number panicle per plant at 21, followed by chemical and organic method at 18, 15, respectively, all the treatments were significanty difference compared with control at 9 as seen in Table 19. The chemical method at harvesting time showed the best panicle length at 21.25cm followed by GAP method at 19.67cm, and organic method at 19.45cm respectively, all treatments were significantly difference compared with control at 16.85cm as seen in Table 19. The chemical method at harveting time showed better significantly in panicle weights at 2.35g/panicle, followed by GAP and organic method at 2.25g/panicle, 2.10g/panicle respectively, all treatments were significantly difference compared with control at 1.65g/panicle as seen in Table 19. The chemical and GAP method at harvesting time gave the higher yield in grain weight at 10.77kg/plot and 10.26kg/plot, followed the organic method at 7.37kg/plot respectively, all treatments were significantly difference when compared with control at 4.12kg/plot as shown in Table 20 and figure 27. The chemical method gave the better in dried hay yields at 17.21kg/plot, and followed by GAP method at 16.42kg/plot and organic method at 9.40kg/plot respectively, all treatments were significantly difference when compared with control at 6.17kg/plot as shown in Table 20 and figure 26. A previous report found similar results when comparing organic, GAP and chemical methods for rice cultivation in Cambodia and also reported on the effect of good agricultural practice and organic methods on rice cultivation under the system of rice intensification in Cambodia (Tann *et al.* 2012b). This research stated that all growth parameters and yields from chemical, GAP and organic methods could be affected by variable factors such as water management, weeding, soil type and soil fertility in each location. Kaewchai *et al.* (2009) also stated that bio fertilizer and bio products of *Chaetomium* could be applied instead of chemical ones. As can be observed in this study, the organic method was significantly better than the non-treated control in the field trials.

Table 17. Disease index of leaf spot on leaves at 80 days and grain on harvesting time caused by *Curvularia lunata* on rice variety Sen Pidoa in field experiment

Treatments	[1]Disease Index on leaves	Disease Index on grains	Disease reduction on leaves (%)	Disease reduction on grains (%)
Non-treated control	5.00 a[2]	5.00 a	-	-
Organic method	3.00 b	3.00 c	40.00	40.00
GAP method	3.00 b	3.33 b	40.00	33.40
Chemical method	2.00 c	1.66 b	60.00	66.80
CV (%)	12.92	16.74	-	-

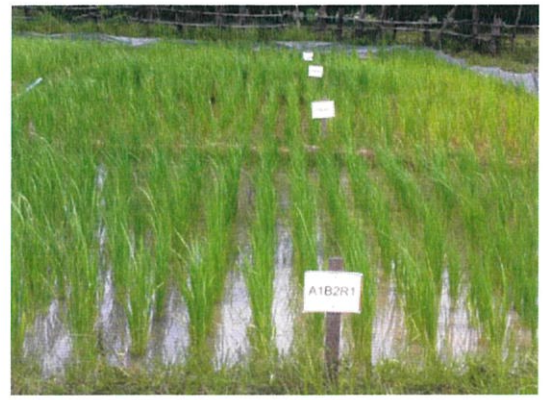
[1]Disease index(DI) was rated using the following scheme: 1= no symptoms 0%, 2= small blighted spots 1-25%, 3= dead cells in the area of blighted spots 1-2mm and turning brown 26-50%, 4= expanded oval-shaped lesions 1-2 cm and cell death in the center of lesion 51-75%, and 5= diseased area over 76% [modified from Soytong and Quimio. (1989)]. [2]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01

Table 18. Plant heights and number tiller of rice variety Sen Pidoa at 50 and 80 days at the field experiment.

Treatments	Plant heights 50 days	Plant heights 80 days	Number tiller 50 days	Number tillers 80 days
Non-treated control	39.50 ab[1]	61.40 b	6 b	9 b
Organic method	44.75 a	63.40 ab	11 a	16 a
GAP method	41.20 ab	66.05 ab	12 a	21 a
Chemical method	44.25 a	69.20 a	13 a	22 a
CV (%)	6.12	3.97	9.77	9.62

[1]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01

Non -treated control

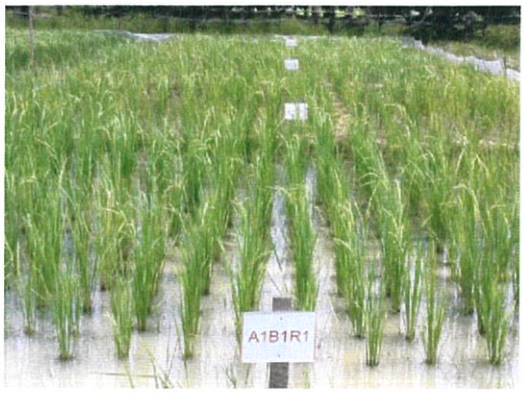


Organic method

GAP method

Chemical method

Fig. 24 Plant heights and number tillers comparison between each treatment at 50 days



Non-treated control

Organic method

GAP method

Chemical method

Fig. 25 Plant heights and number tillers comparison between each treatment at 80 days

Table 19. Number of panicles per plant and panicle length and weight(g) of rice variety Sen Pidoa at 80 days and harvesting time.

Treatments	Panicle number/plant	Panicle length (cm)	Panicle weight (g)/panicle
Non- treated control	9 c[1]	16.85 d	1.65 d
Organic method	15 b	19.45 c	2.10 c
GAP method	21 a	19.67 bc	2.25 bc
Chemical method	18 a	21.25 a	2.35 ab
C.V (%)	7.06	2.24	6.21

[1]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01.

Table 20. The grains and dry hays weight per plot ($20m^2$) of rice variety Sen Pidoa at 14% of moisture contain (MC).

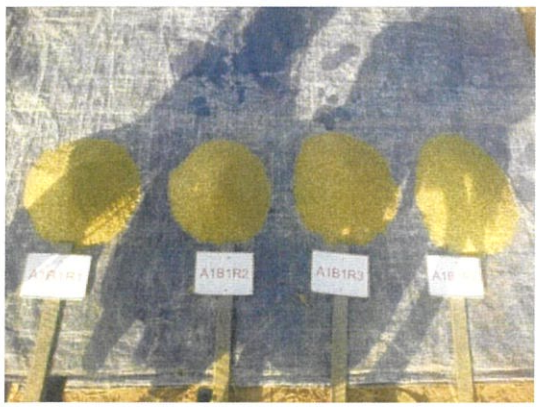
Treatments	Grains weight(kg)/plot	Dry hays weight(kg)/plot
Non-treated control	4.12 c[1]	6.17 c
Organic method	7.37 b	9.40 b
GAP method	10.26 a	16.42 a
Chemical method	10.77 a	17.21 a
C.V (%)	6.54	14.44

[1]Means of four replications. Means followed by a common letters were not significantly different by DMRT at P=0.01.

Non-treated control Organic method

GAP method Chemical method

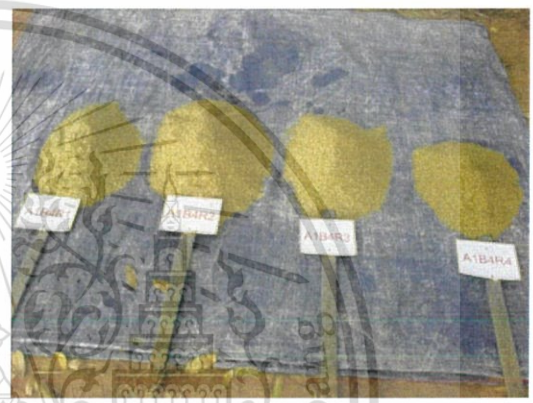
Fig. 26 Dry hays yield comparison between each treatment



Non-treated control

Organic method

GAP method



Chemical method

Fig. 27 Grains yield comparison between each treatment

CHARPTER 5

CONCLUSION

Leaf spots on rice variety IR66 caused by *Curvularia lunata* is found to be the first report in Cambodia. *Chaetomium cupreum* significantly inhibited sporulation of *C. lunata* when compared to the control in bi-culture test. *Curvularia lunata* is reported for the first time to cause leaf spots of rice var. Sen Pidoa in Cambodia. *Chaetomium cupreum* CC3003 can be significantly inhibited *C.lunata* in bi-culture test. The antifungal metabolites from *C. cupreum* expressed antifungal activity against *C. lunata* at the ED_{50} values of hexane, ethyl acetate and methanol crude extracts were 6.417.81μg/ml, 0.837.81μg/ml and 7.81μg/ml, respectively. The nano product from *C. globosum* KMITL-N 0805 was yellowish in color and the particle size was 241 nm. The nano-CGH from *C. globosum* KMITL-N 0805 showed significanty antifungal activity against *C. lunata* at ED_{50} values of 1.21 ppm/ml and followed with nano-CGE at 1.19 ppm/ml and nano-CGM 1.93 ppm/ml, respectively.

The incidence of brown leaf spot caused by *C. lunata* was reduced after application of a spore suspension of *C. cupreum*, *Chaetomium*-biofungicide and chemical fungicide (tebuconazole) to rice seedlings inoculated with *C.lunata* in pot experiment. In pot experiment, It was shown that treatment with a spore suspension of *C. cupreum*, bio formulation of *C. cupreum*, nano particles of *C. cupreum* and the chemical fungicide (tebuconazole) gave higher growth parameters than the inoculated controls. Rice seedlings treated with a spore suspension of *C. cupreum*, biofungicide of *C. cupreum*, nano particles of *C. cupreum* and the chemical fungicide (tebuconazole) showed significantly lower disease incidence than the inoculated control. It was concluded that nano-CGH, nano-CGE and nano-CGM from *C. globosum* KMITL-N0805 decreased leaf spot of rice var. Sen pidoa caused by *C. lunata* and significantly increased in plant height and number of tillers when compared to the non-treated control. This research finding represents the first report that nano-particles derived from *C. globosum* KMITL-N0805 can be controlled *C. lunata* causing leaf spot of rice.

In the field experiment for IR66, the chemical method gave the best results in all plant parameters, followed by the GAP and organic methods, accepted for disease incidence on leaves which lower leaf spot disease in organic method than GAP and chemic methods. The experiment for Sen pidoa variety in the field revealed that the chemical method was better reduction leaf spot

disease caused by *C. lunata* than organic and GAP methods. The chemical and GAP methods gave higher in grain weight than the organic method when compared to the non-treated control. Generally, it is suggested that the organic and GAP methods must concern to soil fertility in each location and other environmental factors involved when compared the chemical method.

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- Feb.7-18,2005 : Project Design, Monitoring and Evaluation (By SILAKA).
- August 01-05,2005 : Bees and Beekeeping in Cambodia.

- 07-12/05/2005 : Visit and Study at Thailand on: Beekeeping, EM, IMO Compost, Fermented Plant Juice, Fermented Fruit Juice, Organic Vegetables, and Organic pig raising.
- 23 – 27 /01/2006 : Single-country Study Mission on Bee-Keeping and Organic Pig Farming (Special Program for Agriculture Productivity Enhancement in Asian Least Developed Country).
- 18-22 /12/2006 : Training and Culture Exchange Program in Organic farming and Handicraft development at Mc KEAN-Chaing Mai-Thailand.
- March 11 – 14 ,2010 : Single-country Study Mission on Guideline Nursery Management on Tree seedling as Acacia crassicarpa, at Southern Forest Seed Joint Stock Company-Ho Chi Minh City,Guynong-Viet Nam
- May 15, 2010 : The Annual Meeting of Thai Phytopathological Society (TPS) and Conference on Plant Pathology in Thailand At Department of Plant Pathology Faculty of Agriculture Kasetsart University, Bangkok, Thailand
- May 5 – 8 /01/2010 : Single-country Study Mission on Guideline Nursery Managemen t on Tree seedling as Acacia crassicarpa, manguim at Southern Forest Seed Joint Stock Company-Ho Chi Minh City-Viet Nam.

Research and International Publications :

1-International Publication:

-Tann, Huyly[1*], Soytong, Kasem[2], Chaiwat Makhonpas[1] and Adthajadee Aram[1] (2011). Comparison between organic, GAP and chemical methods for cultivation of rice varieties in Cambodia. was publication at Journal of Agricultural Technology 7(5): 2235-2241.

-Tann, Huyly[1*], Chaiwat Makhonpas[1], Aram Utthajadee[1], and Soytong, Kasem[2]. 2012. Effect of good agricultural practice and organic methods on rice cultivation under the system of rice intensification in Cambodia. was publication at Journal of Agricultural Technology. 2012. Vol 8(1):289-303.

-Tann, Huyly[1], Soytong, Kasem[2], (2016). Bioformulations and nano product from *Chaetomium cupreum* CC3003 to control leaf spot of rice var. Sen pidao in Cambodia.

International Journal of Plant Biology, Country(Italy), Paper # 6413. The paper was accepted for publication in Vol 8 No 1, 2017

- Tann, Huyly[1] and Soytong, Kasem[2], (2016). Biological control of brown leaf spot disease caused by curvularia lunata and field application method on rice variety ir66 in cambodia agrivita, Journal of Agricultural Science, Country (Indonesia). The paper was accepted for publication in vol.39 no. 1 February 2017.

2-Internatiol Oral Presentation:

- Comparison between chemical, GAP and organic methods for cultivation of rice varieties from Cambodia. 2011, RMUTTO Chanthaburi, Thailand .
- Investigation of rice pathogens and applications of different methods for cultivation of rice var IR66 in the field in Cambodia. The second International Conference on Integration of Science and Technology for Sustainable Development (ICIST) "Biological Diversity, Food and Agricultural Technology" Faculty of Agriculture, KMITL, Bangkok, Thailand, November 28-29, 2013.
- Biological control of rice brown leaf spot caused *Curvularia lunata* and application method in the field of rice Variety IR66 in Cambodia . The Thirdth International Conference on Integration of Science and Technology for Sustainable Development (ICIST 2015) "Biological Diversity, Food and Agricultural Technology". November 28-29, 2015. Champasak Hotel, Lao PDR.
- Bioformulations and nano product from *Chaetomium cupreum* cc3003to control leaf spot of rice var. Sen pidoa in Cambodia. The Fourth International Conference on Integration of Science and Technology for Sustainable Development (ICIST 2015) "Biological Diversity, Food and Agricultural Technology" . November 27-28, 2015. Center for Woman and Development (CWD) Hotel, Vietnam.

Reward/Decoration :

-Year 2007 got the Silver Medal from Ministry of Agriculture, forestry and fishery, Royal of Cambodia.

-Year 2008 got the Golden Medal from Royal of Cambodia

-Year 2014 got the Royal Order Of Cambodia-Grand Cross from Royal of Cambodia.

APPENDICES

International Journal of Plant Biology

Country	Italy
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Publisher	PagePress
Publication type	Journals
ISSN	20370156, 20370164
Coverage	2010-ongoing

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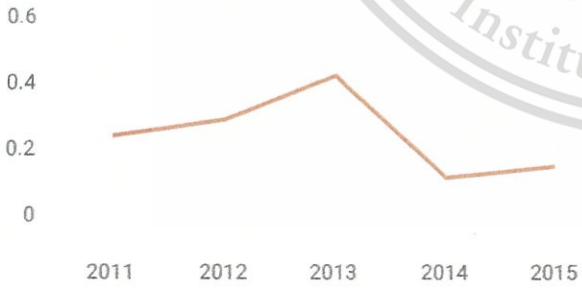

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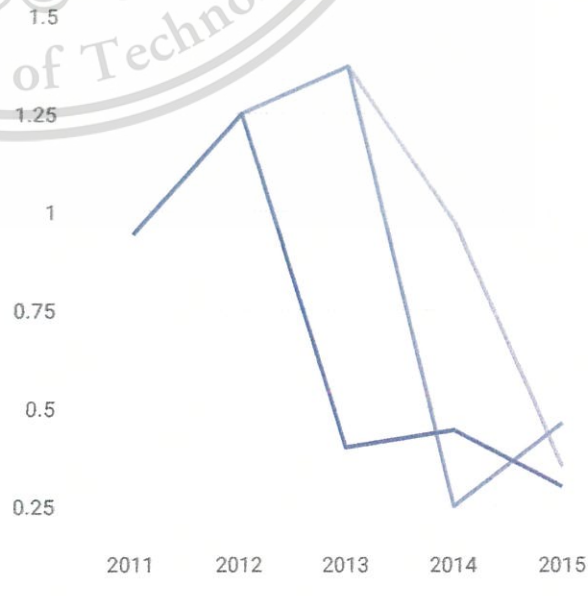

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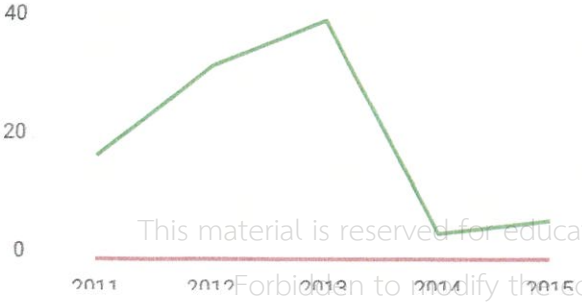

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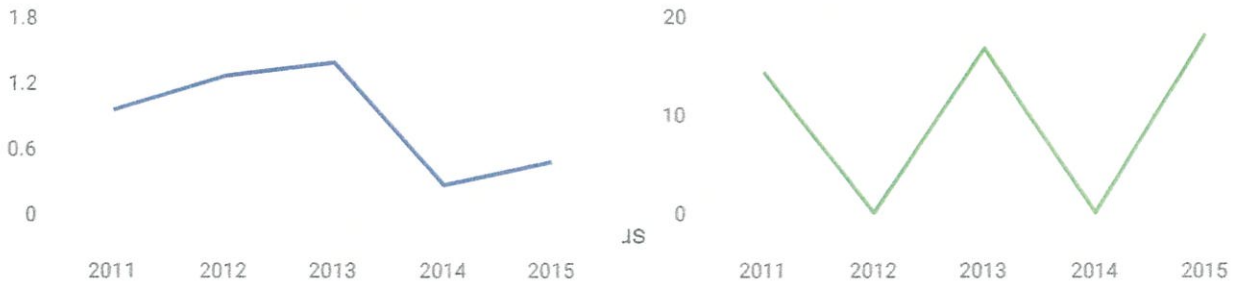

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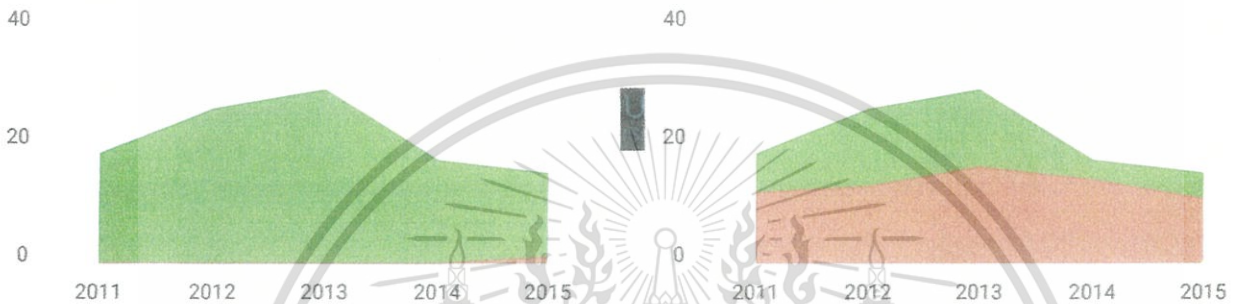

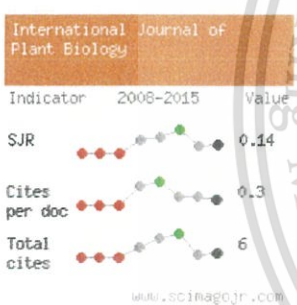

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We are pleased to inform you that your paper entitled "**Bioformulations and nano product from Chaetomium cupreum cc3003to control leaf spot of rice var. Sen pidao in Cambodia**" has been accepted for publication in our journal (Vol 8 No 1, 2017).

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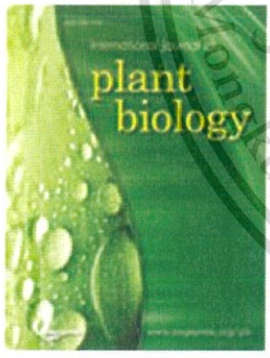

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Bioformulations and nano product from *Chaetomium cupreum* cc3003 to control leaf spot of rice var. Sen pidao in Cambodia

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Abstract

Curvularia lunata was isolated from leaf spot of rice variety Sen Pidoa and tested for pathogenicity. *Chaetomium cupreum* CC3003 expressed antifungal activity against *C. lunata* in Bi-culture test. Crude hexane extract, crude ethyl acetate extract and Crude methanol extract from *C. cupreum* inhibited sporulation of *C. lunata* with ED_{50} of 6.41µg/ml, 0.83µg/ml and 7.81µg/ml, respectively. Pot experiment revealed that plant heights in treated with a spore suspension of *C. cupreum*, biofungicide of *C. cupreum*, nano particle of *C. cupreum* and chemical fungicide(tebuconazole) were not significantly different when compared to the inoculated control. Disease reduction compared to the inoculated control from treatment with a spore suspension of *C. cupreum*, biofungicide of *C. cupreum*, nano particle of *C. cupreum* and chemical fungicide (tebuconazole) ranged between 41.67% to 58.33%. Field experiment indicated that chemical method was decreased leaf spots by 60%, followed by organic method and GAP methods which decreased infection by 40% of both, respectively. The chemical and GAP methods were significantly higher in grain weight which were 10.77kg per plot, 10.26kg per plot than the organic method at 7.37kg per plot when compared to the non-treated control at 4.12kg per plot.

Keywords: brown leaf spot, *Chaetomium* sp., rice

Introduction

Rice (*Oryza sativa* L) is one of the major food crops in Asia where it is the daily diet more than in other regions of the world. The major problems causing reductions in the quality and quantity of rice include pathogens and insect pests. Observation and preliminary disease diagnosis found that a leaf spot of rice caused by *Curvularia lunata* has become one of the most serious diseases of this crop in Cambodia especially in the rice variety Sen Pidoa. [1] Reported that *C. lunata* caused leaf spot for the first time in India, and that symptom showed brown leaf spot and finally blight. Moreover, it has been reported that *C.lunata* caused many symptoms in rice e.g. grain discoloration,[2] leaf spot,[3] black kernel and seedling blight,[4]. Sheath rot of rice was reported for the first time in Tamil Nadu, India,[5]. *Curvularia lunata* causing leaf spots on *Sorghum bicolor* was also reported for the first time in Pakistan,[6] . Biological control of plant diseases has widely contributed to the reduction of the use of toxic chemical fungicides by farmers that pollute the environment and Harm on-target organisms. *Chaetomium* sp., belonging to the Ascomycota ,has been reported as a biocontrol agent against several plant pathogens,[7,8] . *Chaetomium globosum* and *C. cupreum* have been successfully applied to control rice blast caused by *Pyricularia oryzae,*[9].

The objective was to evaluate *Chaetomium cupreum* CC3003 as a biocontrol agent to control leaf spot of rice var. Sen Pidoa caused by *Curvularia lunata*.

Materials and methods

Isolation of pathogen and pathogenicity test

Leaf spots of rice var. Sen Pidoa were isolated from leaf symptoms by tissue transplanting method of [10]. The mycelia on water agar (WA) were transferred onto potato dextrose agar (PDA) until get pure culture. All isolates were identified by morphologically observation under compound microscope. All isolates were tested for pathogenicity test followed the method of Koch's Postulate. The pathogen inoculums was prepared as spore suspension of $1x10^6$ spore/ml. The inoculums was inoculated to 20 days old rice seedlings planted in pots of 12cm diameter by spraying to seedlings, then covered with plastic bags to maintain moisture content. The appeared symptom was re-isolated to be pure culture and identified to confirm species.

Dual culture antagonistic test

Chaetomium cupreum CC3003 was offered by Assoc. Prof. Dr. Kasem Soytong. *C. cupreum* was tested against *Curvularia lunata* causing brown leaf spot in Dual culture plates. The test was performed by using the method of [11,12]. The fungal antagonists and the virulent isolate of *C. lunata* were made Dual culture on potato dextrose agar (PDA) and incubated at the room temperature (28-30°C). The edge of actively growing colony of *C. lunata* and *Chaetomium cupreum* was cut with 0.5mm diameter by the sterilized cork borer and one agar plug of each fungus was transferred to the opposite sides on the PDA plates of 9cm diameter and separately culture of *Chaetomium cupreum* and *C. lunata* served as a controls, then incubated at the room temperature(28-30°C) for four weeks. Data were collected as colony diameter (cm) and sporulation which counted on Haemacytometer under compound microscope. The experiment was done using completely randomized design (CRD) with four replications.

In vitro antifungal metabolites from *Chaetomium cupreum* CC3003 against *Curvularia lunata*

The crude hexane, ethyl acetate and methanol extract from *Chaetomium cupreum* CC3003 were made. The *C. cupreum* extraction were explained as follows: *Chaetomium cupreum* was cultured in potato dextrose broth (PDB) and incubated at room temperature (28-30°C) for 4 weeks. Fungal biomass were removed from liquid by cheesecloth filtration and dried over night at room temperature (28-32°C) for 3days. The extraction was performed by the method described by [13]. The air-dried fungal biomass was ground and extracted with hexane (1:1vol/vol) and incubated by shaking for 3 days at room temperature. The solvent was separately taken out the marc by filtration through filter paper (Whatman No.4). The marc from hexane extraction was extracted with ethyl acetate (EtOAc) and followed with methanol (MeOH) using the same procedure as hexane. The solvents were separately evaporated to yield crude hexane, ethyl acetate and methanol extracts, respectively.

Antifungal metabolites from *Chaetomium cupreum* against *Curvularia lunata* in PDA plate was done by using the method [14]. The experiment was done by using two factors factorial experiment in Complete Randomize Design (CRD) with four replications. Factor A represented crude hexane, ethyl acetate and methanol extracts. Each crude extract was dissolved with 2% dimethyl sulfoxide (DMSO), mixed into PDA before autoclaving at 121°C for 30min and factor B represented the concentrations of 0, 10, 50, 100, 500 and 1000µg/ml of each crude extract. The agar plug (3 mm dia.) of *Curvularia lunata* was cut from the advance margin of the 7 days old colony and sub-cultured to the middle of PDA plate in each concentration of crude extract and incubated at room temperature for 4 days. Data were collected as colony diameter (cm) and spore production of *C. lunata*. Data were computed analysis of variance (ANOVA) and treatment means were compared using the Duncan's multiple range test (DMRT) at P=0.01. The effective dose of ED50 values was computed using probit analysis.

Testing fungal metabolites from *Chaetomium cupreum* CC3003 to inhibit *Curvularia lunata* causing leaf spot of rice var. Sen Pidoa in a pot experiment.

The experiment was performed by using Complete Randomized Design (CRD) with four replications. Treatments were set up as follows: Inoculated with *Curvularia lunata* (T1), spore suspension of *C. cupreum* CC3003 1 x 10^6 spore/ml (T2), Bio fungicide (*C. cupreum*) at 20g/20L of water (T3), nano-particle of *C. cupreum* (T4) at 10ppm/ml and chemical fungicide (Tebuconasole) 0.1ml/L of water (T5). All treatments were inoculated with *C. lunata* and followed the treatments above. Rice seeds var. Sen Pidoa were soaked in clean water for 24 hours in moisten paper until germination, then planted into pot (3 seedlings per pot). The 15 days old rice seedlings were inoculated to wounded leaves with 1 x 10^6spore/ml, three wounded leaves/seedlings were done. Each treatment was applied as mentioned above at every 15 days until harvest.

Data were collected as plant heights (cm), number of tillers at 65 days. Disease index of leaf spot at 65 days was recorded as follows: level 1= no symptoms 0%, level 2= small blighted spot and still healthy tissue 1-25%, level 3= dead cells in the area of blighted spot 1-2mm and turn brown colour 26-50%, level 4= expanded lesion in oval shape 1-2cm and cell death in the centre of lesion 51-75% and level 5= diseased area over 76% which modified from [9]. Data were computed analysis of variance (ANOVA) and treatment means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and P=0.01.

Field Experiment

The field experiment was conducted at Ourung Village, Pongro kroum commune, Chi kreng District, Siem Reap province Cambodia which in the area of disease epidemic or infestation to the rice. The rice in experiment was naturally infected by *Curvularia lunata* causing leaf spot symptoms and observed. The experiment was conducted by using a Randomized Completely Design (RCD) with 4 replications and the treatments were done as follows: The non-treated control (T1), organic method (T2), GAP method (T3) and chemical method (T4). The non-treated control was not used any bio-products and chemicals. Organic method was used *Chaetomium cupreum* (10cc/20L of water), applied organic fertilizer 4.5kg/plot, liquid biofertilizer at 40cc/20L, bioinsecticide (*Metarhizium* and *Beauveria*) (50cc/20L of water) every 20 days until harvest. GAP method (good agricultural practice) was applied the chemical-organic biofertilizer (12-3-3) 1.5kg/plot, alternative spraying between bio-insecticide together with *Chaetomium cupreum* at 10 cc/20L and chemical insecticide (Buprofezin 25%WP 30g/20L) together with chemical fungicide (Tebuconasole 20cc/20L) every 20 days until harvest. Chemical method was applied urea 46-0-0 (0.75kg/plot) in early stage and 15-15-15 before flowering stage (0.75kg/plot) and spraying with Buprofezin 25%WP (30g/20L) together with Tebuconasole (20cc/20L) every 20 days until harvest. Data were collected as plant heights (cm), number of tillers per plant at 50days and 80 days. The length and weight of panicles, number of panicle per plant and grains weight per panicle at 80 days. The grains and dry hays yield per plot at harvesting time. The disease index on leaves at 80 days and on grain at harvesting time were scored as follows: 1= no symptoms 0%, 2= small blighted spots 1-25%, 3= dead cells in the area of blighted spots 1-2 mm and turning brown 26-50%, 4= expanded oval-shaped lesions 1-2cm and cell death in the centre of lesion 51-75%, and 5= diseased area over 76%. and disease reduction were calculated by followed the formula (% disease reduction = (disease index in control - disease index in treatment) / (disease index in control x 100%) by[9]. The data were statistically computed by ANOVA .Data were computed analysis of variance (ANOVA) and treatment means were compared using Duncan's Multiple Range Test (DMRT) at P=0.05 and P=0.01.

Results

Isolation of pathogen Pathogenicity test

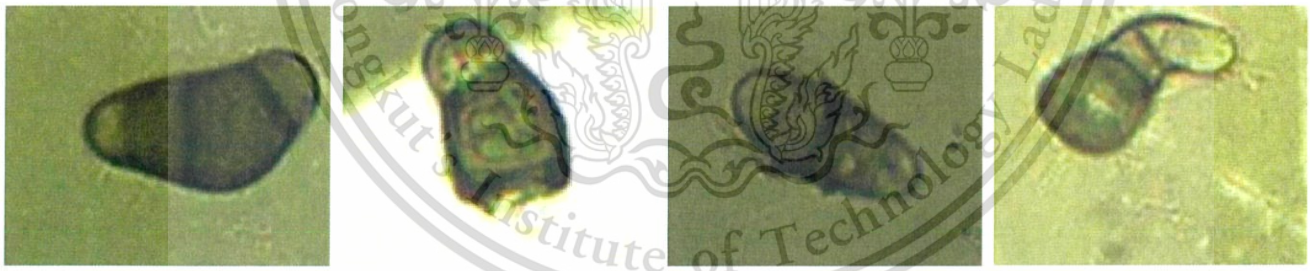
Curvularia lunata was found to be the causing agent to infect rice variety Sen Pidoa. All isolates were tested for pathogenicity to 20 days old seedlings by inoculating spore suspension at concentration of 1 x 10^6spores/ml. The rice seedlings showed clearly symptom of leaf spot and re-isolation to confirm species. The most virulent isolate was used for further experiment. The symptoms were observed from leaves spots were brown in colour, the maximum infection was recorded as leaf sheath. This is a first reported of rice leaf spot caused by *C. lunata* in Cambodia. Result showed that *C. lunata* causing leaf spots of rice var. Sen Pidoa which were isolated and proved for pathogenicity.

Dual culture antagonistic test

Chaetomium cupreum also resulted to inhibit *Curvularia lunata* causing leaf spot of rice variety Sen Pidoa in Dual culture test which significantly suppressed spore reduction of *C. lunata* of 41.03% when compared to the control plate.

In vitro antifungal metabolites from *Chaetomium cupreum CC3003* against *Curvularia lunata*

The extraction of antifungal metabolites from *C. cupreum* using hexane, ethyl acetate and methanol at 1,000µg/ml inhibited spore production of *C.lunata* by 95.13%, 94.92% and 87.90%, respectively. The ED_{50} values of hexane, ethyl acetate and methanol extracts from *C. cupreum* CC3003 were 6.41µg/ml, 0.83µg/ml and 7.81µg/ml, respectively (Table 1). The application method of these antibiotic substances was sprayed onto the plant surface or directly to the pathogen cells which it is high molecular weight substances. With this,[15] reported that crude extracts of *C. cupreum* express antifungal activity against *Dreschera oryzae* causing leaf blight of rice. Moreover, it is clearly shown under compound microscope that the pathogen spores were abnormal due to antagonistic substances extracted with hexane, ethyl acetate and methanol extracts from *C. cupreum* released into pathogen cells and resulted to broken the pathohen cells (Fig.1). [9] also reported this phenomenon namely antibiosis and lyses that antagonistic substances could destroyed the pathogen cells leading to the pathogen loss of pathogenicity. It was interested that *C. cupreum* was reported to antagonize *P. oryzae* causing rice blast in the Philippines,[16].



0 (µg/ml) | Crude Hexane 1,000 (µg/ml) | Crude EtOAC 1,000 (µg/ml) | Crude Methanol 1,000 (µg/ml)

Figure 1. Effects of hexane, ethyl acetate and methanol extracts from *C. cupreum* CC3003 on spores of *C. lunata.*

Table 1. Antifungal metabolites of hexane, ethyl acetate and methanol extracts from *Chaetomium cupreum* against *Curvularia lunata*

Crude extracts	Conc. (µg/ml)	Colony diameter (cm)	Colony inhibition (%)	Spore number (10^6 spore /ml)	Spore inhibition (%)	ED_{50} µg/ml
Crude hexane	0	5.00 a[1]	-	77.00 a	-	6.41
	10	4.38 bc	12.40 hi	64.25 c	16.56 k	
	50	4.23 def	15.40 efg	46.88 d	39.12 j	
	100	4.14 fg	17.20 de	30.00 f	61.04 h	
	500	4.05 gh	19.00 cd	9.88 j	87.17 c	
	1000	3.84 i	23.20 b	3.75 l	95.13 a	
Crude ethyl acetate	0	5.00 a	-	71.50 b	-	0.83
	10	4.48 b	10.40 i	21.50 g	69.93 f	
	50	4.34 cd	13.20 gh	12.63 i	82.34d	
	100	4.28 cde	14.40 fgh	9.63 j	86.53 c	
	500	4.01 h	19.80 c	6.88 k	90.38 b	
	1000	3.60 j	28.00 a	3.63 l	94.92 a	
Crude methanol	0	5.00 a	-	78.5 a	-	7.81
	10	4.46 b	10.80 i	37.38 e	52.38 i	
	50	4.30 cde	14.00 fgh	28.50 f	63.69 g	
	100	4.21 ef	15.80 ef	18.63 h	76.27 e	
	500	4.13 fg	17.40 de	14.00 i	82.17 d	
	1000	4.01 h	19.80 c	9.50 j	87.90 c	
C.V (%)		1.74	10.74	4.47	2.23	

[1/] Means of four replications, means followed by a common letter were not significantly different by DMRT at P = 0.01

Testing fungal metabolites to inhibit *Curvularia lunata* causing leaf spot in rice variety Sen Pidoa in pot experiment.

The results showed that the heights of plants treated with a spore suspension of *Chaetomium cupreum* (28.49cm), biofungicide- *Chaetomium spp.* (28.92cm), nano product from *C. cupreum* (28.04cm) and Chemical fungicide -tebuconazole (27.16 cm) were not significantly differed from the inoculated control (26.91cm) at 65 days after planting (Table2). The Plant heights increased after treated with spore suspension of *C. cupreum,* biofungicide (*C. cupreum),* nano-particles of *C. cupreum* and Chemical fungicide (Teboconazole) which were 5.55%, 6.95%, 4.03% and 0.92%, respectively, when compared with inoculated controls (Table 2.). [14] stated that metabolites produced by *Chaetomium* spp. inhibited several plant pathogens including *C. lunata*. It was clearly demonstrated that treatment with spore a suspension of *Chaetomium cruprem*, biofungicide (*C. cupreum),* nano product from *C. cupreum* and tebuconazole gave higher number of tillers which were 9.25, 9.63, 9.88 and 9.94, respectively, than the inoculated controls at 6.00 at 65 days after planting. All treatments increased the number of tillers from 35.14% to 39.64% when compared with inoculated control (Table 2). Similarly [15] found that the metabolites from *Chaetomium* spp. could inhibit *Drechslera oryzae* which causes leaf spot of rice. Rice seedlings treated with a spore a suspension of *Chaetomium cruprem*, biofungicide (*Chaetomium cruprem*), nano product from *C. cupreum* and Chemical fungicide (tebuconazole) showed significantly lower disease indices(DI) of 1.75, 1.50, 1.50 and 1.25, respectively, than the inoculated control of 3.00. In the treatment of spore a suspension of *Chaetomium cruprem*, biofungicide (*Chaetomium cruprem*), nano-product from *C. cupreum* and Chemical fungicide (tebuconazole) showed significantly disease reduction which were 41.67%, 50%, 50%, and 58.33%, respectively, when compared with inoculated control. (Table 2). [14] stated that metabolites produced from *Chaetomium* spp could inhibited several plant pathogens including *C. lunata*. The number of tiller increased in treatment of spore a suspension of *Chaetomium cruprem* CC3003, biofungicide of *Chaetomium cruprem,* nano-product from *C. cupreum* and chemical fungicide (tebuconazole) which were 35.14%, 37.69%, 39.27% and 39.64%, respectively, when compared with inoculated control in Table 2. Similar report was found from the work of [15] who stated that the metabolites from *Chaetomium* spp could inhibit *Drechslera oryzae* which causing leaf spot of rice and enhanced plant growth parameters.

Table 2. Number of tillers, plant height and disease index of rice var. Sen Pidoa in a pot experiment at 65 days.

Treatments	Number of tillers	% NT increase	Plant heights (cm)	% PH increase	[2]Disease Index	Disease reduction (%)
Inoculated control	6.00c[1]	-	26.91a[1]	-	3.00a[1]	-
Spore suspension of *C. cupreum*	9.25ab	35.14	28.49a	5.55	1.7ab	41.67
Biofungicide (*C. cupreum*)	9.63ab	37.69	28.92a	6.95	1.5b	50.00
Nano-particles of *C. cupreum*	9.88a	39.27	28.04a	4.03	1.5b	50.00
Chemical fungicide (Tebuconazole)	9.94a	39.64	27.16a	0.92	1.25b	58.33
C.V (%)	14.36%	-	7.35%	-	3.11	-

[1]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01. Disease Index [2](DI) was rated using the following scheme: 1= no symptoms 0%, 2= small blighted spots 1-25%, 3= dead cells in the area of blighted spots 1-2 mm and turning brown 26-50%, 4= expanded oval-shaped lesions 1-2 cm and cell death in the center of lesion 51-75%, and 5= diseased area over 76% [modified from ,[9]].

Field Experiment

The results showed that the chemical method were significantly in reduced leaf spot disease by 60%, followed by organic method and GAP method by 40% of both, respectively (Table 3). [17] also found that a bio-formulation of *Chaetomium cochliodes* gave good controlling of brown leaf spot of rice caused by *Curvularia lunata*. Moreover, blight symptom caused by *Curvularia lunata* in grains were significantly reduced by the chemical method at 66.80% compared to organic and GAP methods which reduced disease by 40% and 33.40%, respectively when compared to the non-treated control as seen in Table 3. This research found that the plant heights at 50 days in all treatment were not significantly different when compared with control. The plant height at 80 days in organic and GAP method were not significantly difference with control, but the chemical method gave the plant heights at 69.20cm and was significantly difference compared with control at 61.40cm

(Table 4). The chemical method at 50 days showed the number tiller at 13, followed by GAP method at 12, and organic method at 11, all treatments were significantly difference compared with control at 6. The rice plant at 80days in chemical method showed the number tillers at 22, followed by GAP method at 21, and organic method at 16, all treatments were significantly difference compared with control at 9, as seen in Table 4. The rice seedling at 80days in chemical and GAP methods gave significantly in higher number panicle per plant at 18 and 21, followed by the organic method at 15, all treatments were significantly difference when compared with non-treated control 9, as shown in Table 5. The chemical method gave the best panicle length at 21.25cm, followed by GAP method at 19.67cm and organic method at 19.45cm, respectively, all treatments were significantly when compared with non-treated control at 16.85cm. The chemical and GAP method gave significantly in higher grains weight at 2.35g and 2.25g per panicle, followed by organic method at 2.10g, all treatments were significantly difference compared with control at 1.65g, as seen in Table 5. The chemical and GAP method gave the best grains weight yield at 10.77kg/plot and 10.26kg/plot, and followed by Organic method at 7.37kg/plot, all treatments were significantly difference compared with control at 4.12kg/plot. The chemical and GAP method gave significantly in dry hays yield at 17.21kg/plot and 16.42kg/plot, followed by organic method at 9.40kg/plot, all treatments were significantly difference when compared with control at 6.17kg/plot as seen in Table 6. A previous report found similar results when comparing organic, GAP and chemical methods for rice cultivation in Cambodia and also reported on the effect of good agricultural practice and organic methods on rice cultivation under the system of rice intensification in Cambodia, [18]. This research stated that all growth parameters and yields from chemical, GAP and organic methods could be affected by variable factors such as water management, weeding, soil type and soil fertility in each location. [19] also stated that biofertilizer and bio products of *Chaetomium* could be applied instead of chemical ones. As can be observed in this study, the organic method was significantly better than the non-treated control in the field trials.

Table 3. Disease index on leaves and grain of leaf spot disease caused by *Curvularia lunata* on rice variety Sen Pidoa at 80 days and harvesting time in field experiment

Treatments	[1]Disease index on leaves	Disease index on grains	Disease reduction on leaves (%)	Disease reduction on grains (%)
Non-treated control	5.00 a[1]	5.00 a	-	-
Organic method	3.00 b	3.00 c	40.00	40.00
GAP method	3.00 b	3.33 b	40.00	33.40
Chemical method	2.00 c	1.66 b	60.00	66.80
CV (%)	12.92	16.74	-	-

[1/]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01

Table 4. Plant height and number tiller of rice variety Sen Pidoa at 50 days and 80 days

Treatments	Plant heights 50 days	Plant heights 80 days	Number tillers 50 days	Number tillers 80 days
Non-treated control	39.50 ab[1]	61.40 b	6 b	9 b
Organic method	44.75 a	63.40 ab	11 a	16 a
GAP method	41.20 ab	66.05 ab	12 a	21 a
Chemical method	44.25 a	69.20 a	13 a	22 a
CV (%)	6.12	3.97	9.77	9.62

[1]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01

Table 5. Number of panicles per plant and panicle length and weight(g) of rice variety Sen Pidoa at 80 days.

Treatments	Panicle number/plant	Panicle length(cm)	Panicle weight(g)
Non- treated control	9 c[1]	16.85 d	1.65 d
Organic method	15 b	19.45 c	2.10 c
GAP method	21 a	19.67 bc	2.25 bc
Chemical method	18 a	21.25 a	2.35 ab
C.V (%)	7.06	2.24	6.21

[1]Means of four replications. Means followed by a common letters are not significantly different by DMRT at P=0.01.

Table 6. The grain and dry hay weight per plot ($20m^2$) of rice variety Sen Pidoa at 14% moisture contain (MC).

Treatments	Grains weight(kg)	Dry hays weight(kg)
Non-treated control	4.12 c[1]	6.17 c
Organic method	7.37 b	9.40 b
GAP method	10.26 a	16.42 a
Chemical method	10.77 a	17.21 a
C.V (%)	6.54	14.44

[1]Means of four replications. Means followed by a common letters were not significantly different by DMRT at P=0.01.

Discussion and Conclusions

Curvularia lunata was found to seriously infected rice var.Sen Pidoa in the field in Cambodia and this research finding is confirmed by isolation of pathogenic isolate and proved pathogenicity test. It is reported for the first time in Cambodia. [20] Stated that *C. lunata* is one of the most commonly found fungi in rice seeds leading to grain discoloration and,[1,21] reported that *C. lunata* is caused leaf spot or leaf blight of rice and other hosts. In this study showed that *C. cupreum* significantly inhibited *C. lunata* isolated from leaf spot of rice variety Sen Pidoa. [22] Reported that *Chaetomium cupreum* was antagonistic to the rice blast pathogen caused by *Pyricularia oryzae* in the Philippines.

Fungal metabolites released from *C. cupreum* CC3003 used isolate in this study was reported by,[13] who reported that it produces three new azaphilones named rotiorinols A-C (1-3), two new stereoisomers, (-)-rotiorin (4) and epi-isochromophilone II (5), and a known compound, rubrorotiorin (6). Compounds 1, 3, 4, and 6 exhibited antifungal activity against *Candida albicans* with IC_{50} values of 10.5µg/ml, 16.7µg/ml, 24.3µg/ml, and 0.6µg/ml, respectively. It is suggested that *C. cupreum* could produce and release these bioactive compounds against *C. lunata*. Moreover,[15] reported that Crude extracts of *C. cupreum* CC3003 expressed antifungal activity against *Dreschera oryza* causing leaf blight of rice. It was shown that the pathogen spores were abnormal due to metabolites from *C. cupreum* CC3003 extracted with hexane, ethyl acetate and methanol could destroy the pathogen cells. As a result,[9] reported this phenomenon, namely antibiosis and lyses, that fungal-derived antagonistic substances could destroy the pathogen cells leading to loss of pathogenicity. Moreover,[7] stated that metabolites produced by *Chaetomium* spp. inhibited several plant pathogens including *C. lunata.* Similar result

reported by,[18] stated that the metabolites from *Chaetomium* spp. could inhibit *Drechslera oryzae* which causes leaf spot of rice.

The results demonstrated that rice seedlings variety Sen Pidoa treated with a spore suspension of *C. cupreum*, biofungicide of *C. cupreum,* nano particles of *C. cupreum* and the chemical fungicide (tebuconazole) revealed significantly lower disease indices than the inoculated control. The plant heights treated with a spore suspension of *C. cupreum*, biofungicide of *C. cupreum*, nano particles of *C. cupreum* and the chemical fungicide (tebuconazole) were significantly higher than the inoculated controls at 65days after planting. Similar report stated by,[17] that a biofungicide of *Chaetomium cochliodes* gave good controlling of brown leaf spot of rice caused by *Curvularia lunata*.

In the field trial, result showed that the chemical method gave better reduction of leaf spot disease caused by *C. lunata* than organic and GAP methods. But mostly growth parameters at 80 days were not significantly different among the organic, GAP and chemical methods when compared to the non-treated control. As a result, the chemical and GAP methods were significantly higher in grain weight than the organic method when compared to the non treated control. This result is contradicted to previous study by,[18] who reported that organic method trended to be higher yield than GAP and chemical methods. It is recommended that it could be affected by variable factors such as water management, weeding, soil type and soil fertility in different location.

Curvularia lunata is reported for the first time to cause leaf spots of rice var. Sen Pidoa in Cambodia. *Chaetomium cupreum* CC3003 can be significantly inhibited *C.lunata* in bi-culture test. The antifungal metabolites from *C. cupreum* expressed antifungal activity against *C. lunata* at the ED_{50} values of hexane, ethyl acetate and methanol Crude extracts were 6.41µg/ml, 0.83 µg/ml and 7.81µg/ml, respectively. In pot experiment, It was shown that treatment with a spore suspension of *C. cupreum*, biofungicide of *C. cupreum*, nano particles of *C. cupreum* and the chemical fungicide (tebuconazole) gave higher growth parameters than the inoculated controls. Rice seedlings treated with a spore suspension of *C. cupreum*, biofungicide of *C. cupreum,* nano particles of *C. cupreum* and the chemical fungicide (tebuconazole) showed significantly lower disease indices than the inoculated control. Field experiment showed that the chemical method was better reduction of leaf spot disease caused by *C. lunata* than organic and GAP methods. And the chemical and GAP methods gave higher in grain weight than the organic method when compared to the non-treated control.

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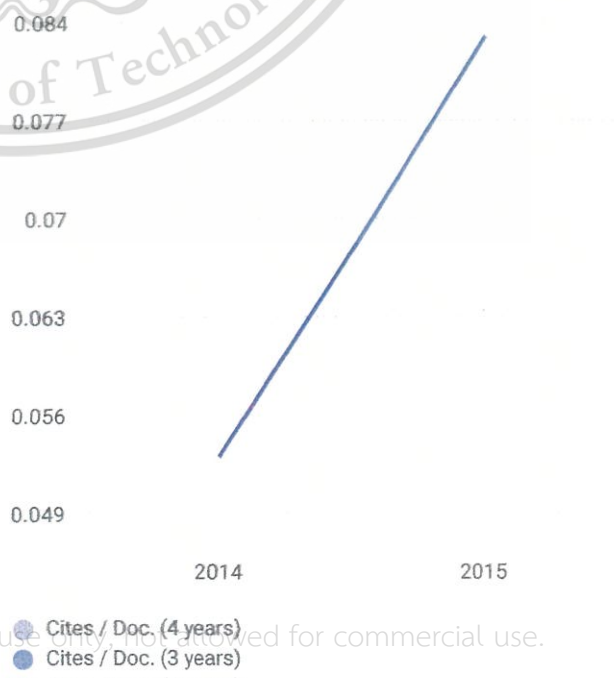

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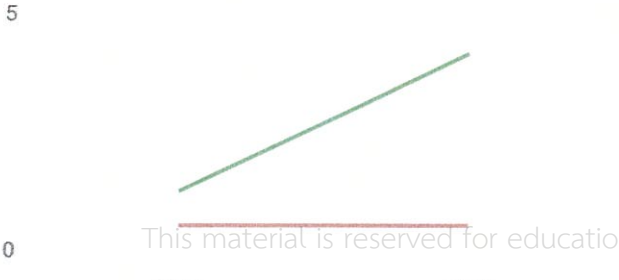

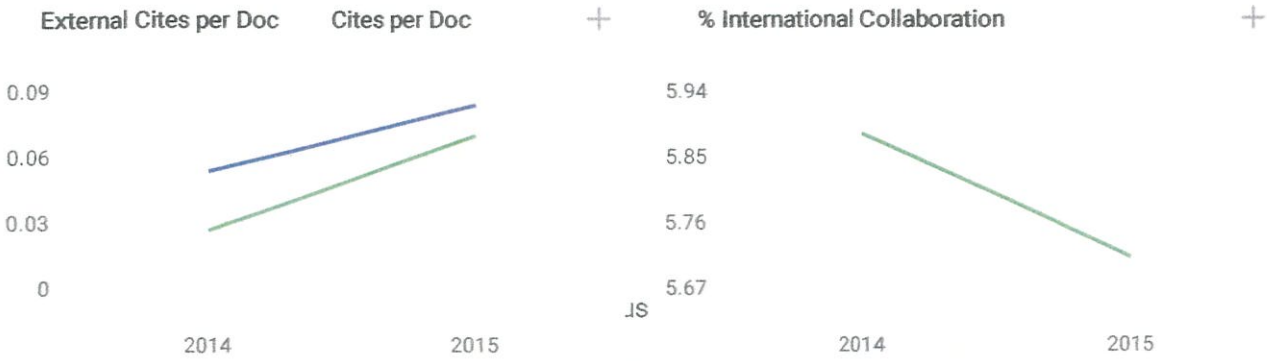

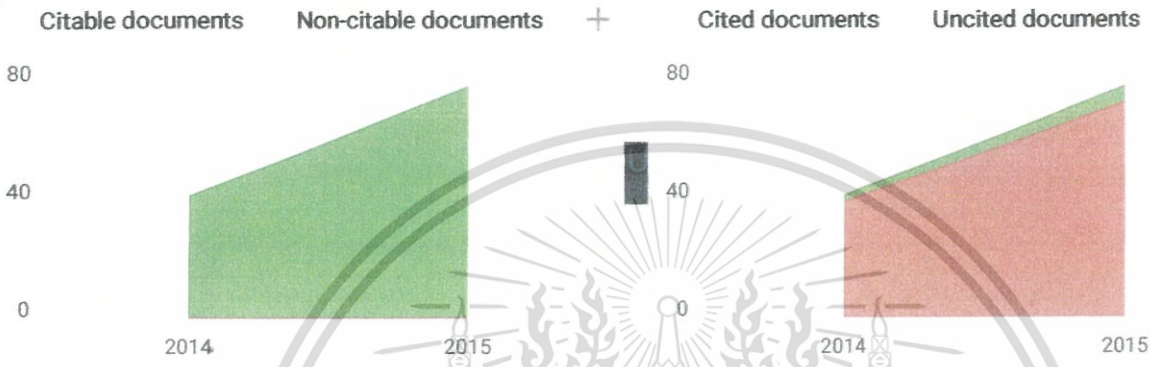

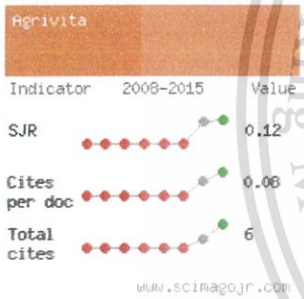

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LETTER OF ACCEPTANCE

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On behalf of The Editor in Chief of Agrivita Journal of Agricultural Science declare that:

Manuscript Title : Biological Control of Brown Leaf Spot Disease Caused by *Curvularia lunata* and Field Application Method on Rice Variety IR66 in Cambodia

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Institution : Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang Bangkok

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This letter is made to whom it may concern.

15 AUG 2016

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BIOLOGICAL CONTROL OF BROWN LEAF SPOT DISEASE CAUSED BY *CURVULARIA LUNATA* AND FIELD APPLICATION METHOD ON RICE VARIETY IR66 IN CAMBODIA

ABSTRACT

Curvularia lunata was found to cause a serious rice brown leaf spot in Cambodia. This is the first report of brown leaf spot on rice in Cambodia. All isolates were tested for pathogenicity. Dual culture antagonistic tests showed that *Chaetomium cupreum* significantly inhibited sporulation of *C. lunata* when compared to the control. In a pot experiment, *C. cupreum* significantly reduced the incidence of brown leaf spot caused by *C. lunata*. After application of a spore suspension of *C. cupreum*, *Chaetomium*-biofungicide and chemical fungicide (tebuconazole) to rice seedlings inoculated with *C. lunata*, the disease was reduced by 68.79%, 75.80% and 72.41%, respectively. In a field trial, the chemical method gave the best results in all plant parameters, followed by the good agricultural practice (GAP) and organic methods. The chemical method gave the highest panicle/plant, panicle length, panicle weight, grain weight/plant which were significantly differed from the GAP and organic methods. The chemical method also gave the best results in filled grain/panicle, unfilled grain/panicle, grain weight/plot, dry hay weight/plot, biomass weight/plot and harvest index, and was significantly better than the GAP and organic methods.

Keywords: biological control, *Chaetomium cupreum*, *Curvularia lunata*, rice

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to Gramineae and is the most economically important food crop in many developing countries (Matsuo *et al.*, 1995). Rice is a stable food to billions of people around the world. It is about 2.5 billion people who consume and the growing areas are mostly in Asia. It serves about 21% of global human per capita energy and about 15% of per capital protein. The world's rice production is traded only 6-7 % of the world market. Thailand, Vietnam, China and the United States are the largest exporters. The United States reported to produce approximately 1.5% of the world's rice crop in Arkansas, California and Louisiana. Rice production in the world is directly consumed about 85 % (IRRI, 2001). About 57% of rice is grown on irrigated land, 25% in rain-fed lowland, 10% in the uplands, 6% in deep-water, and 2% in tidal wetlands (Chopra and Prakash, 2002). Rice is cultivated in many different environmental conditions in Asia (Alford and Duguid, 1998; Chaudhary, 2001). In Cambodia, rice planted in rain-fed lowland areas without irrigation. Total cultivated area for rice production in 24 provinces in 2009 was 2,719,080 hectares and in 2010 was 2,795,892 hectares, but the harvested area in 2009 was 2,674,603 hectares and in 2010 was 2,777,323 hectares only. The total yield production of rice in 2009 was 7,175,473 tons, average yield was 2.84 tons per hectares and the total yield production of rice in 2010 was 8,249,452 tons, and average yield was 2.97 tons per hectares (Nesbitt, 1995; Pracilio *et al.*, 1997; Maeder, 2002; MAFF, 2010).

Brown leaf spot is one of the problems associated with rice disease of many varieties in Cambodia, and is caused by *Curvularia lunata* especially in the last few years. The pathogen not only infects leaves but also infects rice seeds (Simon and Lal, 2013). Other problems for rice production are pathogen resistance to chemical fungicides and their toxic residues, which can negatively impact the environment and human health. Biological control of plant pathogens has successfully provided a relatively recent strategy for integration with other control measures. It helps to reduce the heavy application of chemical fungicides, build up agro-ecosystem and reserve natural balances. There are several reports on the potential use of biological control agents against plant pathogens (Kaewchai *et al.*, 2009). *Chaetomium cupreum* is a strictly saprophytic antagonist which is effective against several plant pathogens (Soytong and Quimio, 1989) e.g. *Phytophthora palmivora* (Pechprome and Soytong, 1996) and *Colletotrichum gloeosporioides* (Noiaium and Soytong, 1999) and *Pyricularia oryzae* (Soytong and Quimio, 1989; Soytong, 1992a; 1992b). It is challenging to find alternative methods which are safe agricultural inputs like bio-fertilizer and bio-pesticides to be used instead of toxic chemicals in rice production representing of good agricultural practice (GAP) and organic agriculture (Soytong *et al.*, 2001).

The objectives of this research were to study biological control of rice brown leaf spot caused by

C. lunata, and application methods in a pot experiment and in the field cultivated rice variety IR66 in Cambodia.

MATERIALS AND METHODS

Isolation, identification and pathogenicity test of the rice pathogen

Brown leaf spot of rice variety IR66 was isolated from leaf symptoms by the tissue transplanting method (Soytong and Quimio, 1989). The mycelia on water agar (WA) were transferred onto potato dextrose agar (PDA) until pure cultures were obtained. All isolates were identified by morphologically observation under a compound microscope. All isolates tested for pathogenicity followed Koch's Postulates. The pathogen inoculums was prepared as a spore suspension of 1 x 10^6 spores/ml. Twenty-day-old rice seedlings planted in pots were inoculated by spraying then covered with plastic bags to maintain moisture content. The pathogen was re-isolated from symptomatic tissue, returned to pure culture and identified morphologically to confirm species.

Dual culture antagonistic test against rice pathogen

Chaetomium cupreum was tested against *C. lunata* in dual culture plates. The test used the method of Soytong (1992a). The fungal antagonists and a virulent isolate of *C. lunata* were cultured on potato dextrose agar (PDA), and incubated at the room temperature (28-30°C). The edge of actively growing colony was cut with 5 mm diameter by the sterilized cork borer and one agar plug of each fungus was transferred to the opposite sides on PDA plates of 9 cm diameter and separately cultured *C. cupreum* and *C. lunata* served as controls, then incubated at room temperature (28-30°C) for 4 weeks. Data were collected as colony diameter (cm) and number spore production, which counted using a Haemacytometer under a compound microscope. The analysis of variance (ANOVA) was computed, and treatment means were compared using Duncan's Multiple Range Test (DMRT) at $\alpha = 0.05$ and 0.01.

Efficacy of *C. cupreum* for control of brown leaf spots caused by *C. lunata* on rice variety IR66 in pot experiment

The experiment used a randomized complete block design (RCBD), four replications and treatments were done as follows: the inoculated control with *C. lunata*, spore suspension of *C. cupreum* 1 x 10^6 spores/ml, biofungicide (*C. cupreum*) 20 g /20 L of water, chemical fungicide (tebuconazole) 0.1 ml/ 1 L of water. Rice seeds of variety IR66 were soaked in sterile water for 24 hours, put in moisten paper until

germination, and then planted into pots (3 seedlings per pot). The 15 days old seedlings of rice variety IR66 were inoculated by *C. lunata* with 1 x 10^6 spores/ml (three-wounded leaves/seedling) to all treatments and immediately applied the products as mentioned above every 15 days until harvest. The collected data included plant height (cm), number of tillers, disease index, and disease reduction.

Application of *C. cupreum* to control brown leaf spots on rice variety IR66 in the field

The field experiment was conducted at Toek Vil Agriculture Research Station, located in Siem Reap province Cambodia which in the area of disease epidemic or infestation to the rice. In experiment, the used rice was infected by *C. lunata* naturally. The experiment was conducted by using RCBD with four replications and treatments were as follows: the non-treated control, organic method which applied organic fertilizer 4.5 kg/plot, liquid biofertilizer 40 cc/20L, bioinsecticide (*Metarhizium and Beauveria*) at the rate of 40 cc/20L of water, biofungicide (*C. cupreum*) at the rate of 10 g/20L of water every 20 days until harvest. Good Agriculture Practice (GAP) method applied the chemical-organic, biofertilizer (12-3-3) at the rate 1.5 kg/plot, for disease and insects were controlled by alternated spraying with a bioinsecticide plus biofungicide and a chemical insecticide (Buprofezin 25%WP 30 g /20L) plus chemical fungicide 20 cc/20L) every 20 days until harvest. The chemical method applied urea 46-0-0 at the rate of 0.75kg/plot in the early stage and 15-15-15 before the flowering stage at the rate of 0.75 kg/plot and spraying with chemical insecticide (Buprofezin 25%WP 30 g /20L) plus chemical fungicide 20 cc/20L) every 20 days until harvest. The plot size was 6 x 5 m^2 (30 m^2). Each replication was separated by a 0.5 m bund. Twenty-day-old seedlings of rice variety IR66 were transplanted at a spacing of 25 × 25 cm. Fertilizer application was done according to the above-mentioned treatments for individual experimental plots. Manual weeds control was used in all treatments. The necessary water management was maintained for rice cultivation. At the panicle initiation phase to ripening stage, a water level of 5 -10 cm was maintained and drained off from the field 10 days before harvesting. Harvested plants were left in the field for 4-5 days for sun drying. Threshing was done manually, and grains were obtained and weighed at a 14% moisture content. The collected data included plant height (cm), number of tillers per plant, length and weight of panicles, number and weight of grains per panicle, numbers of filled and unfilled grains and grain and straw yields.

RESULT AND DISCUSSION

Isolation of rice pathogen and pathogenicity test

C. lunata was found to be the causal agent of brown leaf spot on rice variety IR66 in Cambodia.

C. lunata was tested for pathogenicity to 20 days old seedlings by inoculating with a spore suspension at a concentration of 1 x 10^6 spores/ml. The rice seedlings showed clear symptoms of brown leaf spot and the pathogen was re-isolated to confirm species. The most virulent isolate was used for further experiments. Ou (1985) stated that *C. lunata* is one of the most commonly encountered fungal genera which may infect rice varieties up to 80 %. The research finding is reported by Simon and Lal (2013) who discovered *C. lunata* causing blight disease of rice in Uttar Pradesh in India. The symptoms were observed on leaves, brown spots, and the maximum infection was recorded on the leaf sheath.

Dual culture antagonistic test

C. cupreum actively expressed antifungal activity against *C. lunata* isolated from rice variety IR66 in dual culture after 28-days incubation. In dual culture the *C. cupreum* significantly inhibited spore production of *C. lunata* at 28.55 % when compared to the control plate. *C. lunata* on dual culture with *C. cupreum* plate produced 183.44 spores / ml compared to control plate at 256.72 spores/ml. *C. cupreum* significantly inhibited colony growth of *C. lunata* in dual culture plate by 21.78 % at 28 days. The colony diameter of *C. lunata* in dual culture plate was 7.04 cm compared to the control plate at 9.00 cm in Table 1.

Table 1. *C. cupreum* to inhibit colony growth and spore production of *C. lunata* which isolated from rice variety IR66 in dual culture at 28 days

Treatments	Colony diameter (cm)	[2/] Colony diameter (cm) (%)	Number of spore production (10^6 spore / ml)	spore production Inhibition (%)
Control	9.00 a [1]	-	256.72 a	-
Dual culture	7.04 b	21.78	183.44 b	28.55

[1] Number followed by a common letter are not significantly different by DMRT at $\alpha = 0.01$. [2/] % Inhibition of Colony diameter (cm) or spore production = (R1 - R2) / R1 x 100 ; R1 = colony diameter or spore production of *C. lunata* in control plate and R2 = colony diameter or spore production of *C. lunata* in dual culture plate.

Similar reports, *C. cupreum* has been recorded to control rice blast caused by *Pyricularia oryzae* in the Philippines (Soytong, 1992b). Moreover, the *C. cupreum* isolate used in this study was reported by Kanokmedhakul *et al.*, (2006) to produce rotiorinols A, C, rotiorin, and rubrorotiorin expressed antifungal activity to inhibit *Candida albicans* with IC_{50} values of 10.5, 16.7, 24.3, and 0.6 ug/mL, respectively. It was

concluded that the control mechanism of *Ch. cupreum* could be antibiosis. However, Soytong (2014) reported that *Chaetomium cochliodes* actively against brown leaf spot on rice var. Pittsanulok 2 caused by *Drechslera oryzae* in Thailand. It showed good inhibition of mycelia growth of 38.18% and inhibited inoculums production by 71.55%.

Efficacy of *C. cupreum* to control brown leaf spot of rice variety IR66 caused by *C. lunata* in a pot experiment

Rice seedlings treated with biofungicide (*C. cupreum*), chemical fungicide (tebuconazole) and spore suspension of *C. cupreum*, showed significantly lower disease index (DI) of 1.75, 2.00, and 2.25, respectively than the inoculated control with *C. lunata* of 7.25. With this spraying the spore suspension of *C. cupreum*, chemical fungicide and biofungicide to inoculated rice seedlings with *C. lunata* the disease reduced of 68.97, 75.86 and 72.41%, respectively Table 2.

Table 2. Effect of treatment on disease index , and disease reduction on rice variety IR 66 at 95 days

Treatments	[1] Disease index	[2] Disease reduction (%)
Inoculated control with *Curvularia lunata*	7.25 a[3]	-
Spore suspension of *Chaetomium cupreum*	2.25 b	68.97
Biofungicide (*Chaetomium cupreum*)	1.75 bc	75.86
Chemical fungicide (tebuconazole)	2.00 bc	72.41

[1] Disease index was modified from Soytong (2014) which level 1 = leaf spot 0%, 2 = leaf spots 1-10%, 3 = leaf spots 11-20%, 4 = leaf spots 21-30%, 5 = leaf spots 31-40%, 6 = leaf spots 41- 50%, 7 = 51-60%, 8 = 61-70%, 8 = 71-80%, 9 = 81-90% and 91-100%. [2] Disease reduction (%) was disease index of inoculated control - disease index in each treatment / disease index of inoculated control x 100. [3] Average of four replications. Means followed by a common letter in each column are not significantly different by DMRT at α =0.01.

After application with a spore suspension of *C. cupreum*, biofungicide (*C. cupreum*) and chemical fungicide to rice seedlings inoculated with *C. lunata*, the results showed that plant heights were non-significantly differed which were 18.77 cm, 18.34 cm and 18.94 cm, respectively but significantly differed when compared to the inoculated control (14.16 cm). Moreover, the number of tillers was also not significantly differed after application with a spore suspension of *C. cupreum*, biofungicide and chemical fungicide which were 4.94, 5.50 and 4.94 respectively but significantly differed when compared to the inoculated control (3.25) Table 3.

Table 3. Efficacy of treatments on plant height and number tillers of rice variety IR66 at 35 days in a pot experiment.

Treatments	Plant heights (cm)	Number of tillers
Inoculated control with *C. lunata*	14.16 a[1]	3.25 b
Spore suspension of *C. cupreum*	18.77 a	4.94 ab
Biofungicide (*C. cupreum*)	18.34 ab	5.50 ab
Chemical fungicide (tebuconazole)	18.94 a	4.94 ab

[1] Number followed by a common letter in each column are not significantly differed by DMRT at α =0.01.

With this, Soytong (2014) reported that testing *C. cochliodes* in different formulations resulted to control brown leaf spot of rice caused by *Drechslera oryzae*. The results of that study showed that a biopowder formulation gave the significantly highest to control leaf spot and highest plant growth when compared to the non-treat control, followed by crude extract of *C. cochliodes*, and spore suspension of *C. cochliodes.* Moreover, the bio-powder formulation resulted in a significantly increased plant growth of over 44 %, followed by a crude extract of *C. cochliodes*, spore suspension of *C. cochliodes* and benlate. The current research represents the first report of control of brown leaf spot of rice caused by *C. lunata* by application of *Chaetomium* sp.

Application of *C. cupreum* to control *C. lunata* caused brown leaf spot of rice variety IR66 in the field

The organic, good agricultural practice (GAP) and chemical methods were tested for rice cultivation of variety IR 66 in a field trial in Cambodia. Result showed that the chemical method gave the highest plant height at 80 days which was 72.55 cm, followed by the GAP method (67.2 cm) and organic method (62.35 cm) which were significantly different from the non-treated control (53.19 cm). The chemical and GAP methods gave the best results in number of tillers at 80 days which were 15 tillers and 14 tillers, respectively, followed by organic method (12 tillers) which was significantly different from the non-treated control (6 tillers) as seen in Table 4.

Table 4. Efficacy of treatments on plant height and number of tillers per plant of rice variety IR66 at 80 days in the field trial.

Treatments	Plant heights (cm)	Number of tillers/plant
Non-treated control	53.19 c[1]	6 c
Organic method	62.35 d	12 ab
GAP method	67.2 bc	15 a
Chemical method	72.55 a	14 a

[1] Number followed by a common letter in each column are not significantly different by DMRT at α =0.01.

The chemical method also gave the best results in panicle/plant, panicle length (cm), panicle weight (g), grain weight(g)/plant were 13 panicles/plant, 26.09 cm, 4.70 g and 4.05 g, respectively, which were significantly differed when compared to GAP were 18 panicles/plant, 25.38 cm, 4.24 g and 3.60 g, respectively, and the organic method were 11 panicles/plant, 24.83 cm, 3.36 g and 2.90 g Table 5.

Table 5. Efficacy of treatments on panicles and grains of rice variety IR66 at 80 days in the field trial

Treatments	Panicle/ Plant	Length of panicle(cm)	Panicle weight(g)	Grains weight(g)
Non-treated Control	6 c[1]	23.25 bc	2.56 c	2.25 c
Organic method	11 b	24.83 ab	3.36 b	2.90 b
GAP method	18 a	25.38 a	4.24 a	3.60 ab
Chemical method	13 b	26.09 a	4.70 a	4.05 a

[1] Number followed by a common letter in each column are not significantly differed by DMRT at α =0.01.

Number of filled grain and unfilled grain per panicle, grain and dry hay weight (kg) per plot (20 m^2) at 14% MC were gathered. Chemical method gave the best results in filled grain/panicle, unfilled grain/panicle, grain weight(kg)/plot, dry hay weight(kg)/plot, biomass weight(kg)/plot and Harvest Index (5%) were 111 filled grain/panicles, 15 unfilled grain/panicles, 10.55 kg, 25.97 kg, 41.04 kg and 0.31, respectively which were significantly differed from GAP were 106 filled grain/panicles, 12 unfilled grain/panicles, 9.65 kg, 28.49 kg, 35.62 kg and 0.27, respectively and the organic method which 104 filled grain/panicles, 7 unfilled grain /panicles, 6.34 kg, 16.52 kg, 22.61 kg and 0.27, respectively (Table 6).

Table 6. Efficacy of treatments on grains, dry hay, biomass and harvest index of rice variety IR66 per plots (20 m^2 planted area) at 14% moisture content.

Treatments	Filled grain/ panicle	Unfilled grain/panicle	Grains weight (kg)/plot	Dry hays weight (kg)/plot	Bio mass weight (kg)/plot	Harvest Index (5%)
Non-treated Control	79 c[1]	16 a	4.35 c	8.89 d	13.24 d	0.33 a
Organic method	104 b	7 c	6.34 b	16.52 c	22.61 c	0.27 b
GAP method	106 b	12 b	9.65 a	28.49 a	35.62 b	0.27 b
Chemical method	111 a	15 a	10.55 a	25.97 b	41.04 a	0.31 ab

[1] Numbers followed by a common letter in each column are not significantly different by DMRT at α =0.01.

This experiment revealed that chemical and GAP application gave better result than organic method. This contradicts the previous experiment of Tann *et al.* (2011) reported that the organic method revealed better rice straw weight than non-treated control, and followed by GAP and chemicals at harvesting of 115 days. The organic method can be increased in plant height and number of tillers per plant by 3.06% and 57.69%, respectively in 60 days after planting. The GAP method increased in plant height and tiller number by 11.23% and 69.44%, respectively while the chemical method increased plant height and tiller number by 6.73% and 62.71%, respectively. The grain weight (yield) increased by the GAP, chemical and organic methods by 59.15%, 55.38% and 44.23%, respectively. This may due to different location of experimental sites, soil fertility, disease and different tested variety (Stanhill, 1990; Maeder, 2002). The organic method requires evaluation of many factors for completely successful cultivation (Paull, 2011).

CONCLUSION

Brown leaf spot on rice variety IR66 caused by *C. lunata* is found to be the first report in Cambodia. Based on dual culture test, *C. cupreum* inhibited spore production of *C. lunata*. The brown leaf spot was reduced by application of a spore suspension of *C. cupreum*, biofungicide (*C. cupreum*) and chemical fungicide to rice seedlings inoculated with *C. lunata* in pot experiment.

In field, the chemical method gave the best results in all plant parameters, followed by the GAP and organic methods. It concluded that spraying a spore suspension of *C. cupreum*, biofungicide (*C. cupreum*)

and chemical fungicide to rice seedlings inoculated with *C. lunata* reduced the disease. The experiment showed that chemical and GAP application gave better result than organic method.

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