DEVELOPMENT OF AN INQUIRY-BASED LEARNING UNIT FOR HIGH SCHOOL STUDENTS TO PROMOTE CONCEPTUAL UNDERSTANDING ON PLANT DEFENSE RESPONSES

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
(SCIENCE AND TECHNOLOGY EDUCATION)
FACULTY OF GRADUATE STUDIES
MAHIDOL UNIVERSITY
2009

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entitled

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was submitted to the Faculty of Graduate Studies, Mahidol University
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ACKNOWLEDGEMENTS

First and foremost, I would like to express my deepest gratitude to my major advisor, Assoc. Prof. Dr. Pintip Ruenwongsa, for her valuable guidance, advice, encouragement and numerous opportunities that she has provided over the years throughout this research study. I also would like to thank my co-advisors, Assoc. Prof. Dr. Bhinyo Panijpan and Dr. Arun Chanchaichaovivat, for their useful suggestions and generous supports. Without the brilliant supervisions from the advisors, this dissertation could not be done.

My sincere appreciation is extended to Dr. Pisarn Soydhurum for his helpful comments and valuable time spent for serving as my chair of the dissertation committee. My special thanks go to Prof. Dr. Charles R. Barman, my wonderful mentor during my time at the School of Education, Indiana University Purdue University at Indianapolis, USA, for his warmly support, unfailing guidance, and careful comment on my educational research and manuscripts. I would like to especially thank Prof. Dr. Joseph Chappell at the Deparment of Plant and Soil Sciences, University of Kentucky, USA, for kindly providing the standard capsidiol. His support allowed me to continue carrying out my scientific research. I am also particularly grateful to Dr. Wanida Tanaprayothsak at Department of Biology, the Institute for the Promotion of Teaching Science and Technology, and Dr. Teerada Wangsomboondee at Department of Botany, Chulalongkorn University, for valuable comments and advices.

I am extremely thankful to the Institute for the Promotion of Teaching Science and Technology (IPST) for providing me a great opportunity to continue my doctoral study and a financial support during my study. It is definitely clear that, since I started to work as a staff at IPST, I was inspired to dedicate myself for education and continue my doctoral study in education. I would like to say a special thank you to all of teachers and students involving in this research study. This research study would not have been completed without their co-operating. My sincere thanks are due to all the staff at the Fundamental Science Department, IPST, and the Institute for Innovative Learning, Mahidol University, for helpful support in many ways. Many thanks are also extended to all my wonderful friends especially a number of lovely friends in the Institute for Innovative learning for their truth friendship, powerful support and encouragement.

I wish to express my deepest thank to my parents, my brother, and my families for their love, care and support, and for helping me to focus on my education without any distractions. This work would not have been my possible without their entirely love and support. Finally, I dedicate this dissertation to all meaningful people who have touched my life, particularly my parents, my families, all teachers and all friends.

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ABSTRACT

This research study was conducted with two research aspects in mind including a scientific aspect and an educational aspect. The scientific research dealt with the study of the induced resistance by a yeast, *Pichia guilliermondii* strain R13, in harvested chili. Then, some of the findings of the present scientific study were used to develop a laboratory experiment and an inquiry-based learning unit for high school students to promote students' learning in the concept of plant defense responses.

The scientific involved the *P. guilliermondii* strain R13, the yeast isolated from Thai rambutan, which has been shown to suppress the fungal pathogen, *Colletotrichum capsici*, in harvested chili. Its multiple modes of action include nutrient competition, tight attachment to the fungus, and hydrolytic enzyme secretion. This study investigated the ability of the *P. guilliermondii* strain R13 to induce resistance against *C. capsici* in chili fruit. The pretreatment of chili with the yeast antagonist, physically separated from the fungus by specified distances, significantly reduced disease incidence and lesion diameter caused by *C. capsici*. Compared to the controls, the yeast treatment also significantly enhanced the activities of phenylalanine ammonia-lyase, chitinase, and β-1,3-glucanase, and the accumulation of capsidiol phytoalexin in chili tissue. Scanning electron micrographs showed that the morphology of *C. capsici* spores and hyphae were abnormal and that the pathogen had restricted growth on chili tissue adjacent to the yeast-inoculated sites. The results indicated that the induction of resistance may be another mechanism by which the yeast antagonist suppresses *C. capsici*. Some of the results were then used to develop the learning unit for high school students.

In science education, several researchers have reported about the plant neglect problem in biology curriculum. Additionally, most students think animal studies are more interesting than those of plants because they believe that plants are inferior to animals in that they are passive and unable to respond to external challenges, particularly biological invaders such as microorganisms and insect-herbivores. The purpose of this educational study was to develop the inquiry-based learning unit which focuses on promoting students' conceptual understanding of plant defense responses to biological invaders as well as raising students' awareness in studying plants. The study also investigated students' perceptions of the learning unit implemented in a constructivist classroom. A total of 31 Thai science-majors from Grade 12 participated in this study. Multiple data-gathering techniques of quantitative and qualitative methods were employed; conceptual testing, written documents, a questionnaire and interviews. Based on the data analysis, it was found that students who actively participated developed a better conceptual understanding of plant defense mechanisms. In addition, they appeared to have positive attitudes toward the learning unit as evidenced by their preference for inquiry-based activities in a constructivist learning environment. Finally, their perspectives on plant study had been favorably changed by the active learning experience.

KEY WORDS: INDUCED RESISTANCE/ PLANT DEFENSE/ INQUIRY APPROACH/ CONCEPTUAL UNDERSTANDING

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DEVELOPMENT OF AN INQUIRY-BASED LEARNING UNIT FOR HIGH SCHOOL STUDENTS TO PROMOTE CONCEPTUAL UNDERSTANDING OF PLANT DEFENSE RESPONSES

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บทคัดย่อ

การศึกษาวิจัยนี้ประกอบด้วยการวิจัยในเชิงวิทยาศาสตร์และการศึกษา งานวิจัยวิทยาศาสตร์ได้ศึกษาเกี่ยวกับการชักนำให้เกิดความต้านทานในพริกหลังเก็บกี่ยวโดยใช้ยีสต์ *Pichia guilliermondii* สายพันธุ์ R13 จากนั้นผลการศึกษาของงานวิจัยเชิงวิทยาศาสตร์ได้ถูกนำมาพัฒนาเป็นบทปฏิบัติการทดลองและหน่วยการเรียนแบบสืบเสาะความรู้สำหรับนักเรียนมัธยมศึกษาตอนปลาย เพื่อพัฒนามโนทัศน์เรื่องการตอบสนองแบบต้านทานของพืช

งานวิจัยวิทยาศาสตร์นี้ได้ศึกษาเกี่ยวกับยีสต์ *P. guilliermondii* สายพันธุ์ R13 ที่ได้จากการคัดแยกจากเงาะของไทย ซึ่งมีประสิทธิภาพในการควบคุมราโรคพืช *Colletotrichum capsici* ในพริกหลังเก็บเกี่ยว กลไกที่ยีสต์ชนิดนี้ใช้ในการควบคุมราก่อโรค ได้แก่ การแย่งสารอาหาร การเกาะติดแน่นบนราและ การหลั่งเอนไซม์ย่อยสลายเส้นใยรา งานวิจัยในครั้งนี้ได้ศึกษาความสามารถของ *P. guilliermondii* สายพันธุ์ R13 ในการชักนำให้เกิดความต้านทานต่อ *C. capsici* ในผลพริก จากการทดลองใส่ยีสต์ปฏิปักษ์ชนิดนี้บนเนื้อเยื่อพริก ในบริเวณที่แยกห่างจากรา พบว่ามีการลดลงของทั้งการเกิดโรคและขนาดของรอยแผลที่เกิดจาก *C. capsici* อย่างมีนัยสำคัญ เมื่อเปรียบเทียบกับกลุ่มทดลองยังพบว่าการใช้ยีสต์บำบัด ส่งผลต่อการเพิ่มขึ้นในการทำงานของเอนไซม์ phenylalanine ammonia-lyase, chitinase, และ β-1,3-glucanase และเพิ่มการสะสมของปริมาณ capsidiol phytoalexin ในเนื้อเยื่อพริกอย่างมีนัยสำคัญ นอกจากนี้ภาพอิเล็คตรอนแบบส่องกราดยังแสดงให้เห็นถึงความผิดปกติด้านสัณฐานวิทยาในสปอร์และเส้นใยของ *C. capsici* ซึ่งถูกจำกัดการเติบโตในเนื้อเยื่อพริกบริเวณที่ใกล้กับเนื้อเยื่อที่ถูกบำบัดด้วยยีสต์ ผลการทดลองชี้ให้เห็นว่าการชักนำให้เกิดความต้านทาน อาจเป็นอีกกลไกหนึ่งที่ยีสต์ปฏิปักษ์ใช้ในการควบคุม *C. capsici*

ในทางวิทยาศาสตร์ศึกษา งานวิจัยหลายชิ้นได้รายงานถึงปัญหาด้านการละเลยเรื่องพืชในหลักสูตรชีววิทยา นอกจากนี้ นักเรียนส่วนใหญ่คิดว่าการศึกษาเกี่ยวกับสัตว์มีความน่าสนใจมากกว่าการศึกษาเกี่ยวกับพืช โดยนักเรียนเชื่อว่าพืชนั้นอยู่ในชั้นที่ต่ำกว่าสัตว์ เพราะพืชเป็นเพียงผู้ถูกกระทำและไม่สามารถตอบสนองต่อสิ่งกระตุ้นภายนอก โดยเฉพาะต่อผู้บุกรุกชีววิทยา เช่น จุลินทรีย์และแมลงกินพืช การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อพัฒนาหน่วยการเรียนแบบสืบเสาะความรู้ เพื่อพัฒนามโนทัศน์เรื่องการตอบสนองแบบต้านทานของพืชต่อผู้บุกรุกชีววิทยา พร้อมกับการส่งเสริมความตระหนักของนักเรียนในการเรียนเรื่องพืช นอกจากนี้งานวิจัยได้รวมไปถึงการศึกษาทัศนคติของนักเรียนที่มีต่อหน่วยการเรียนดังกล่าวซึ่งนำไปทดลองใช้ในห้องเรียนโดยเน้นการสร้างความรู้ด้วยตนเอง ผลการทดลองบางส่วนที่ได้จากงานวิจัยทางวิทยาศาสตร์อันเป็นส่วนหนึ่งของการศึกษาในครั้งนี้ ถูกนำมาใช้พัฒนาปฏิบัติการทดลองซึ่งใช้ในหน่วยการเรียนที่พัฒนาขึ้นด้วย หน่วยการเรียนนี้ได้ทดลองใช้กับนักเรียนระดับชั้นมัธยมศึกษาปีที่ 6 สายการเรียนวิทยาศาสตร์ จำนวน 31 คน และใช้กระบวนการหลากหลายที่ผสมผสานระหว่างวิธีเชิงคุณภาพและวิธีเชิงปริมาณ ได้แก่ แบบทดสอบ ผลงานนักเรียน แบบสอบถาม และการสัมภาษณ์ ในการเก็บข้อมูล ผลจากการวิเคราะห์ข้อมูลพบว่า การเรียนเชิงรุกของนักเรียนส่งผลให้นักเรียนสามารถพัฒนามโนทัศน์เรื่องการตอบสนองแบบต้านทานของพืชได้เป็นอย่างดี นอกจากนี้ผลการประเมินยังชี้ให้เห็นว่านักเรียนมีทัศนคติที่ดีต่อหน่วยการเรียนจากการแสดงถึงความพอใจต่อรูปแบบการเรียนการสอนแบบสืบเสาะความรู้ที่เน้นสิ่งแวดล้อมในการเรียนรู้แบบการสร้างความรู้ด้วยตนเอง และจากการที่ได้รับประสบการณ์การเรียนเชิงรุกดังกล่าว จึงส่งผลต่อการเปลี่ยนแปลงทัศนคติของนักเรียนต่อการเรียนเกี่ยวกับพืชในทางที่ดีขึ้น

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LIST OF ABBREVIATIONS

AAAS	American Association for the Advancement of Science
ANOVA	analysis of variance
AT	attitude
AU	area in absorbance unit
^{o}C	degree Celsius
CHI	chitinase
CLE	constructivist learning environment
CLES	Constructivist Learning Environment Survey
CM-Chitin-RBV	carboxymethylchitin-ramazol brilliant violet
cm	centimeter
CV	critical voice
DOA	Department of Agriculture
DOAC	DAO culture collection
EC	enzyme commission
EDTA	ethylene diaminetetraacetic acid
e.g.	for example
et al.	*et.alli* (Latin), and others
FAO	Food and Agricultural Organization of the United Nations
FPLU	Fighting Plant Learning Unit
g	gram
h	hour
i.e.	*ed est* (Latin), that is
IPST	The Institute for the Promotion of Teaching Science and Technology
IR	induced resistance
ISR	induced systemic resistance

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LIST OF ABBREVIATIONS (cont.)

L.	Linnaeus
min.	minute
mRNA	messenger ribonucleic acid
µg	microgram
µl	microliter
µm	micrometer
ml	milliliter
mm	millimeter
mM	millimolar
mU	milli-unit
ng	nanogram
nm	nanometer
NRC	National Research Council
NSES	National Science Education Standard
NYDA	nutrient yeast dextrose agar
NYDB	nutrient yeast dextrose broth
ONEC	Office of the National Education Commission
p.	page
PAL	phenylalanine ammonia-lyase
PDA	potato dextrose agar
pH	potential of hydrogen ion
pp.	pages
PR-protein	pathogenesis-related protein
PR	personal relevance
rDNA	recombinant deoxyribonucleic acid
Rf	retention factor
RH	relative humidity

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LIST OF ABBREVIATIONS (cont.)

SAR	systemic acquired resistance
SC	shared control
SN	student negotiation
SU	scientific uncertainty
SD	standard deviation
SEM	scanning electron microscope
spp.	two or more species
TLC	thin layer chromatography
U	international unit
UV-C	ultraviolet C
v	volume
var.	varietas (Latin)
VOCs	volatile organic compounds
w	weight

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CHAPTER I
INTRODUCTION

Overview

This chapter introduces the overall details of the research study including scientific the study which is related to the educational study. Each research aspect contains the background and rationale of the study, purpose of the study, significance of the study, research questions and definition of term. The chapter ends with the organization of the thesis which includes the details offered in chapter one to six.

1.1 Background and Justification for Scientific study

It is essential to control plant diseases for maintaining the quality and abundance of food produced by agriculturists in worldwide. Recently, different strategies are normally used to manage and control plant diseases. However, the horticultural practices are heavily relied on the use of chemical fertilizers and pesticides (Pal & Gardener, 2006). To date, current concern about the environment indicates the limitation of using chemicals for plant disease control. A board use of pesticides leads to the presence of undesirable problems such as chemical residues in ecology, toxic to many living organisms and the development of pesticide resistant pathogens. Among the trends to reduce the use of chemicals, several physical and biological means have been suggested as the safer alternative methods for plant management (Droby et al., 2003).

The well known term of “biological control” and its abbreviated synonym “biocontrol” (Pal & Gardener, 2006) is one of the most encouraging techniques for preharvest and postharvest protection by using antagonistic microorganisms or even natural plant- and animal-derived compounds (Janisiewicz & Korsten, 2002). A variety of microbial antagonists have been reported about their potential to control several pathogens on various fruits and vegetables (Fravel, 2005). In particular, several natural yeast isolates appear to be the potential microbial antagonists. They do

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not produce allergenic spores and mycotoxins in their biocontrol activity (Droby et al., 1993) and their modes of action to control pathogens are likely to produce fewer hazards to the consumers. Additionally, yeast isolates have simple nutritional requirements and are able to colonize on dry surface for a long period of time as well as sustain many pesticides used in post-harvest environment. Yeasts can also grow rapidly on inexpensive substrates resulting in being easy and cost effective to produce yeast product in large quantities (Druvefors, 2004; Raacke et al., 2006).

A numerous reports from many laboratories indicated the potential use of yeast antagonists to control several postharvest plant pathogens on a variety of harvested commodities. Some of yeast isolates have been patented and developed into commercial products. Currently, three yeast strains, *Candida oleophila*, *Cryptococcus albidus*, and *Metschnikowia fruticola*, are available as commercial products under the trade name Aspire® (Ecogen Inc., Langhorn, PA), Yield Plus® (Anchor Yeast, Cape Town, South Africa), and Shemer® (M. Keren Zu, Agogreen Minrab Group, Ashdod, Israel, pers. comm.), respectively (Blachinsky et al., 2008; Droby et al., 2002; Kurtzman & Droby, 2001).

The benefit of understanding the mode of action by which an antagonist inhibits the pathogen growth is to optimize the organism formulation and application for effective plant management (Janisiewicz, Tworkoski, & Sharer, 2000). According to the biocontrol actions of yeast antagonists, control of plant pathogens by these yeasts often involves several mechanisms including competition for space and nutrients (Janisiewicz, Tworkoski, & Sharer, 2000; Ghaouth, Wilson & Wisniewski, 1998), production of cell-wall degrading hydrolytic enzymes (e.g. chitinase and β-1-3-glucanase) (Bar-Shimon et al. 2004; Masih & Paul, 2002) and direct interactions with the pathogen (Chan & Tian, 2005; Wisniewski et al., 1991), induction of host resistance (Ippolito et al., 2000; Yao & Tian, 2005a; Zhao et al., 2008; Xu & Tian, 2008), and resistance to oxidative stress (Castoria et al., 2003).

Additional mode of action of antagonistic yeast, particularly, *induced resistance*, has been reported in various studies and shown to protect a variety of harvested commodities (Droby et al., 2002; Ghaout, Wilson, & Wisnieski, 2003, Ippolito et al., 2000, Sticher, Mauch-Mani, & Metraux, 1997; Yao & Tian, 2005a; Zhao et al., 2008). Induced resistance is generated after the activation of the host

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plant's defense system. As described by Ozeretskovskaya (1995), this phenomenon is frequently referred to as immunization, sensitization, vaccination or acquired immunity. Upon treatment with suitable elicitors, plant tissues often reacts by activating various biochemical and structural defense system in that these actions help to suppress the spread of pathogens in plant tissues. Induced resistance reaction can be developed within a limited area of plant tissue close to the site of induction or can be expressed in the plant tissue distant from the treated site (Ozeretskovskaya, 1995). Site restricted defense responses involve the rapid death of cells (hypersensitive response), the deposition of lignin, callose and hydroxyproline-rich glycoproteins in cell wall as well as the accumulation of phytoalexins. In contrast, the systemic defense responses include the production and accumulation of antifungal pathogenesis-related (PR) protein such as chitinase (CHI), β-1,3-glucanase, chitosanase, peroxidase and phenylalanine ammonia lyase (PAL) (Fajardo et al., 1998; Ghaout, Wilson & Wisnieski, 2003; Ozeretskovskaya, 1995).

As reported by many researchers, antagonistic yeasts induce several structural and biochemical defensive responses in surface wound of tested harvested fruit. Droby et al. (2002) demonstrated that *C. oleophila* was capable of inducing resistance to *Penicillium digitatum* in grapefruit. Application of *C. oleophila* to grapefruit peel tissue increased ethylene biosynthesis, PAL activity, phytoalexin accumulation, and increased CHI and β-1,3-glucanase protein level. In apple fruit, *Candida saitoana* induced CHI activity and caused the deposition of structural barriers (papillae) in apple surface wound (Ghaouth, Wilson, & Wisniewski, 1998). In the year 2000, Ippolito et al. (2000), also illustrated that *Aureobasidium pullulans*, caused increases in CHI, β-1,3-glucanase and peroxidase activite in apple wound. These increases were markedly started at 24 h after yeast treatment and reached maximum levels at 96 h after yeast treatment. In jujube fruit *Cryptococcus laurentii* caused transient increases in the activity of β-1,3-glucanase and the expression of the *Glu-1* gene in fruit tissue was highly induced (Tian et al., 2006, 2007). In addition, when peaches were treated with the yeast *Pichia membranefaciens,* several defensive proteins have been triggered and accumulated in plant tissues including antioxidant proteins and pathogenesis-related proteins (Chan et al., 2007).

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Anthracnose caused by *Colletotrichum* species is a major damaging disease of tropical vegetables especially chili (*Capsicum annuum* L. var. *acuminatum* Fingerh.). In Asia, for example, *Colletotrichum capsici* (Syd.) Butler & Bisby is a major causal agent of chili anthracnose and is widespread among red chilies (Kim, Park & Lee, 1989; Kim, Oh, & Yang, 1999). When infected by *C. capsici*, chili fruit first develops water-soaked and small black circular spots on the surface, and then the spots turn diffuse and black. After that, the typical anthracnose symptoms develop gradually from the infected area and appear as expanded necrosis (Roberts, Pernezny, & Kucharek, 2001). This disease limits production and fruits showing blemishes have reduced marketability (Manandhar, Hartman, & Wang, 1995).

In 2004, the Food and Agricultural Organization of the United Nations (2004) reported about the increasing year by year of the demand for chilies in the world. The good quality chilies for import and export should be absent of disease appearance or fungal toxins (The Chili Pepper Institute, 2004). However, for many years, control of anthracnose disease on chili fruit still relies mainly on the use of chemical fungicides (Parry, 1990). With the current concerns about the harmful effects of chemical pesticides to organisms and to the environment, several studies have focused on the use of microbial antagonists e.g. *Trichoderma harzianum* (Oanh et al., 2006), *Bacillus amyloliquefaciens* (Intanoo & Chamswarng, 2007), *Pseudomonas fluorescens* (Srinivas et al., 2006), and *Pichia guilliermondii* (Chanchaichaovivat, Panijpan, & Ruenwongsa, 2007, 2008) to manage chili anthracnose.

To focus on the use of yeast antagonist, Chanchaichaovivat, Panijpan, & Ruenwongsa (2007) reported on the potential use of the yeast antagonist *P. guilliermondii* strain R13 isolated from Thai rambutan (*Nephelium lappaceum* L.) for control of *C. capsici* (Syd.) Butler & Bisby on chili fruit (*Capsicum annuum* L. var. *acuminatum* Fingerh.). They also provided evidence that suppression of *Colletotrichum capsici* by *P. guilliermondii* strain R13 results from competition for nutrients, tight attachment of the yeast to the fungus, and secretion of hydrolytic enzymes including CHI and β-1,3-glucanase (Chanchaichaovivat, Panijpan, & Ruenwongsa, 2008). However, whether *P. guilliermondii* strain R13 also suppresses the pathogen by inducing host resistance in chili fruits has not yet been investigated. Thus, this study was undertaken to assess the possibility that the yeast antagonist *P.*

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guilliermondii strain R13 induces resistance against *C. capsici* in chili fruit. A better understanding of the multiple defense mechanisms involved in the suppression of *C. capsici* by *P. guilliermondii* strain R13 could provide guidance for the use of *P. guilliermondii* strain R13 for the postharvest biological control of chili anthracnose.

1.1.1 Scientific Purposes

Natural yeast isolates are biocontrol agent suitable for preventing and controlling postharvest rots, particularly fungal rot, on different fruits and vegetables (Lima et al., 1997). Yeasts are potentially useful antagonists because they are biodegradable, cost effective, and not toxic to humans or even other animals (Raacke et al., 2006). Therefore, managing plant diseases by antagonistic yeast appears to be recognized as one of promising methods for postharvest plant diseases management (Ghaouth, Wilson, & Wisniewski, 1998; Droby et al., 2002; Paster et al., 1993).

In the previous study of Chanchaichaovivat, Panijpan, & Ruenwongsa (2007, 2008), the potential and the mechanism of action of the yeast antagonist *P. guilliermondii* strain R13 to control anthracnose fungus *C. capsici* in chili fruits were evaluated. However, the mechanism by which *P. guilliermondii* strain R13 induces host resistance in chili fruits has not yet been elucidated. Thus, a main objective of this study aimed to evaluate the ability of *P. guilliermondii* strain R13 in the eliciting induced disease resistance against *C. capsici* in chili fruits.

To investigate the induced host resistance phenomenon by the yeast strain R13, percentage of anthracnose disease incidence and wound lesion diameter of yeast-treated chili were primarily studied compared to the controls. Various induced biochemical defensive responses were also determined and quantified including the production of defense-related enzymes (CHI, EC 3.2.1.14; β-1,3-glucanase, EC 3.2.1.39 and PAL, EC 4.3.1.24) and the accumulation of capsidiol phytoalexin in chili tissue after treatment with the strain R13. In addition, anthracnose fungus infected in chili tissue adjacent to the yeast-treated site was observed to investigate the morphological changes under scanning electron microscope.

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1.1.2 Significances of the Scientific Study

This study expected to provide additional scientific evidence of another possible defense mechanism of *P. guilliermondii* strain R13 against fungal pathogen of chili anthracnose. Clarifying the mechanism of this biocontrol agent may provide a better control of postharvest chili anthracnose by using the yeast isolate *P. guilliermondii* strain R13 as an alternative agricultural practice for harvested chili.

1.1.3 Definition of Term

Induced resistance is taken to mean the heightened resistance in a plant body against pathogens due to the pretreatment with microorganisms, microbial products, or even inorganic and organic compounds. The activity of the inducing agents is due to its own ability in the induction of resistance, but not due to its antimicrobial activity or ability to be transformed into antimicrobial agent (Kuc', 2000). It is found that when plant resistance is induced, several types of biochemical and/or physiological changes related to pathogen resistance are occurred in plant tissues. Additionally, induced resistance may be localized at the site of induction or it may be systemic in all or some parts of plant distant from the inducing agent-treated area (Ozeretskovskaya, 1995).

1.2 Background and Justification for Educational study

Biology is a fundamental subject in the natural science that integrates many branches of knowledge to explain the phenomena of the natural world and the living things around us. Regarding biology education, plant science is considered as a critical component of learning content for students in all grade levels as observed in science curricula of several countries (Curriculum Corporation, 1994; IPST, 2001; Ministry of Education, 1993; National Research Council, 1996). The importance of plant study has been stressed by several authors. For example, the National Research Council (NRC, 1992, p. 1) stated that “Our knowledge about the world around us is incomplete if we do not include plants in our discoveries and it is distorted if we do not place sufficient emphasis on plant life.” Carter (2004) also suggested that people of all ages must be taught about plants in our lives. If they develop their understanding and appreciation for the plant science, they would see value of it as a part of science

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education, a part of conservation and survival, a part of the national economy, and an ethical and esthetic life.

However, even though plant science is considered an integral part of the biology curriculum, it appears that many students perceive plants to be less interesting organisms than animals (Green, 1958; Hoekstra, 2000; Strgar, 2007; Tamir & Jungwirth, 1974; Tunnicliffe, 2001; Wandersee, 1986). Some students have extreme opinions about plants such as “plants are boring” (Rowland, 2007). They seem to prefer and value the study of other organisms more than the study of plants (Senchina, 2008). For many years, this lack of attention of students has been mentioned from various researchers (Hershey, 1996; Uno; 1994; Wandersee & Schussler, 1999). Some authors concluded that the reason of neglecting plant learning probably lies on the way that people perceive plants as inferior organisms to animals (Flannery, 2002; Hoekstra, 2000). Likewise, people believe plants lack the unique characteristics as animals such as movement, communication by sounds, feeding and various behaviors resembling to human.

Over the years, many researchers reveal that students’ alternative conceptions about plant science are common and persistent. Many research studies have examined students’ misconceptions about plants in many areas (Hershey, 2004). For centuries, plants have been considered as static and passive organisms (Trewavas, 2002). Some children, the very young or even older ones, regard plants as non-living as a result of their immobility (Wood-Robinson, 1991). These views have been demonstrated in studies of students’ concepts of plants. Several researchers found that students possess alternative conceptions about plant-pathogen interactions and plant-herbivore interactions related to plant defenses (Raven et al., 2005; Stamp, 2004, 2005). The students in these studies perceived plants as helpless and passive organisms that cannot defend themselves against invaders. Even though plants are perceived by people as passive creatures, they are neither helpless nor passive and can respond to many environmental stimuli such as light, gravity, temperature, chemicals, mechanical disturbances, etc. In addition, plants also possess self-defense mechanisms to biological stimuli such as microorganisms and insects.

Unlike animals, plants cannot run away from their enemies, have no other organs to push away the invaders, and have no immune system. Instead, plants

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develop their own defense strategies against their challenges. The self-defense responses of plants can be demonstrated in many interesting ways. For example, all plant cells have the ability to detect invading pathogens and then produce the inducible defenses such as the production of antimicrobial chemical compounds, microbial-defensive proteins and enzymes, and plant cell suicide to restrict the growth of pathogens (Veronese et al., 2003). To resist insect herbivores, plants also have evolved specific defense mechanisms. When they are attacked by pests, they release some volatile organic compounds (VOCs) that later promote the action of natural enemies of pests. In addition, VOCs from the damaged plants can work as the warning sign triggering the neighboring plants to prepare their defense responses prior to insect attacks (Dicke, Agrawal, & Bruin, 2003; Flannery, 2002).

In Thailand, we have carried out a preliminary investigation about the opinion of Thai high school students on learning about plants and animals. After surveying 148 grade 12 students from three urban science high schools in the central part of Thailand, the results indicated that they had a preference for learning about animals (77%) over plants (21%). Only very few students (2%) had no preference in this aspect. The exploratory investigation also indicated several reasons why students tend to prefer learning about animals over plants. Some felt plants generally look the same and that most plant laboratory activities are uninteresting. In addition, most of the students believed that plants are inferior to animals, lacking many of the unique characteristics of animals and that plants are passive organisms, unable to run, move, make noises, fight or respond to stimuli, such as plant invaders and humans. Students' perceptions of plants help us realize the reasons behind their lack of interest in learning about them. Another part of the preliminary investigation focusing on students' conceptions of plant self-defenses revealed that most of the participants held similar alternative conceptions about plant defenses to biological invaders such as pathogens and insect herbivores. Students thought plants were passive organisms unable to defend themselves against any invaders (Table 1.1). These findings are similar to those cited by Raven et al. (2005), Stamp (2004, 2005).

According to the *National Science Curriculum Standard* (IPST, 2001) of Thailand, standard 1.1 focuses on students' understanding of the fundamental unit of a living organism. Included in this standard, is the response of plants to their

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environment (IPST, 2003). The *Science Education Standards* (NRC, 1996) in the United States also emphasize this fundamental concept in its high school life science Content Standard C. However, Thai high school curriculum materials for this topic tend to focus on plant responses to physical stimuli such as light, gravity, humidity and chemicals with little or no emphasis placed on plant responses, particularly defense responses, to biological stimuli (e.g. microorganisms and insect-herbivores) which are equally influential as physical stimuli. As proposed by the American Society of Plant Physiology Education Foundation (2009), "the topic of plant defense to the biological invaders is listed to be a general principle which every student and literate citizen should know". With this topic missing from the Thai curriculum, the students may not understand how plants produce defense responses to biological invader for their survival like other organisms. As a consequence, students may not see plants as an active and viable option in biology education.

Many educators believe that the notion of "plants being boring" or "plants are not interesting passive organisms", should be changed (Flannery, 2002; Rowland, 2007). They believe the studying plants can be engaging and interesting. For example, Bowker (2004) noted that the children showed interest in plants that were relevant to their lives and that they could change their perceptions of plants with appropriate classroom experiences. Therefore, it is time to have students re-examine their views related to learning about plants as well as the alternative conception that plants are passive organisms incapable of defending themselves from invaders. We need to find ways to present plants in classroom instruction so students are able to experience the complexity and uniqueness of this organism. Moreover, developing students' understanding of plant self-defense mechanisms may provide them with the basic knowledge needed to investigate other areas of biology such as plant pathology, plant physiology, plant molecular biology and entomology.

In science education, the common aim is to create learning exercises that can help students learn science in the most effective way. For many years, educators have emphasized a constructivist perspective in learning science (Appleton, 1997; Baviskar, Harter, & Whitney, 2009; Palmer, 2005). This perspective is based on the idea that the knowledge cannot be transferred, but is individually constructed through an interaction of ideas and social experiences (Lesgold, 2004; Liang & Gabel, 2005).

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Specifically, constructivist view represents the importance of addressing students' existing concepts, understanding what they are, and creating situations to stimulate the students to inquire and construct their own knowledge based on what they already know (Baviskar, Harter, & Whitney, 2009; Cakir, 2008). From this point of view, it lends theoretical support for teachers to facilitate students' ownership of the learning experience through a process of active, cooperative, and student-centered instruction (Driver et al., 1994).

Table 1.1 Comparison of 12th grade students' view on the defensive responses of plants against pathogens and insect herbivores

Survey questions	**Percentage of students' answer to the question (n = 148)**			**Students' alternative conceptions and some samples of students' explanation**
	Yes	**No**	**Not Sure**	
Can plants defend themselves against pathogens?	23	73	4	Alternative conception: *Plants cannot defend themselves against pathogens because (most common incorrect explanation):* - Plants have no brain and no circulation system, therefore they are not able to produce antibody to resist pathogens. - Plants are at the lower evolutionary level than animals. They may not have any system to resist pathogens as the immune system in animals. - The plant body is very fragile and different from the animal body. Also, plant cells have less organelles than animal cells. These might affect the function of the plant defense system. - All I have seen is plants are invaded by many living invaders such as insect-herbivores or animal-herbivores and even humans. Hence, it's impossible for them to have any defense against such violent pathogens.
Can plants defend themselves against insect herbivores?	37	60	3	Alternative conception: *Plants cannot defend themselves against insect herbivores because (most common incorrect explanation):* - All plants are incapable of movement and have no organs for defending themselves. Hence, they would not resist insects. - Plants have no such organ systems like in animals, e.g., the brain to control and command how plants should do to defend themselves. - Plants are just passive organisms and I have only seen them sit still and never react to anything. They are just foods for animals.

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Currently, a positive classroom environment is viewed as an integral goal for education, equally as important as the content stressed in instruction (Fraser, 2002). Researchers have found that students achieve better when the classroom has a greater congruence between the actual classroom environment and that preferred by students (Chang, Hsiao, & Barufaldi, 2006: Fraser & Fisher, 1983). Several studies note that a constructivist classroom has proven to be a powerful tool in science education which offers a more appealing environment for students (Kim, Fisher, & Fraser, 1999; Tobin & Tippins, 1993; Tsai, 2000). It was found that a plausible, better understanding as well as interpreting experience of learning and teaching are generated from such appeal of constructivism. To construct the constructivist classroom environment, the teachers need to focus on certain core items including; personal relevance, scientific uncertainty, critical voice, shared control, student negotiation and attitude (Salish I Research Project, 1997; Taylor, Fraser, & Fisher, 1997). In this way, constructivist instruction can act as a powerful strategy to build a classroom environment that maximizes student learning.

Researcher suggests that various methods based on a constructivist perspective have been used to promote the conceptual understanding of science for most students (Akkus et al., 2003; Posner et al., 1982; Tobin & Tippins, 1993). Of those methods, the inquiry approach appears to be an effective means for promoting meaningful science learning (Moyer, Hackett, & Everett., 2007; NRC, 1996, 2000). The essential features of inquiry include effective questioning, answering, collecting information, analyzing and drawing conclusions as well as problem solving by means of the scientific processes (Soydhurum, 2001). The *National Science Education Standards (NSES)* promote the use of inquiry within the classroom (NRC, 1996). The NSES describes that inquiry teaching enables students to develop abilities to do scientific inquiry, gain understanding about scientific inquiry, and acquire and develop scientific concepts by teacher facilitation. In Thailand, the National Education Act (ONEC, 1999), recommends the use of inquiry-based approaches in science teaching to help students develop their own learning process. In addition, the Institute for the Promotion of Teaching Science and Technology (IPST) (IPST, 2000) also stressed that inquiry approaches can be an effective tool in science instruction to help students construct their own knowledge.

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As mentioned earlier, the findings of the preliminary study on plant learning of Thai high school students and the incompleteness of the biology curriculum with respect to plant defense mechanisms indicates a need to include this topic in biology instruction. Therefore, this study attempted to serve this need by developing an inquiry-based learning unit that promotes students' understanding on plant defense responses to biological stimuli in the environment as well as their positive perception to studying about plants.

1.2.1 Educational Purposes

Based on the preliminary investigation, Thai high school students view plants as an uninteresting passive organism different from other organisms such as animals. They also possess an alternative conception that plants cannot defend themselves against the invaders such as microorganisms and insects. In addition, the Thai biology curriculum does not implement the topic of plant self defense for high school students. Thus, it is deemed necessary to provide the chance for students to understand the concept of this topic area and experience the active uniqueness of plants by learning through an effective and appropriate teaching strategy.

The principal objectives of this educational research are as follows:

1) To develop a guided-inquiry learning unit based on a constructivist perspective for high school students to help them understand the concept of plant defense responses to biological stimuli in the environment and promote their perception of studying of plants.
2) To investigate the effectiveness of the designed learning unit on students' conceptual understanding on plant defense responses and students' perception of plant study.
3) To study students' perception of the guided-inquiry learning unit based on a constructivist perspective.

This guided-inquiry learning unit was developed based on the findings from the scientific part of induced-resistance in chili fruit by a yeast antagonist to construct hands-on activities to promote students' conceptual understanding on plant defenses. An inquiry approach and a constructivist perspective were employed in the learning unit development and implementation. The learning unit was designed to

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supplement the regular biology curriculum for use with high school students in Thailand. The lessons created for the unit relate to the students' everyday life and all of the learning materials required to implement the unit are easily found in the local area. Through the use of the unit, the students acquired new knowledge about plant defense responses to biological invaders by actively investigating through hands-on activities, by critical discussion, and by making additional inquiries through scientific articles, news releases, academic websites, and text-books.

1.2.2 Research Questions

This study attempted to answer four research questions:

1) Can the inquiry-based learning unit be effectively implemented within a constructivist environment to promote students' understanding on the concept of plant defense responses to biological invaders and to alter students' perception of plant study?
2) How can the inquiry-based learning unit promote students' conceptual understanding of plant defense responses to biological stimuli?
3) How can the inquiry-based learning unit affect to students' perception of plant study?
4) What are students' perceptions of the learning unit implementation?

1.2.3 Significances of the educational study

A new inquiry-based learning unit of plant defense responses may promote Thai high school students' understanding of plant defense responses to biological invaders as well as positive perceptions of the studying of plants. In addition, this learning unit can be used as a teaching model which represents a procedure of how to develop and implement the unit, and how to assess the effectiveness of using the unit based on the inquiry approach and constructivist epistemology.

1.2.4 Definition of Term

Students' conceptual understanding of the plant defense response means students construct their fundamental understanding that plants are generally able to produce their own defense mechanisms resist to the biological invaders e.g.

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microorganisms and insects in many ways for the purpose of their survivals like other organisms.

Inquiry–based approach means a teaching and learning strategy which allows students to actively investigate their learning tasks through the process of questioning and doing inquiry activities. To address the problem, students need to gather, analyze, and interpret data, give priority to evidence to formulate reasonable explanation that are communicated to others, and then initiate the questions that are interested to them.

Guided-inquiry activity means a set of inquiry-based activities which students perform an investigation to answer the questions posted by the teacher. The main responsibility of the students is figuring out how to answer the given questions. From their own investigation, the students need to collect data, interpret data and construct some kind of answer based on the evidences to the question.

Constructivism means a learning theory that explains about the nature of human learning. This theory views that knowledge cannot be transferred but must be constructed by the learner themselves. The student is viewed as an active learner who actively constructs the knowledge based on their individual process and social interaction process.

Constructivist Learning Environment (CLE) means a classroom environment which is consistent with constructivist epistemology and is emphasized on 6 key dimensions: personal relevance, scientific uncertainty, critical voice, shared control, student negotiation and attitude. In CLE-classroom, teacher's role is being a facilitator, the learning unit focuses on central concepts, and assessment is based on authentic student works.

1.3 Organization of the Thesis

This study is divided into two main research aspects: scientific study and educational study. Some of the ideas and laboratory techniques used in the scientific study of the induction of chili resistance by the yeast antagonist were adapt to develop the new hands-on activities incorporated in an inquiry-based learning unit to promote students' understanding in the concept of plant defense responses. So, the new designed learning unit can thus be used to promote students' understanding in a plant

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science concept for high school students as well as students' perception of plant study. This thesis is organized in six sequential chapters as follows:

Chapter One provides background of the research problem and the purpose of this study. This part also specifies the significance of the research project and the definition of terms relevant to the study.

Chapter Two presents the literature reviews related to the scope of the present study in each research aspect. The scientific review covers the information of yeast biocontrol, mechanism of yeast antifungal activities, induced disease resistance in plants, chili and chili anthracnose, and then ends with a topic of antagonistic yeast *Pichia guilliermondii*. In the section of the educational review, it provides the summaries of relevant literatures regarding learning theories, inquiry-based instruction, and classroom assessment.

Chapter Three describes the methodology and methods used to conduct the research in both scientific and educational study. The first part includes the methods employed to investigate the ability of the yeast antagonist to induce disease resistance in the chili. The latter part deals with the educational research design to conduct a learning unit based on an inquiry approach to promote students' conceptual understanding in a plant science concept as well as their perception of studying about plants.

Chapter Four reports the main findings of the scientific research regarding the effects of the antagonistic yeast on the induced resistance in chili based on various scientific evidences. Another part provides the results of the effect of the inquiry-based learning unit on student achievement as well as their perception of the plant study and the learning unit implementation.

Chapter Five presents a discussion of the major findings of the scientific and educational study in relation to other research studies. The limitations and implications of the study are also mentioned at the end of each part of research aspect.

Chapter Six draws the overall findings of the research study.

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CHAPTER II
LITERATURE REVIEW

Overview

This chapter illustrates a review of relevant literature on both scientific aspect and educational aspect. The scientific review describes the role of yeast biocontrol in the agricultural practice, mechanisms of yeast antagonist to control plant pathogens, and the induction of plant disease resistance by several elicitors. The reviews of the importance of chili, anthracnose disease caused by *Colletotrichum* species, and anthracnose disease management are also mentioned. Then, the scientific review ends with the literature review of the yeast antagonist *Pichia guilliermondii* to control fungal pathogens.

The educational review begins with the literatures relevant the plant biology education. This is followed by the review of learning theory including the theoretical basis of constructivism used to ground for this research study. The detail of inquiry-based instructional models based on constructivist perspective and classroom assessment are also then covered. At the end of this chapter, the summary of the educational review is included.

Scientific review

Concerns regarding environmental risk and human health from using chemicals have been the main purposes for investigating alternative control strategies for agricultural products. Since the beginning of the 1990s, several studies reported the effectiveness of a variety of microbial antagonists against a wide range of postharvest pathogens growing on a variety of harvested fruits and vegetables (Droby & Chalutz, 1994; Droby et al., 1993; Fravel, 2005). To date, biological control by antagonistic microorganisms, particularly, natural yeast isolates is widely recognized as a promising approach for control of postharvest plant diseases (Ghaouth, Wilson, & Wisniewski, 1998, 2003b; Droby et al., 2002; Paster et al., 1993). The success of

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application with some yeast antagonists in large-scale studies has generated the development of postharvest biological control product to control fruit and vegetable rots (Droby et al., 2003) and by early 2000, there were some yeast biocontrol products available on the market (Wisniewski et al., 2007).

2.1 Yeast Biocontrol

During the last few years, several research studies mainly focused on the selection and characterization of the effective yeast antagonists to control plant diseases. Several important properties of natural yeast isolates make them potentially useful antagonists for biocontrol management. For example, yeasts are biodegradable, cost effective, and not toxic to humans or other animals (Raacke et al., 2006). From many reports, a variety of yeast and yeast-like isolates shows high inhibitory capacity against postharvest pathogens on various fruits. For example, *Candida sake* (CPA-1) protects apples and pears against *Penicillium expansum* and *Botrytis cinerea* (Nunes et al., 2002; Viñas et al., 1998). Several species of *Cryptococcus* e.g. *Cryptococcus laurentii* and *Cryptococcus albidus* have been shown a biocontrol activity against gray and blue mold rot on various commodities such as apple, peach, pear, kiwifruit and strawberry (Helbig, 2002; Roberts, 1990; Zhang, Zheng, & Yu, 2007). A pilot testing of the biocontrol efficacy of the yeast *Pichia guilliermondii* (US-7) showed that the strain US-7 generated potential reduction of decay development caused by *Penicillium digitatum* on several citrus fruits (Droby et al., 1993). Zhao et al. (2008) have also been reported that *P. guilliermondii* (CNM 2.1801) was effective in controlling postharvest disease in tomato fruit caused by *Rhizopus nigricans*. *P. membranaefaciens* (Hansen) Hansen showed a potential effect on controlling post-harvest brown rot caused by *Monilinia fructicola* in peach fruit (Xu et al., 2008). In addition, *Aureobasidium pullulans* (de Bary) Arnold isolated from the surface of table grape was effective in suppressing gray and blue mold decay of apple, table grape, kiwifruit and strawberry (Ippolito et al., 2000; Lima et al., 1997; Schena et al., 1999).

Among a number of yeast biocontrol, some potential yeast antagonists have been patented and are commercially available on the market for control the postharvest decays in fruits. Three recent commercial yeast biocontrol products are Aspire®, Yield Plus® and Shemer® (Wisniewski et al., 2007). In some countries, commercial

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formulation based on the yeast *Candida oleophila* Montrocher (Aspire®, Ecogen, Inc., Langhorne, Pa), has been used as postharvest treatments on pome and citrus fruits controlling the fruit decay caused by *Penicillium itallicum* Wehmer and *P. digitatum* Sacc. (Janisiewicz & Jeffers, 1997; Janisiewicz & Korsten, 2002). In 1996, the product Aspire® was introduced commercially in United State. Yeast product Yield Plus® with *C. albidus* (Saito) Skiner was introduced to South African market in 1997. It was used effectively as the biocontrol product resists to pathogen strains like *Botrytis*, *Penicillium* and *Mucor* on apples, pears and other crops (Lisa Picard, Anchor Yeast, Cape Town, South Africa, pers. comm.) (Druvefors, 2004). Another commercial yeast product, Shemer®, registered in Israel, is based on yeast *Metschnikowia fruticola*. It has been tested in field and showed effectiveness against several plant pathogens (i.e., *R. stolonifer, B. cinerea, Sclerotinia sclerotium*) of grape, strawberry and sweet potato (M. Keren Zu, Agogreen Minrab Group, Ashdod, Israel, pers. comm.) (Blachinsky et al., 2008; Kurtzman, & Droby, 2001).

The mechanism of action of biocontrol agent that inhibits the target pathogens is still less understood and has not been fully elucidated (Druvefors, 2004). To date, biocontrol systems of yeast antagonists have involved various modes of actions. All of the mechanisms described below are likely to be operating to control plant pathogens as reported by various studies.

2.2 Mechanism of Yeast Antifungal Activities

The mechanism by which the biocontrol agent resists the plant pathogens has been researched extensively in relation to plant associated with pathogen, antagonist and other microorganisms (Droby & Chalutz, 1994). Understanding the mode of action that an antagonist inhibits the pathogen growth is needed for optimizing organism formulation and application for effective plant management. Druvefors (2004) mentioned that, due to the complex biocontrol environment, it is very difficult to construct experimental situation that can exclude all other possible mode of actions. However, it was found from various studies that no one single mode of action have been shown to be responsible for biocontrol activity (Janisiewicz & Korsten, 2002).

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Commonly, biological control of plant pathogens by yeast antagonists appears to be involved multiple antagonistic mechanisms. Mechanisms of yeast that have been suggest to play a key role in controlling fungal pathogens include competition for space and nutrients (Janisiewicz, Tworkoski, & Sharer, 2000; Ghaouth, Wilson & Wisniewski, 1998), production of cell-wall degrading hydrolytic enzymes (Bar-Shimon et al. 2004; Masih & Paul, 2002) and direct interactions with the pathogen (Chan & Tian, 2005; Wisniewski et al., 1991), induction of host resistance (Ippolito et al., 2000; Yao & Tian, 2005a; Zhao et al., 2008), and resistance to oxidative stress (Castoria et al., 2003).

2.2.1 Competition for Nutrients and Space

Competition for nutrients and space has been suggested as the main mechanism of action of biocontrol agent. Several studies have been investigated in relation to this kind of mechanism on the interaction model of yeast antagonist with fungal pathogen such as *Aureobasidium pullulans* with *P. expansum*, *B. cinerea*, *R. stolonifer*, or *Aspergillus niger* (Castoria et al., 2001; Janisiewicz, Tworkoski & Sharer, 2000), *Rhodotorula glutinis* and *C. laurentii* with *B. cinerea* and *P. expansum* (Droby et al., 1989), *C. laurentii* and *Sporobomyces roseus* with *B. cinerea* (Filonow, 1998). *P. guilliermondii* with *P. italicum* (Arras et al., 1998), *C. oliophila* with *P. digitatum* (Brown et al., 2000), and *Metschnikowia pulcherrima* with *B. cinerea* (Piano et al., 1997). These investigations demonstrated that the competition for nutrients and space could be relevant in part of the inhibitory mechanism. For example, the study of Filonow (1998) revealed that the yeast antagonist *C. laurentii* and *Sporobolomyces roseus* showed stronger sugar consumption than the mold *B. cinerea*. The conidia germination of *B. cinerea* was suppressed when these two yeast biocontrol agents were co-cultured with *B. cinerea* conidia in diluted solutions of fructose, glucose or glucose or in diluted apple juice. Filonow also found that there was no difference in sugar consumption between yeast with and without biocontrol activity indicating that the additional factors are one part of inhibitory mechanism. In addition, the rapid colonization and reproduction of yeast could help them to outcompete fungal pathogens by exhausting the limited available substrate. So that, none are available for pathogen to grow. Like during *in vitro* co-cultivation of cells of

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M. pulcherrima and *B. cinerea* on a synthetic medium, the spore germination of *B. cinerea* was inhibited completely as shown in the study of Spadaro et al. (2002). However, competition for nutrients and space is difficult to prove directly in the experiment due to a lack of appropriate methods. Therefore, this mechanism is likely required to have further investigation.

2.2.2 Killer Toxin Effect

In 1963, it was first reported about killer phenomenon generated by yeast by Makower and Beavan (1963). They observed that the brewing yeast strains of *Saccharomyces cerevisiae* produced toxins that killed susceptible strains of the same species. Since the study of Makower and Beavan was reported, the killer toxin phenomenon then has been shown to occur in a large number of yeast isolate in environment such as *Candida*, *Cryptococcus*, *Pichia*, *Debebayomyces*, *Kluveromyces*, etc. (Marquina, Santos, & Peinado, 2002). Many killer toxins can affect to other yeast strains belonging to the same or related species, bacteria and even those of filamentous fungi (Izgu & Altinbay, 1997; Santos & Marquina, 2004). Since the discovery in the killer phenomenon, killer yeasts and their toxins have been extensive studied in several other genera and species. The importance of this discovered knowledge is contributing to several applications in environmental, medical and industrial biotechnology. In addition, there are some reports of inhibitory effects of killer yeasts against some plant pathogenic fungi. For example, based on the results of competition plate biossays, Walker McLeod & Hodgson (1995) have shown that certain killer yeasts such as *S. cerevisiae* 28 and *Pichia anomala* NCYC 435 produced inhibitory effect against a range of plant pathogenic fungi such as *Rhizoctonia solani*, *Fusarium equiseti*, *Botrytis fabae*, *Phytophthora infestans*, and *B. cinerea*. The investigation of Santos & Marquina (2004) in small scale trial revealed that the postharvest application of *Pichia membranifaciens* CYC 1106 inhibited *B. cinerea* CYC20010. The finding was shown that purified killer toxin generated from *P. membranifaciens* inhibited *B. cinerea* indicating that this certain yeast, or its toxins have the potential to control *B. cinerea*. Thus, the yeast killer toxins might have the potential usefulness as the biocontrol agent against plant fungi of environment. Most of killer proteins are stable and act actively within a very narrow temperature and pH range. However, it was

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suggested in some reports that toxins from all killer strains are sensitive to protease and heat-labile macromolecule (Marquina, Santos, & Peinado 2002; Suzuki & Nikkini, 1999).

2.2.3 Antibiosis

It is commonly recognized that antibiotic production of bacterial biocontrol agent plays a significant role in suppression target microorganisms (Raaijmakers & Vlami, 2002). To date, several reports have indicated that some yeast isolates on fruits and leaf surface produced antibiotic substances in the interaction of fungal pathogens (Avis & Bélanger, 2002; Avis, Boulanger, & Bélanger, 2000; Benyagoub, Bel Rhlid, & Bélanger, 1996; Choudhury, Traquair, & Jarvis, 1994; Hajlaoui et al., 1994; Spadaro et al., 2002; Urquhart & Punja; 2002). For instance, Choudhury, Traquair, & Jarvis (1994) confirm the phenomenon of antibiosis by using liquid culture filtrate of yeast-like fungus *Sporothrix flocculosa* and *Sporothrix regulosa* with bioassay against plant pathogenic fungi and bacteria. Choudhury et al. isolated two unusual extracellular fatty acids (4-Methyl-7,11-heptadecadienal and 4-methyl-7,11-heptadecadienoic acid) with antimycotic and antibacterial activity. As reported by Hajlaoui et al. (1994), treatment of *B. cinerea* and *Fusarium oxysporum* with antibiotic produced by *S. flocculosa* resulted in reduction of spore germination and mass production. Urquhart and Punja (2002) also reported that they found a fatty acid ester from the yeast-like fungus *Tilletiopsis pallescens*. The active fraction of this fatty acid inhibited germ tube development of a powdery mildew fungus *Podosphaera xanthii* and fungal spores appeared damage by plasmolysis. Collapse of hyphae and conidiospores was exhibited on the leaves treated with the active fraction of antifungal compound. Purified antifungal compound at high concentration (200 µg ml^{-1}) was also active against other fungal plant pathogens such as *F. oxysporum*, *Pythium aphanidermatum* and *Phoma* sp. However, according to the current debate about the antibiotic resistance of human pathogens, the mode of action of suitable antagonist should not rely on the production of unsafe compounds like antibiotics (Wisniewski et al., 2007). Therefore, it is under decision whether an antibiotic-producing biocontrol agent is suitable and should be registered for use on food or feed (Druvefors, 2004).

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2.2.4 Hydrolytic Enzyme Production and Parasitism

Fungal cell wall degrading enzymes are associated with degradation of hyphae of many pathogens (Elad, 1996). Expression and secretion of these enzymes by different microorganisms result in significant suppression of plant fungal pathogens. Currently, it is well documented that involvement of fungal cell wall degrading enzymes is suggested to play a role in yeast biocontrol mechanism (Bar-Shimon et al., 2004). Numerous antagonist yeasts have been shown to produce β-1-3-glucanase and chitinase (CHI) against several plant fungal pathogens (Bar-shimon et al., 2004; Castoria et al., 2001; Chan & Tian, 2005; Ghaout, Wilson, & Wisniewski, 1998; Jijakli & Lepoivre, 1998; Masih & Paul, 2002; Urquhart & Punja, 2002; Wisniewski et al., 1991). In relation to the lysis of plant fungal cell wall, β-1-3-glucanase produced by *P. anomala* strain K has been studied by Jijakli and Lepoivre (1998). They have also shown in the *in vitro* study that the activity of exo-β-1-3-glucanase produced by *P. anomala* was enhanced when cell wall preparation of *B. cinerea* was supplied as the carbon substrate. The β-1-3-glucanase was shown to suppress germ tube development of *B. cinerea* and induced leakage of cytoplasm and cell swelling. Masih and Paul (2002) also demonstrated that the secretion of β-1-3-glucanase produced by yeast *P. membranifaciens* resulted in destruction of *B. cinerea* hyphae. Additionally, CHI production by antagonistic yeasts has been also reported in medium co-culturing yeast antagonist and fungal pathogen such as *A. pullulans* and *P. expansum* (Castoria et al., 2001), *T. albescens* and *T. pallescens* and *P. xanthii* (Urquhart & Punja, 2002), and *Candida saitoana* and *B. cinerea* (Ghaout, Wilson, & Winiewski, 1998)

Direct attachment of living yeast cells to spores and hyphae of fungal pathogens have been studied by several investigators indicating possible role in biological activity of antagonist yeast to control fungal pathogens (Arras et al., 1998; Castoria et al., 1997; Chan & Tian, 2005; Cook et al., 1997; Wisniewski et al., 1991). For example, Arras et al. (1998) reported the strong attachment of *P. guilliermondii* cells to the *P. italicum* mycelium and subsequent degradation of the hyphae wall in close contact to the attached yeast cells. Ghaout, Wilson, & Winiewski, (1998) investigated severe cytological injury such as cell wall swelling and protoplasm degeneration on the *B. cinerea* hyphae in proximity of the attached *C. saitoana*. In

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1991, Wisniewski et al. (1991) showed that *P. guilliermondii* attached intensively to *B. cinerea* hyphae. After 24 hours of co-culturing *P. guilliermondii* and *B. cinerea*, pitting and collapse of fungal hyphae were also observed. In addition, Wisniewski et al. found that the yeast *P. guilliermondii* secreted high level of exo-β-1-3-glucanase and CHI when cultured in various carbon sources and on the cell wall of fruit rotting fungi. Similary, the study of Chan and Tian (2005) indicated that *P. membranefaciens* and *C. albidus* had capability of attaching to the test fungal hyphae (*Monilinia fructicola, P. expansum* and *R. stolonifer*). It was found high level exhibition of β-1,3-glucanase and exo-CHI in culture extract of such yeast antagonists supplied with cell wall preparation of pathogens as the carbon source. Therefore, the ability of the antagonist yeast to produce the cell wall degrading enzyme is possibly hypothesized to be associated with the firm attachment of the antagonist yeasts to the fungal pathogens and the partial degradation of fungal mycelia (Chan & Tian, 2005; Wisniewski et al., 1991).

2.2.5 Induction of Host Resistance

In this regard, induction of host resistance by yeast is well-documented to be another mechanism of action in biocontrol activity of a variety of antagonistic yeasts. By using yeast antagonist as a microbial elicitor to induce plant resistance, a numerous study has also shown a possible of biocontrol action in which yeasts can elicit several physical and biochemical defense responses in a variety of postharvest commodities. Droby et al., (2002) reported that *Candida oleophila* Montrocher strain 182 induced disease resistance by causing increases in phytoalexin production (scoparone, scopoletin, and umbelliferone), ethylene biosynthesis, phenylalanine ammonia-lyase (PAL) activity, and the accumulation of chitinase (CHI) and β-1,3-glucanase enzyme in grapefruit. When apples were treated with *C. saitoana* strain 240, specific structural defenses were triggered, such as the formation of papillae and protuberances along the cell walls of wounded apple tissue; this activity coincided with the production of CHI and β-1,3-glucanase (Ghaouth, Wilson, & Winiewski, 1998, 2003). In addition, when *C. laurentii* (Kufferath) Skinner was applied to jujube fruit, β-1,3-glucanase activity increased. The expression of the *Glu-1* gene in fruit tissue was highly induced, and the product of this gene may play a role in the defense

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response against infection by *Alternaria alternate* (Fr.) Keissler and *P. expansum* Link (Tian et al., 2006, 2007). Tomato fruits inoculated with *P. guilliermondii* strain CNM 2.1801 expressed defensive biochemical changes through a number of enzymes including peroxidase, polyphenoloxidase, superoxide dismutase, catalase, PAL, CHI, and β-1,3-glucanase (Zhao et al., 2008). Similarly, proteome analysis of peach fruit and sweet cherry fruit showed that *P. membranefaciens* Hansen enhanced resistance against *P. expansum* Link by inducing the production of a number of antioxidant enzymes and pathogenesis-related (PR) proteins (Chan et al., 2007; Chan & Tian, 2006). In all these cases, pathogen and disease suppression were related to the induction of resistance in the harvested crops.

2.3 Induced Disease Resistance in Plants

2.3.1 Plant Resistance

Generally, plant systems have own defense mechanisms against parasitic organisms. In nature, resistance mechanisms of plant are diversity. At any stages of infection, plants use combination of their pre-existing (passive) structural and chemical defense, and induced structural biochemical defense inhibit pathogens (Sharma, 2006). Several compounds synthesized for pre-existing and induced defense response are listed in Table 2.1. Some compounds have their ability to against directly to invasive pathogens, whereas others form barrier and restrict the development of pathogens (Kuc', 2001). During the invasion by parasitic organisms, plant tissues often react by inducing structural and biochemical defense system that help to suppress the spread of pathogens in host tissue (Ozeretskovskaya, 1995). This induced resistance (IR) has long-lasting resistance to a broad spectrum of pathogen and IR can be local and/or systemic in nature, depending on the type, source and amount of elicitor (Pal & Gardener, 2006).

The process by which the plants become resistant to the invading organisms is active. As described by Benhamou (1996), plant cells can perceive and respond to a stress signal due to the pathogen attack as the sequence of molecular events leading to the establishment of the multi-component plant defense mechanisms (see Figure 2.1). Site restricted defense responses involve various reactions such as the rapid death of cells in hypersensitive response, the deposition of lignin, callose and

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hydroxyproline-rich glycoproteins in cell wall and the accumulation of phytoalexins. Whereas, the systemic defense responses include the production and accumulation of antifungal PR-proteins such as PAL, CHI, chitosanase, β-1,3-glucanase and peroxidase (Fajardo et al., 1998; Ghaout, Wilson, & Winiewski, 2003).

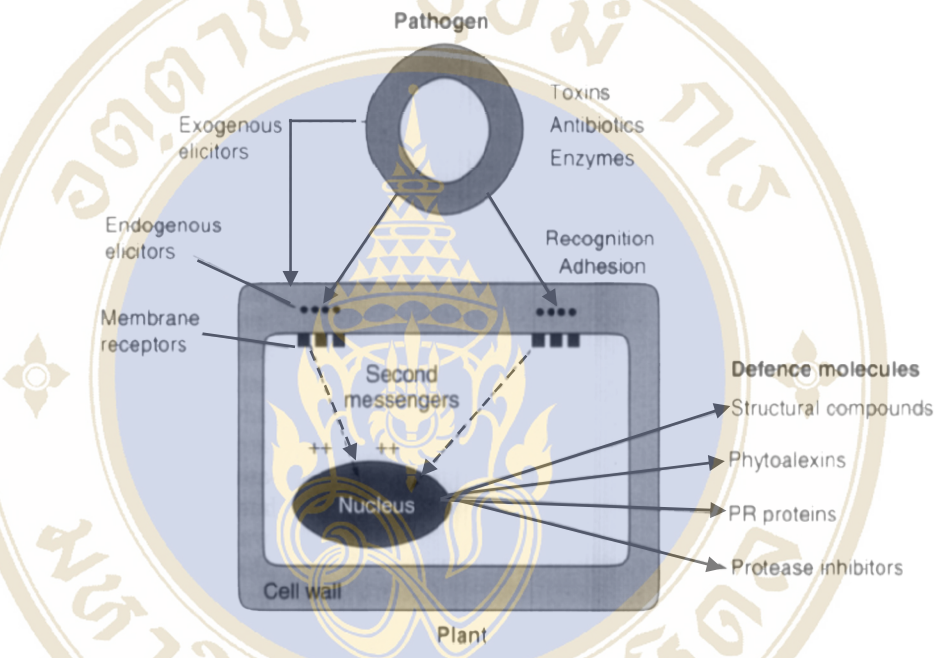

Figure 2.1 Signals and responses in plant-pathogen interactions. Upon recognition between both partners, the pathogen produces an array of metabolites that contributes to the release of signaling pectic polymers (endogenous elicitors). Binding of these elicitors to specific membrane receptors causes membrane depolarization, leading to the activation of second messengers that transducer the signal to the nucleus. Defense genes, encoding structural compounds, enzyme of secondary metabolism, pathogenesis-related proteins and proteinase inhibitors, are then triggered. Among the PR proteins, chitinase and β-1-3-glucanase may cause fungal cell wall hydrolysis, leading to the release of chitin and β-1-3-glucan oligomers (the exogenous elicitors) that bind to specific membrane receptors and trigger a cascade of events similar to that induced by the endogenous elicitors (data from Benhamou, 1996).

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Table 2.1 Putative defense compounds/systems for disease resistance in plants (data from Kuc', 2001)

Passive and/or wound response:
Waxes, cutin, phenolic glycosides, phenols, quinones, steroid, glycoalkaloids, suberin, terpenoids and proteins (thionins)
Increases: after infection:
Phytoalexins, reactive oxygen species/free radicals, calcium, silicone/silicates, polyphenoloxidases, phenolic cross-linked cell wall polymers, hydroxyl proline and glycine-rich glycoproteins, thionins, antimicrobial proteins and peptides, chitinases, β-1-3-glucanases, ribonucleases, proteases, callose, lignin, lipoxygenases and phospholipases

IR can be divided into two categories: systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Walter et al., 2005). SAR is caused by necrotizing pathogen infection, and this kind of resistance can be developed locally and systemically in response to pathogen infection (Oanh et al., 2006). After the formation of necrotic lesion which can be the result of the hypersensitive response or a symptom of disease, the SAR pathway is then activated (Ryals et al., 1996; Sticher, Mauch-Mani, & Metraux, 1997). SAR expression results in development of a broad spectrum resistance against a variety of microorganisms including virus, bacteria and fungi (Guzzo, 2003). The activation of SAR is mediated by a salicylic acid-dependent process (Hammerschmidt, 1999) and is associated with various cellular defense responses. These defense responses include the production of pathogenesis-related (PR) protein and phytoalexins, accumulation of reactive oxygen species, rapid alterations in cell walls and enhance activity of various defense-related enzymes (Ryals et al., 1996; Conrath et al., 2001). In contrast, the activation of ISR expresses as a result of plant-growth-promoting rhizobateria colonization in plant roots. ISR is mediated by a jasmonate- or ethylene-signaling pathway and it can develop without the production of PR-protein in the plant tissue (Walters et al., 2005).

2.3.2 Elicitors of Plant Resistance Response

Currently, it is now widely reported that treatment of plants with various elicitors (e.g. microorganisms, plant extracts and synthetic chemicals) can lead to the

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induction of plant resistance to suppress subsequent pathogen attack both locally and systemically (Walter et al., 2005). Elicitors of defense responses can be divided into two classes; abiotic and biotic elicitors. Abiotic elicitors have been described from abiotic origin such as UV radiation, heavy metal ions, inhibitors of some metabolic step and so on. Meanwhile, many biotic elicitors are of a variety of living materials such as microorganisms (nonpathogenic and pathogenic strains), and microbial products (oligosaccharide, glycoproteins and peptides, and phospholipid from fungi). Many reported have been suggested that some smaller molecules such as arachidonic acid, jasmonic acid and salicylic acid help mediating the induction of plant defense reaction (Benhamou, 1996; Dixon, Harrison, & Lamb, 1994; Ozeretskovskaya & Vasyukova, 2002).

In harvested fruits and vegetables, both biotic and abiotic agents can elicit resistance responses. For example, treatment of peach fruit with nonionizing UV-C (from wavelength 190 to 280 nm) radiation caused a significant increase of CHI, β-1-3-glucanases, and PAL activities starting from 6 h after treatment and the enzyme activities reached highest level after treatment at 96 h. An increase in accumulation of CHI, β-1-3-glucanases, and PAL mRNA were also detected in UV-C treated fruit (Ghaouth, Wilson & Callahan, 2003a). As reported by Rodov et al. (1994) and Mercier et al. (1993), UV-C treatment had potential to enhance plant resistance by induction of various defensive compounds, phytoalexin, e.g. scoparone, scopoletin and 6-methoxymellein in citrus fruit and carrot, respectively. Application of DL-β-amino-n-butyric acid to wound sites on grapefruit peel surface caused the activation of various pathogen defense responses, including activation of CHI gene expression and protein accumulation started at 48 h, and an increase in PAL activity after 72 h of treatment (Porat et al., 2003). Cao, Jiang, & He (2005) found that when yali pear was treated by acibenzolar-S-methly, activity of main defense enzymes including peroxidase, PAL and CHI, and antioxidant enzymes including superoxide dismutase and catalase were enhanced. In various fruits such as strawberry, tomato and pepper, chitosan can elicited the resistance by induction of antifungal hydrolases such as CHI, β-1-3-glucanases, and chitosanases. The level of chitosanase production remained in high level for up to 14 days after chitosan treatment (Ghaouth & Arul, 1992). Studies in bell pepper by Ghaouth et al. (1994) illustrated the potential of chitosan in

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stimulating various structural defense barriers such as thickening of host cell wall, formation of papillae, and plugging of some intercellular spaces with fibrillar material. In 1998, Fajardo et al. (1998) reported the potential of margosan-O (an oil-based plant-derive product from neem seed) combined with the yeast *C. oleophila* in the reduction of fruit decay in orange caused by *P. digitatum*. This elicitor application followed by challenging with the green mold pathogen stimulated the production of CHI, β-1-3-glucanases and peroxidase. In addition, these enzymes appeared to be involved in the defense mechanism against the fungal pathogen. Various citrus species have also been investigated in the resistant responses after elicitation by fungal pathogens. Likewise, Kuniga and Matsumoto (2006) found that the inoculation of gray mold *B. cinerea* induced the accumulation of scoparone (6,7-dimethoxycoumarin) in all citrus used in their study and time-course of scoparone accumulation depended on each citrus species.

2.4 Chili and Chili Anthracnose

Chili is the fruit of the plant genus *Capsicum* and it belongs to the family *Solanaceae*. Globally, chili is considered as an important tropical and subtropical cash crop. The chili has a geographical origin in the American tropics and then spread to cultivate in other places worldwide (Anand et al., 2009). Both fresh chili and dried chili production covered a total area of 3,729,900 ha with a total of chili production of 20,000,000 t (Food and Agricultural Organization of the United Nations, FAO, 2003). Large areas are used for chili production included in China, India, Mexico, Morocco, Pakistan, Turkey, and Thailand (Than et al., 2008).

A variety of chili cultivars range from mild or sweet-fleshed varieties called ‘pepper’ to spicy or pungent-fleshed varieties called ‘chilie’ (Oanh, 2005). In the report of Than et al. (2008) revealed that in different part of the world, the genus *Capsicum* contains approximately 20-27 species, and five species of those *Capsicum*; *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* are recognized as the domesticated cultivar. Among these five *Capsicum* species, *C. annuum* is one of the most common cultivated type including bell, cherry, cayenne, ancho, yellow wax, serrano and jalapeno (Tong & Bosland, 1999; Bosland & Votava, 2003).

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In 2004, FAO (2004) reported about the significant increasing of the demand for chilies in the world. Presently, high demand of global market leads to high yield production with a good quality in size and color of chili commodities. Also, the chilies with the good quality must be free of diseases, fungal toxins and chemical contaminants (Klieber, 2000). However, the main troubles effect to the limitation of chili production is their susceptibility to diseases caused by fungi, bacteria, viruses and nematodes. These diseases lead to limit in the productions, and yield loses can be occurred during in the field, or even in transit and storage process (Hartman & Wang, 1992). To minimize loses due to the disease problem, a variety strategy has been used to control of pre- and postharvest infection of chili commodities such as planting disease resistant varieties, using pathogen-free seed and crop rotation, using sterilized equipment for transplant production and pathogen-free field areas, and treating with chemical or non-chemical agents (e.g. microbial antagonists, natural enemies, plant products).

2.4.1 Causal Organism

Among the chili diseases, anthracnose is one of the most damaging diseases of chili production found in the tropical and subtropical areas (Widjaja, 1991). The disease can occur on leaves, stems, and cause extensive pre- and postharvest decay to chili fruit. The anthracnose rot on chili fruit results in reducing marketable yields in chili production. The main causal agents caused anthracnose disease on a wide range of important tropical and sub-tropical crops is the fungus *Collectotrichum* species. Five species of the genus *Collectotrichum* including *C. acutatum* Simmonds, *C. coccodes* (Walk.) Hughes, *C. graminicola* (Ces.) Wils., *C. gloeosporioides* (Pent.) Penz. & Sacc., *C. capsici* (Sydow) Butl. & Bisby, have been reported to be anthracnose casual agents of chili in many countries (Kim, Park, & Lee, 1989). Additionally, two of those, *C. gloeosporioides* and *C. capsici*, have been frequently reported to be the major species caused the disease in North America and Asia (Hadden & Black, 1987).

In Asia, *C. capsici* generally caused chili anthracnose and is widespread among red chilies (*C. annuum* L.). It primarily infects the chili at green fruit stage but causes anthracnose disease at ripening stage (Kim, Park, & Lee, 1989; Manandhar,

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Hartman, & Wang, 1995). In 1913, *C. capsici* isolate from the chili (*Capsicum frutescens* L.) was first mentioned as the scientific name *Vermicularia capsici* by Sydow H. (Oanh, 2005). Colonies of *C. capsici* are dense, and white to green or gray. Acervuli produced by *C. capsici* are rounded or elongated with diameter approximately 350 μm. Setae are several septates, massive, scattered and dark brown. Conidiophores are septate, and in sometime it was found in branched form. Conidia appear salmon masses and they are nonseptate, hyaline, falcate with an acute apex and narrow truncate base in dimension of 16-30 x 2.4-4 μm (Roberts et al., 2004). The mycelium of *C. capsici* is septate, and inter- and intra cellular. Appresoria produced by *C. capsici* are abundant, clavate to ovate, and become complex and long irregular chain. The study of Pring et al. (1995) also reported that, in cowpea hypocotyls, *C. capsici*'s conidia germinated started within 16 h and then after 24-30 h of germination, appressorium was produced at the end of fungal germ tubes.

Typically, since the arrival of inoculum on the host surface, a series of infection process developed by *Colletotrichum* species starts. When conidia adhere to and germinate on fruit tissue, they then produce germ tubes with adhesive appressoria which grow down directly through cuticle to epidermis and penetrate to epidermal cells. After penetration, the fungal pathogens form an intramural network of hyphae beneath the cuticle and then spread rapidly throughout the tissue with both inter- and intracellular hyphae causing host cell dead and lesion development (Jeffries et al., 1990).

2.4.2 Symptoms and Disease Cycle

Colletotrichum species can affect in all parts of plant at any stages during plant growth. Anthracnose caused by *Colletotrichum* species can occur in field area and develops during postharvest stages. In chili fruit, incidence of anthracnose disease ranged from 5-75% (Roberts et al., 2004). Ripe rot and die-back of chili anthracnose are two common and serious symptoms resulting economic loses (Smith & Crasson, 1959). Anthracnose symptoms on fruit usually appear in ripen stage. When chili fruit is infected with *C. capsici*, the decayed fruit first shows water soaked and small black circular spot on the fruit surface that later become soft, slightly sunken and light tan (Roberts, Pernezny, & Kucharek, 2001). After that, the typical anthracnose symptoms

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develop gradually from the infected area and appear as expand necrosis. The multiple lesions may occur and can cover most of the fruit surface. Within a lesion, the concentric rings of the acervuli are normally found. Depending upon *Colletotrichum* species the lesions are brown and then black from the formation of satae and sclerotia (Roberts et al., 2004). In some cases, infected mature chilies loose their red color and turn to straw or pale white colored (Srinivas et al., 2006).

The primary source of inoculum comes from infested debris in soil, weed host or contaminated seeds. When acervuli of infected tissue produce new spores, secondary spread can take place by rain splashing and wind-blown rain to other sites. Generally, infection occurs during warm and wet weather. In rainy weather, the extensive dispersal of spores to other fruits usually results in severe lose. The temperature around 27^{o}C with high humidity (a mean of 80%) is optimum for disease development (Than et al., 2008). The infection can also occur in a range o temperature of 10-30^{o}C. Mature fruits are more likely to be infected by the fungus. Likewise, in chili fruits, the red chilies are infected more frequently than the green chilies. This might possibly due to the difference in physical and chemical properties of the cuticle and exocarp (Kim, Oh, & Yang, 1999). However, immature chilies are also susceptible to the infection and infected immature chilies may not express anthracnose symptoms until the fruits mature (Roberts et al., 2004).

2.4.3 Disease Management

The integrated management, not only one specific technique, is recognized as an effective program for control chili anthracnose (Agrios, 2005). Since the process of seed transplantation to after fruit harvest, several strategies have been currently used to manage the anthracnose disease. The primarily common practices are the use of pathogen-free chili seeds and transplants. In addition, in the field, it should be free of infected debris and have a good drainage (Than et al., 2008). In case of the disease was previously present, the chilies should be rotated away from other solanaceous plants for at least 2 years (Roberts, Pernezny, & Kucharek, 2001). Additionally, the use of resistant cultivars can be selected as an alternative practice to reduce yield losses from the damaging disease. Many studies from different countries and regions have been reported about some resistant cultivars to chili anthracnose (Hong &

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Hwang, 1998; Ullasa et al., 1981; Yoon & Park, 2001). Some resistant cultivars are commercially available such as Bengal Green, Lorai and Perennial (Mehrotra & Aggarwal, 2003). Applying biofungicides from the plant products also has been tested to control chili anthracnose fungus. For example, biofingicides like the crude extract from rhizome, leaves and creeping branches of sweet flag (*Acorus calamus* L.), palmorosa (*Cymbopogon martini*) oil, *Ocimum sanctum* leaf extract, and neem (*Azadirachia indica*) oil expressed their inhibitory effect against the fungus *Colletotrichum* spp. in chili fruit (Jeyalakshmi & Seetharaman, 1998; Korpraditskul et al., 1999).

For many years, the use of chemical fungicides is the most common and traditional practice to control chili anthracnose (Parry, 1990). Some recommended fungicides include Manganese ethylenebisdithiocarbamate (Maneb), strobilurin fungicide enebisdithiocarbamate (Quadris), trifloxystrobin (Flint) and pyraclostrobin (Cabrio) are applied for anthracnose management (Smith, 2000; Than et al., 2008). However, the use of chemicals for disease management causes the harmful effect to organisms and environment, and also arises fungicide resistance in plant species.

With the current concerns about the harmful effects of chemical pesticides, several studies have thus focused on the use of microbial antagonists as an alternative strategy to control chili anthracnose. For example, Boonratkwang et al. (2007) reported that *Trichoderma harzenianum* strain PM9 has effective inhibitory effect against *C. gloeosporioides* in chili. Oanh et al. (2006) also reported that foliar spraying with *T. harzenianum* strain PM9 reduced chili anthracnose in field experiments. In 2007, Intanoo and Chamswarng (2007) found that two bacterial antagonists, *Bacillus amyloliquefaciens* strain DGg13 and BB133, controlled chili anthracnose caused by *C. capsici* in Thailand. In India, treatment of chili seed with *T. harzenianum* and *Pseudomonas fluorecens* isolated from native soil reduced infection of the seed by *C. capsici* (Srinivas et al., 2006).

2.5 Antagonistic Yeast *Pichia guilliermondii*

P. guilliermondii is a yeast species belonging to genus *Pichia*. Its asexual and anamorphic form is known as *Candida guilliermondi*. *P. guilliermondii* has been investigated as a possible biological control agent in several postharvest commodities

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such as grapefruit, apple, soybean, and tomato against a variety of plant fungal pathogens such as *P. digitatum*, *B. cinerea*, *P. expansum*, *Aspergillus flavus*, and *R. nigrican*, respectively (Droby et al., 1989; Paster et al., 1993; Winiewski et al., 1991; Zhao et al., 2008). Several mechanisms of actions, not only one single mode, have been shown to involve in control of the plant pathogens. For instance, based on the study of Droby et al. (1989) in grapefruit, it was found that nutrient competition may play role in the mechanism of action in which *P. guilliermondii* strain US7 suppressed *P. digitatum*. In addition, Winiewski et al. (1991) indicated that *P. guilliermondii* strain US 7 had a tenacious attach to *B. cinerea* hyphae. They also observed pitting and collapse of the hyphae started within 24 h after yeast treatment together with high level of exo-β-1-3-glucanase produced by this antagonist yeast. Thus, the authors suggested that the strong attachment along with the secretion of cell wall hydrolysis enzyme might involve in biocontrol activity of the strain US7. Another possible mode of action of *P. guilliermondii*, induced resistance, was discussed in the study of Rodov et al. (1994) and Zhao et al. (2008) who investigated this kind of phenomenon in citrus fruit and tomato fruit, respectively. Rodov et al. found that *P. guilliermondii* elicited the production of phytoalexins scoparone and scopoletin in the citrus peel even the absence of plant fungal pathogen. Zhao et al. illustrated that inculation of *P. guilliermondii* prior to *R. nigricans* resulted in enhanced biocontrol activity. This yeast did not produce any of antifungal compounds, however, competition for nutrients and space on wound appeared to play a role in biocontrol activity. Their result indicated that the yeast-treated fruit exhibited changes in several defensive enzymes related to the onset of induced resistance such as peroxidase, superoxide dismutase, catalase, phenylalanine ammonia-lyase, chitinase and β-1,3-glucanase activity. Moreover, the investigation of Arras, Nicolussi, & Ligios (1999) also showed that *P. guilliermondii* did not secret any hazardous antibiotics. Therefore, treatment of fruits with this kind of organism is safe for human consumption.

Regarding to this study, *P. guilliermondii* R13 originally isolated from Thai rambutan (*Nephelium lappaceum* L.) was identified by using rDNA sequence comparison technique together with physiological and morphological characteristic identification. Colonies of *P. guilliermondii* R13 are tannish-white and butyrous. The yeast cells can be found in single, pair or short chain of ovoidal-shaped cell measuring

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1.5-4.8 x 2.0-15.0 µm. Spores of this yeast species are hat-shaped and located in asci. Each ascus contains 1-4 spores.

Biocontrol activity of *P. guilliermondii* R13 was tested against the anthracnose pathogen, *C. capsici*, in red chili fruit by Chanchaichaovivat, Panijpan, & Ruenwongsa (2007). The *in vivo* test revealed that *P. guilliermondii* R13 inhibited *C. capsici* growth with biocontrol efficacy of 93.3% relative to the controls and reduced disease incidence of fungal infection to as low as 6.5%. Additionally, the application of the antagonist *P. guilliermondii* R13 was more effective than chemical treatment with chlorinated water in preserving chili fruits (Chanchaichaovivat, Panijpan, & Ruenwongsa, 2007).

The mechanisms of action of *P. guilliermondii* R13 against *C. capsici* was undertaken both *in vitro* and *in vivo* test. Observation on co-culture of yeast and fungus under the light microscope illustrated that this yeast antagonist attached to the fungal spore and hyphae and the attachment associated with restricted proliferation of *C. capsici*. Studies of competition for nutrients in chili juice revealed the suppression of spore germination and germ tube length of *C. capsici* after the strain R13 treatment. It was found that the suppression was exhibited significantly by addition glucose, sucrose and some nitrate substrates showing that the strain R13 was competing with the *C. capsici* for these sources. In both solid and liquid media, *P. guilliermondii* R13 also produced hydrolytic enzymes including chitinase and β-1,3-glucanase. These enzyme activities were expressed in highest production when the *C. capsici* hyphal cell wall was used as the carbon source compared to laminarin or glucose. Based on these scientific evidences, Chanchaichaovivat, Panijpan, & Ruenwongsa (2008) concluded that strain R13 suppressed the chili pathogenic fungi by using several modes of actions including tight attachment to fungal pathogen, competition for nutrients and secretion of cell wall hydrolytic enzymes.

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Educational review

2.6 Plant Biology Education

> "Plants are the most important, least understood, and most taken-for-granted of all living things. Either directly or indirectly they feed every other form of life. They created and control our atmosphere and our weather. Moreover, they protect the land we live on, harness the power of the distant sun, and not only feed but also shelter, clothe and warm our teeming population" (Wilkins, 1988)

Plants surrounding us are a key member in our global environment. They are critically important for the survival of many organisms in environment particularly humankind. Based on several reasons that plants are critical to all living things, the need for understanding fundamental concept of plants is therefore very essential for all people in society as included in the science curriculum of various countries. For instance, according to the *National Science Education Standards* (National Research Council, 1996), NRC emphasizes several fundamental concepts of plant science which student grade K-12 should know and understand in its Life Science Content Standard. In Thailand**,** the need of Thai student grade 1-12 to understand the core concept of plant science has been also shown in the *National Science Curriculum Standard*, particularly, as stated in the science standard of sub-strand Living Things and Living Processes, and Life and the Environment (IPST, 2001).

Even though, plant science is emphasized to be one of basic science needed to study for students in all grade levels as well as animal science, it is unfortunate that most of students have less understanding and appreciation of plants than those of animals (Carter, 2004). Based on many reports, many students and other older people have mistaken perception and alternative understanding about plants (Reinsvold, 1999). Several studies mention that animal study frequently captures student attention rather than those of plant (Wandersee, 1986; Wandersee & Schussler, 1999; Hoekstra, 2000; Tunnicliffe, 2001). In addition, people also generally possess

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the plant blindness phenomenon which Wandersee and Schussler (1999) defined this term as (a) the inability to see or notice the plants in one's environment; (b) the inability to recognize the importance of plants in the biosphere and in human affairs; (c) the inability to appreciate the aesthetic and unique biological features of the life forms that belong to the Plant Kingdom; and (d) the misguided anthropocentric ranking of plants as inferior to animals and thus, as unworthy of consideration. Considering to these conditions, people do therefore neglect plant leading to decline interest and appreciation in plant study (Wandersee & Schussler, 1999).

In biology teaching, the problem of neglecting plant is often mentioned by several authors (Hershey, 1996; Steward, 1967; Uno, 1994). Main purposed explanation regarding to neglect plant study includes the weakness of plant science curriculum and textbook and incompetence of teacher. For example, textbook and science curriculum have not placed much emphasis on plant science relative to animal science (Carter, 2004; Hershey, 1996). Plant concepts taught in the classroom are not relevant to students' live and connected to real world problem (Hershey, 1992). In addition, there are too few plant hands-on activities developed and presented in textbook. Also, most of plant activities used in biology classroom appears to be less attractive and exciting (Hershey, 1996; Uno, 1994). Another concerning issue of plant teaching is teacher's perception of plant which can impact to student's attitude and student achievement. Despite the main responsibility of plant science teachers is to convey and help students to develop botanical knowledge, teachers also perceive plants as the less interesting organisms than those of animals (Flannery, 1991). Unfortunately, it is not only students who usually possess that the material in botany is boring, but the teachers also express negative attitude in that plants are boring (Honey, 1987; Tamir, 1974). Additionally, the teachers also possess inadequate content knowledge of plant science and they are well trained in animal teaching rather than plant teaching (Honey, 1987).

Due to the concern on the imbalance of students' preference of plant study compared to animal study as well as the misunderstanding in plant uniqueness and characteristic, several authors have been launched the ways to help alleviating this plant paucity problem. For example, Hershey (1992) suggested the development of the new curriculum that promotes the use of plants in biology education. This new

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curriculum should emphasize the plant biology topics and hands-on exercises that show how plant science is relevant and connects to students' daily experience. Furthermore, biology teachers and student teachers who become to teach plant science need to have well training to develop their correct understanding in plant science topic along with using an appropriate teaching technique and strategy. In biology classroom, teachers should use plants as model organisms to teach biological concepts and frequently implement classroom session with plant activities (Reinsvold, 1999). More importantly, teachers should avoid animal chauvinism which is a bias for animals and against plants as well as avoid using teaching materials contain misconception about plants (Hershey, 2005). Additionally, plant scientists should also be a part to help promoting plant education and increasing public awareness of plant science such as speaking to school science classes, having project with teachers and curriculum developer to improve plant teaching and learning, and writing articles for popular magazine which reach much larger audiences (Hershey, 1989; Uno, 1994).

Using plants as the learning model in hands-on experiment and demonstration offer several advantages for teaching purposes. Plants are low-cost, readily available, minimal space requirement, and easy and safe to handle. In addition, working with plants avoids ethical problems in using animals. Importantly, study of plants is essential to fundamental understanding of life and our relationship to environment (Hershey, 2005; Reinsvold, 1999). A longstanding problem of plant neglect in biology education needs to be considered and resolved by using suitable approaches (Hershey, 1996). As recommended earlier by several authors about the ways to alleviate plant neglect problem, in doing that students might understand the uniqueness and importance of plants and perhaps become interested and appreciated in plant study.

Regarding to this study, we focus on promoting students' understanding in a plant science concept as well as promoting students' awareness in plant study by developing a new learning unit of plant science. Therefore, this new instruction is deem necessary to frame by suitable learning and teaching theories.

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2.7 Learning Theories

Theory is a set of principles that help us understand about the theorist's basic beliefs in natural events and phenomena. Regarding to teaching and learning, theories of learning provide principles that help us to understand in learning phenomenon and how learning generates by a learner (Bybee, 2000, p.4). In addition, learning theories also serve as a theoretical framework used to guide an instructional development and implementation in classroom and to conduct an educational research.

'Learning' is quiet a common term used widely in education, but it seems always difficult to define the meaning and explain about its process. Several researchers have devised the definition of learning to describe what this term is. According to definition, for example, Tobin (1990) defined learning as an active, interpretive and iterative process. Illeris (2003) described more that, in psychology and education, learning is a very complex process as a result of the integration of three different dimensions; internal cognitive dimension of knowledge and skill, the internal emotional dimension of feeling and motivation, and social dimension of communication and cooperation. When all these three dimensions bring together, learning process will occur and in that it leads the learners constructs their knowledge.

2.7.1 Historic Learning Theory

Over the years, theory developers proposed their contributions of knowledge construction by being placed the ideas on a fundamental understanding and a scientific rationality from their own (Illeris, 2003). The ideas associated with learning have struggled with many philosophers and psychologist for long time ago (Bybee, 2000). Behaviorism and cognitivism are two main categories of learning theories frequently mentioned (Child, 2007). Most of early work on learning theory was contributed by behaviorist views which focus on the interaction of stimulus and a response that can lead to one's learning. A learner is assumed to be passive responding to environmental stimuli (Shuell, 1993). The most prominent view of behaviorism was developed by B.F. Skinner. In addition, view of behaviorism was also included the works of researchers such as Thorndike, Tolman, Guthrie, Hull and Pavlov. Behaviorism views only on the objectively observable aspects of learning and in viewing that it was claimed to be scientific. Gredler (2001) suggested that this theory

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based on three basic assumptions. First, observable reaction of behavior should be focused rather than internal mental events. All behavior can be explained without emphasis placed on mental states. Second, behavior is operated by external stimuli in environment. Third, measurable changes in behavior due to the experience lead to the learning development.

In short, the term 'learning' viewed by behavior theorists is taken to mean the acquisition of new behavior through conditioning based on a stimulus (Hung, 2001). Bybee (2000) cited that learning theory of behaviorism contributed many attractive features such as this view can be use to explain many phenomena associated with learning and it is described based on the empirical research studies. However, the views that a learner is a passive recipient knowledge from external resources as well as less focusing learner's consciousness make this behaviorist view insufficient to explain the process of learning development. Today, many educators consider not only just behavior, but they also focus on other phenomena such as knowing, understanding, and being creative (Bybee, 2000). Some researcher like Bolles (1975) also argued that most empirical research explained in behaviorism was conducted in animals. However, human is more complex organism than those of animals, conducting the research by using animals as the model is therefore more difficult to control variables and generalize to human.

2.7.2 Contemporary Learning Theory

Currently, contemporary learning theory termed 'cognitive' is widely documented. This type of theory provides the comprehensive explanation of how the learners develop their knowledge. Importantly, cognitive theory seeks to understand how incoming information is processed and constructed into knowledge (Coker & White, 1993). Cognitive theory stresses that learning requires the participation in doing activity of the learners and in that the activity of attaining understanding is building knowledge structures. John Dewey believed that teaching should be an active process and student learning takes place when students had actively engaged in meaningful problem solving (Llewellyn, 2002, p.42). Dewey focused on the importance of building students' knowledge from having their own direct experiences. He posted that when the learners had the meaningful experiences, these situations

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allowed them to construct their knowledge. In the late nineteenth and early twentieth century, the Gestalt psychologists viewed learning as an active organized processor of information. Based on this view, the whole ideas were put and fit together like pieces of a puzzle and that existing knowledge play an important role in learning (Bybee, 2000, p.8). Jean Piaget developed a contemporary view on the nature of cognitive development. Piaget's learning theory assumes that children construct cognitive representations from their active experiences. When children experiences new information and if that information fit to the children's prior knowledge, they will use the new information assimilate into existing knowledge structure. If the new knowledge do not fit, children will reorganize existing knowledge so then the new information accommodate and fit into the existing one (Llewellyn, 2002, p.43). These two processes, assimilation and accommodation, are related to the cognitive development followed Piaget's view. Lev Vygotsky emphasized that social relationships influence of cognitive development of the learner. In this view, knowledge is constructed through interactions with other people, such as peers, teachers and parents, particularly, with the one who has specific knowledge (Hakvoort, 2002).

To date, as Bybee (2000, p.9) mentioned, it is difficult to identify a current learning theory with only a single person. New science philosophy stresses on the idea that scientific knowledge is generated from the individual construction based on the main factors including prior knowledge, environment and society. Llewellyn (2002, p.43) cited that "the new era in educational psychology ushered in the cognitive and constructivists". Then, the contemporary learning theory used to ground the framework of this study will be described as follows:

2.7.2.1 Theory of constructivism

Today, constructivism is the most frequently used term in educational literature to describe how people learn and construct the knowledge. Major influence of contemporary science and mathematics education is the theoretical basis of constructivism (Matthews, 2000). However, Baviskar, Hartle, & Whitney (2009) mentioned that constructivism is frequently misunderstood by some educational researcher. As cited by the Institute of Learning Center Education (2009),

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constructivism is not a teaching strategy, but a theory concerning how students learn. The usual constructivism views that the learners actively build their own knowledge based on their current and prior knowledge or experience (Lesgold, 2004). In other words, constructing one's own knowledge on the basis of one's existing experiences leads to the one's learning development.

For the past 20 years, constructivist paradigm has been focusing in the area of science education. Constructivism is an important theory of learning that is used to guide the development of new teaching methods, particularly in science education (Baviskar, Hartle, & Whitney, 2009). To date, several of science education researches have been carried out from a constructivist perspective and researches suggested that this view of learning is a very powerful perspective used to frame the educational studies (Akkus et al., 2003). Basically, constructivism is categorized into two major traditions; cognitive constructivism and social constructivism. These two views see learning similarly as an active process rather than a passive process. However, they emphasize different paths of knowledge construction (Palmer, 2005).

The 'cognitive constructivism' was developed from the idea of Jean Piaget and it stresses on the importance of the cognitive process occurring within the individuals. Therefore, cognitive constructivism emphasizes the personal construction of knowledge (Piaget, 1978). This view purposed that individuals attempt to make sense of the world and their learning can be constructed based on physical, mental, or social experiences. In those of experiences, physical experiences refer to physical interaction with object in environment, mental experience include their thinking about what they have observed, and social experiences can be interactions with adults and peers. Based on these experiences, individuals interpret and relate them to the existing knowledge and finally develop their own personal understanding (Palmer, 2005). According to this view, teachers therefore have important role of providing suitable experiences that help facilitating students' learning. In addition, the interactions of peers and adults also play an integral source of experience that leads cognitive conflict in children and then when the conflict occurs in the learning process, normally, people tend to reduce this conflict and balance the cognitive system. Promoting cooperative learning thus seems to create a learning community for students and enhance the learning process (Liang & Gabel, 2005).

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On the other hand, ‘social constructivism’ was developed from the idea of Lev Vygotsky, and it emphasizes on the importance of key features; society, culture, and language, which effect on children’s cognitive development. Vygotsky’s theory is postulated to the zone of proximal development which referred to the distance between the actual developmental level that reflected in the children’s independent problem solving, and the level of potential development that reflected in the guidance by others. Importantly, the zone of proximal development is created on effective teaching in which interaction between students and others is promoted (Gabel & Liang, 2005). Vygotsky views learners as active persons who construct their own knowledge through problem solving under the guiding by adults or in collaborating with their peers as well as discovering things by their own. Based on this view, knowledge is socially constructed in particular social and cultural contexts (Vygotsky, 1978).

As mention above, in constructivist perspective, meaningful learning and understanding of the learners are constructed as a result of their internal world experiences interact with external world (Saunders, 1992). Constructivist view of learning suggests the teachers in providing more chances for the students to reconstruct their own knowledge through a process of social interaction and engaging in problem solving and critical thinking (Driver et al., 1994). Therefore, constructivist teachers need to understand that knowledge is not acquired passively and cannot be transferred to students only by giving lecture without participating in experiential activities (Yager, 1991). In this perspective, teachers have to be viewed as a facilitator to help students construct the knowledge not the persons who transfer knowledge (Duit & Tragust, 1995). The term ‘constructivist-inform teaching’ refers to the kind of teaching that framed by a constructivist epistemology (Palmer, 2005). Students should be provided meaningful learning through effective learning activities (Akkus et al., 2003). Teaching methodology should help students in actively construction their own knowledge by starting from what they already know. In doing that, students can reconstruct the new knowledge. In addition, teaching strategies should focus on providing students with direct experiences that can induce their cognitive conflict in learning (Driver et al., 1994). Appropriate series of teaching and learning phrases such as the sequence devised in a learning cycle purposed by Karpus (1977) including exploration, concept introduction and concept application should be implemented in

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the instructions. These sequences of consecutive phases are designed based on the way how people learn about the world (Lawson & Thompson, 1988). Furthermore, although the construction of knowledge is a personal process, it can be encouraged by interpersonal process by student-student interaction and student-teacher interaction (Banet & Ayuso, 2003). Therefore, providing opportunity for students to interact with others such as participation in group working is considered to be essential teaching strategy (Yager, 1991). More importantly, science instruction is more meaningful and appealing to students when classroom environment is framed with constructivist perspective (Taylor, Fraser, & Fisher, 1997).

Baviskar, Hartle, & Whitney (2009) stressed that implementing constructivism to ground a practical methodology for teaching is difficult and has created a misconception in developing constructivist-based instruction. They also suggested the four essential characteristics for lessons that claim to be constructivist: eliciting prior knowledge, creating cognitive dissonance, application of new knowledge with feedback, and reflection on learning. The first element, eliciting prior knowledge, is important because the core idea of constructivism stated that all knowledge is developed based on the existing knowledge and experiences of the learners. This step is placed to make sure that the activities assess students' prior knowledge and help relating it to the incoming information. Prior knowledge can be addressed by using diverse strategies, such as formal pre-tests, asking informal questions, formal interviews, or written documents such as concept mapping that require basic knowledge to be applied. The second element refers to creating cognitive dissonance in mind of students, it is very necessary in order to make the students aware of the difference between their prior knowledge and the new one acquired from the learning activities. The students may find a problem and think critically, if they are provided high problematical tasks. The third element is application of knowledge with feedback. In this application step, students are allowed to interpret and modify their prior knowledge and then apply their understanding to the new context. Based on this way, students can also compare their knowledge construction with their peers and other specific person, and apply them into the new situation. The last element is reflection on learning and in that the students will be aware of their learning after they has acquired and verified the knowledge. This kind of reflection focuses on providing

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the chance for students to express what they have learned by using several assessment techniques. Furthermore, another interesting point raised by Baviskar, Hartle, & Whitney (2009) is about the certain element such as group work in constructivist. Regarding to their view, even though group work may be necessary for the teaching and learning strategies, it may not be considered as an essential element of constructivist instruction. Baviskar, Hartle, & Whitney also mentioned that the cognitive constructivist theory does not state that learning occurs only in groups or even that the learning necessarily occurs best in groups. Consequently, group work may be or it may not be a constructivist educational tool, depending on the implementation of the lesson.

To conclude, constructivism is a driving learning theory in modern education today. It is likely to be more effective when classroom teaching practice is implemented by an understanding of how students learn. Therefore, it is important that the implication of constructivist learning theory is implemented in a classroom practice (Palmer, 2005). Additionally, research suggests that reform in science education has much needed to focus on constructivism implementation in teaching and learning. As suggested by Yager (1991), science education would be reformed and moved into next century with enthusiasm and promise, if the constructivist perspective is promoted effectively in teacher education.

2.8 Inquiry-Based Instruction

Since the late 1950s and early 1960s, inquiry was first proposed in science education (Chiappetta, 1997). Over the years, inquiry has been recommended as an essential component of ongoing science education reforms in many countries (American Association for the Advancement of Science [AAAS], 1993; National Research Council [NRC], 1996, 2000; Schwab, 1962). The main reason is that inquiry is assumed to be an effective approach to help students learn science concepts and develop their skills of doing science as well as develop an understanding of the nature of science (Olson & Loucks-Horsley, 2000; Sandoval, 2005).

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2.8.1 The definition of inquiry

The central meaning of the term inquiry can be described as scientific inquiry and inquiry-based teaching and learning. The term 'scientific inquiry' refers to the general process of investigation that scientists use to answer the question of natural world (DeBore, 2004; NRC, 1996). In that inquiry process, it has the characteristics of the way to acquire scientific knowledge including the convention and ethics involved in the development, acceptance, and utility of scientific knowledge (Schwartz, Lederman, & Crawford, 2004). Meanwhile, the term 'inquiry-based teaching and learning' is relied mainly on the pedagogical approach that leads the way for students to develop their understanding in the scientific concepts. This kind of teaching and learning emphasize in the process of questioning, investigation, and problem solving consistent to the process as the scientists do (Bybee, 2004; DeBore, 2004)

'Inquiry-based teaching' is well-known term used in science education for many years. However, many teachers seem not to be exactly understood what inquiry-based teaching means and what the procedures incorporated in this kind of teaching are. For many years, several science educators and researchers have provided the definition and procedures of the inquiry approach in teaching. One definition described in the science standard set by the National Research Council (1996, p.23) defined 'inquiry' as "the diverse ways in which scientists study the natural world and propose explanation based on the evidence derived from their work". In addition, inquiry is characterized in the standard as:

> a multifaceted activity that involves making observations; posing questions, examining books and other sources of information to see what is already know in light of experimental evidence; using tools to gather, analyze, and interpret data; proposing answers, explanations, and predictions; and communicating the results, inquiry requires identification of assumptions, use of critical and logical thinking, and consideration of alternative explanations.

The standard (NRC, 1996, p.23) also emphasized that students in all grade levels and in every domain of science should have the opportunity to use scientific

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inquiry and develop the ability to think and act in ways associate with inquiry. The *National Science Education Standards (NSES)* (NRC, 1996) have identified the essential elements of scientific inquiry as engaging with simple questions, planning and conducting investigations, using tools to gather, analyze and interpret data, giving priority to evidence in responding to formulate reasonable explanations that are communicated to others.

2.8.2 Scientific Inquiry-Based Teaching and Learning

Recently, a numerous research studies in science education have shown the potential of using inquiry-based approaches in teaching and learning to promote active learning for students than those of teaching in traditional approaches (Cunningham et al., 2006). In classroom, Bybee (2000) proposed three elements of inquiry teaching that teachers should focus on: (a) the use of inquiry to assist learners to grasp content, (b) the use of activities to enable learners to develop abilities for conducting inquiry, and (c) the use of activities that enable learners to understand how scientists conduct actual science inquiries. In doing so, developing students' ability to understand nature of learning based on inquiry activities hence needs direct experiences and continues practicing with the inquiry processes (Cohen & Manion, 1995). Therefore, teacher's roles in inquiry teaching are complex and need to be changed from the traditional way of teaching (Crawford, 2000).

In traditional teaching, teachers accustom to lecturing and giving assignment and homework. Teachers' role is to transmit the certain knowledge directly to students and the students are expected to receive and memorize information passively (Akkus, Gunel, & Brian, 2007). This kind of passive instruction, the students seem to be provided less participation in inquiry-based activities and encouragement to use critical thinking. They also rarely have direct experience with the activities presenting the nature of science (Wise, 1996). Commonly, teachers' role in constructivist and inquiry-oriented approaches is often mentioned as a facilitator and a guide. However, Crawford (2000) suggested a more extensive range of teachers' role and in that view teachers need to pay more active and complex participation than being as a facilitator or a guide. Based on inquiry instruction, it demands a high level of teacher involvement in teaching and learning process to engage students in the

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cognitive processes. Those of the cognitive processes involve asking question, making testable hypothesis, conducting investigation, collecting data, formulate explanation and constructing knowledge, and revising knowledge. Additionally, teachers also need to create inquiry-based learning environment and support inquiries while interacting with students (NRC, 1996, pp. 32-33). Inquiry-based teaching strategies need to be aligned with the theories of how students learn science consistent to the constructivist perspective. This view focuses on students actively reconstructing their conceptual understanding through building on students' experience along with teacher's facilitation (Driver et al., 1994; Zion et al., 2004). Based on this view, it means building of the knowledge needs to be relied on the co-construction between students and teacher in the learning process. For the effectiveness of using inquiry teaching in science classroom, the teachers thence have to understand "what inquiry in classroom means" (Bell et al., 2003)

The integration of socio-constructivist perspectives of learning with hands-on exercises enhances knowledge construction of inquiry for students as a result of their learning from firsthand experience (Rogoff, 1994). Many inquiry activities suggested by several researchers focused on using inquiry-based laboratories to enhance student learning, to promote knowledge construction and understand the nature of science as well as to raise students' inquiry learning (Bell et al., 2003; Campbel et al., 2000; Hofstein & Lunetta, 2004; Taitelbaum et al., 2007). In addition, the use of other strategies can also be designed as inquiry-based instruction such as discrepant event demonstrations, inductive and deductive activities, writing, and gathering information by reading (Akkus et al., 2007; Chiapetta, 1997; Osborne, 2002). For instance, Osborne (2002) suggested that active reading from various resources such as articles in books, scientific magazines, or internet could be designed as inquiry-based activities which promote students' learning. Akkus et al. (2007) implemented writing-to-learn tasks designed based on inquiry approach for students to be involved in building scientific arguments and knowledge construction, and in doing so, the students showed the effective use of their own daily language to make connection to scientific concepts which is more meaningful to them.

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2.8.3 Level of Inquiry Instruction

Basically, inquiry-based teaching implements three specific sequences of a learning cycle: an exploration phase, an invention phase, and an application phase. The stage of exploration refers to the first stage that students perform an unstructured investigation. The invention phase involves the stage that a concept is presented to students. Meanwhile, the application phase emphasizes the process that the same concept is applied to other situations (Lewis & Lewis, 2008). Schwab (1962) suggested that laboratories were essential to school science. In the science classroom, an important purpose of inquiry teaching was to help students learn how to ask questions of the materials they studied, and how to address those of answers. A scale of openness of inquiry has been purposed by Schwab (1962). Three scales of inquiry instruction are depended on the amount of student involvement to complete their task, degree of teacher involvement to facilitate the students as well as the scientific background of the students regarding the inquiry subject and working methods. The lowest level of inquiry is referred to a strict instruction given to the students and the teacher provides specific inquiry-oriented question and procedure to students. At the intermediate level, the students are given an inquiry question but they have to design their own method to address the answer. Finally, at the highest level of inquiry, the teachers only provide learning environment and the students need to generate their own inquiry question and find the way to solve the problem by themselves. After Schwab has proposed on the scale of inquiry, later, several researchers also has categorized and suggested similar core idea of the inquiry level which may range from open inquiry to a more guided approach (Hegarty-Hazel, 1986; NRC, 2000; Staer, Goodrum, & Hacking, 1998).

To date, the category of inquiry level stressed by the National Research Council (NRC) (2000) is recognized today as a basis for categorizing type of inquiry teaching. In open inquiry, this method serves the type of experimental work conducted by the scientists and the key of this inquiry type is students' participation in asking inquiry question generated by them. Then, the students make their own decision to find the solution with the assistance by the teachers. This open-inquiry teaching demands high level of thinking of the students and teacher involvement. As for structured inquiry, the teacher posts a problem as well as the specific procedures for

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the students to investigate and address the problem. The students discover relationship between variable or otherwise generated from data collection. This structured-inquiry are quiet similar to those called as cookbook activities but the instruction provides less direction than those of cookbooks (Colburn, 2000). However, many researchers suggest that the students could not well develop scientific and critical thinking, and appropriate attitude in learning when providing this type of structured inquiry in the classroom (Berg et al., 2003; Kaberman & Dori, 2008).

The intermediate stage between open-inquiry and structured-inquiry is suggested as a guided-inquiry. In guided-inquiry, the teachers post the questions and provide materials for the students to address the problem. Later, students devise their own path and solution and come up with the conclusion by themselves (Colburn, 2000; NRC, 2000). In short, Sandoval (2005) defined the term 'guided inquiry' as 'the instructional method that supports students' inquiry by guiding one or more steps to complete the task'. Guided-inquiry teaching promotes a desired goal of learning instruction. Moreover, this kind of inquiry benefits in reduction of a "waste of time" during implementing the new instruction. It also helps reducing frustration of students due to their fear of the unknown and achieving undesirable results (Trautmann, MaKinster, & Avery, 2004). Furthermore, Germann, Haskins, & Auls (1996) indicated that the guided-inquiry can help students make a transition from learning through a structured inquiry stage to an open inquiry level. In science education, guided inquiry approaches have been used in a numerous studies for helping students develop various scientific concepts and these approaches have been proved to be a potential tool for knowledge construction (Jackson et al., 1994; Linn & Songer, 1993; Penner, Lehrer, & Schauble, 1998; Reiser et al., 2001; White, 1993; White & Frederikson, 1998).

In summary, inquiry-based teaching and learning is recognized as an effective teaching and learning method which promotes student-centered learning and provides many educational benefits in classroom (DeBore, 2004). The main purpose of inquiry is to guide students to construct their own knowledge by direction investigation through the scientific process. Also, inquiry motivates and challenges students to solve an authentic problem in that this way may help students understand themselves and the natural world. For these reasons, developing curricula that employs inquiry learning and shift the focus of science classroom into environment that

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engaged in inquiry is therefore considered as an important challenge for educational reform today (Chiapetta, 1997; Sadeh & Zion, 2009).

During the instructional program provided for students, one of the main concerns is the learning progress of students. Recently, assessment of the learning outcome by multiple assessment instruments becomes a critical mean to monitor student progress during instruction. In addition, assessment can also aid learning in a number of other ways. Next section will therefore describe about classroom assessment related to the inquiry-based instruction.

2.9 Classroom Assessment

As defined by NRC (1996, p.76), assessment is “a systematic, multistep process involving the collection and interpretation of educational data”. The purpose of assessment is not just measure what students have learned, but, more importantly, it is an effective tool to facilitate student learning. In that latter sense of assessment, it facilitates student learning involves improving regular question, providing quality feedback, and encouraging student self-assessment (Coffey, 2003). Furthermore, teachers also can use assessment to inform the effectiveness and then improve their teaching. Enger and Yager (2001) have been suggested an assessment framework for science learning and experiences to promote science literacy which can be divided into six domains: concepts, processes, application, attitudes, creativity, and nature of science. Concept domain includes facts, laws or principles, theories, and the internalized knowledge held by students. Science concept is considered as a central in science instruction (Thagard, 1992). Therefore, students’ understanding in these concepts is considered to be critical to get success in learning. Process domain involves the 13 processes skills used in science as mentioned by the AAAS (1968). The abilities of students to do a set of process are the goal for instruction and assessment. These processes and skills are essential for knowing, doing, and thinking in science. Application domain refers to the elaboration of the concept and processes what students have learned to address a new situation. This domain is important because if students can make application to new situation, it means they have their understanding in the meaning of information and processes. Attitude domain is concerned with the feeling, emotion and opinion of students in regard to their learning.

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In science education, the term ‘attitude’ is generally categorized into (a) attitude toward science (e.g. interest in science, attitude toward scientist) (b) scientific attitude (e.g. open-mindedness, honesty). The positive attitude or feeling may drive students’ attempt to solve problems resulting in the self-directed growth in learning. Torrance (1969) described term ‘creativity’ as ‘the process of becoming sensitive to problems, deficiencies, gaps in knowledge, missing elements, and disharmonies. Creativity is a complex construct and plays an essential role in many processes of science and doing science. To promote creativity domain, the classroom should become more student centered, and the creativity will then be encouraged the richness of experience in performing activities for students. The last domain is nature of science domain which the importance is related to how scientists think and perform their works in the scientific community. When nature of science is promoted for students, they will get a deeper understanding of the meaning of science in the real world.

In the teaching context, assessment is a process of data collection of student outcome. The way to collect the data can range from informal to formal assessment in either formative or summative ways or in the combination of both ways (Enger & Yager, 2001). Assessment is usually thought as an event or strategy that comes after teaching and something done for students, not with students (Coffey, 2003). In traditional way, assessments usually stress in the type of the paper-and-pencil test such as true-false, fill in the blank, multiple choices, and short answer type test. While science instruction today that becomes more student centered and constructivist-oriented classroom need to use a variety of assessment strategies to capture student performance and more parallel to their learning experiences (Enger & Yager, 2001). The new views of assessment stress on measurement of students’ opportunity to learn through the assessment and also shift toward authentic assessment that requires students to apply their knowledge to real world situation (NRC, 1996). The term ‘continuous assessment’ or ‘formative assessment’ has been launched by Carlson, Humphrey, and Reinhardt (2003) to describe type of assessment that use in the real time along student learning not just the end of unit. They suggested that continuous assessment is crucial to student learning in inquiry-based classroom because it is involved daily observations and documentation of students’ work during student inquiry investigation and discussion. This kind of assessment is also rooted in

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the natural setting of the classroom. When the teachers keep tracking this information and then analyze the data, they can understand students' thinking and monitor their growth in the concepts, processes, and dispositions of science. Enger and Yager (2001) have also suggested various strategies of alternative assessments to provide diverse ways for students to express their cognitive development and academic progress. Those assessment strategies include teacher observations of student performances, student interview, student self-assessments, presentations, projects, concept maps, portfolios, and others techniques. Furthermore, the assessment tasks for student should be engaged to students' real lives or are similar to the activities that scientists do their works (NRC, 1996).

Wilson and Scalise (2003) devised four principle of assessment that would be useful in classroom: a developmental perspective, a quality of evidence, a match between instruction and evidence, and a management by teacher. A developmental perspective of student learning refers to making assessment over the learning time to observe the development of student understanding of the concepts and skills. The second principle is establishing the technical quality of classroom assessment which should have comparability or validity of the test, be maintained standards of fairness such as consistency and absence of bias, and be developed on the basis of educational standard. In the third principle, a match between instruction and evidence, it is focused on generating a good match between what is taught and what is assessed. In that, instruction and assessment need to be placed in alignment. The forth principle refers to classroom based assessment which must be handle under teachers' management and this principle is likely most critical. In the assessment process, teachers play an active role in collecting data and interpreting the data to explain results effectively and appropriately. Students must be also involved in the assessment process by providing opportunities for them to reflect their opinion on what they are learning, and to discuss and express their understanding of what establishes quality work. Coffey (2003) indicated that when students play their role in assessment process, they seem acquire the tool that they need to take the responsibility in their learning.

In summary, assessment is a process acting to improve student learning. Both teacher and student can use information from assessment to bridge the gab between the current stage of teaching and learning and where the qualified stage

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should be (Atkin & Coffey, 2003). Currently, many educators emphasize on making changes in classroom assessment from the traditional way to the authentic assessment. Besides conventional paper and pencil test, a variety of alternative assessment strategies can be used for meaningful assessment. These techniques of assessment should base on authentic student's work and occurred within the interaction between student and teacher (NRC, 1996). In doing so, assessment can be viewed as a useful pathway to improve teaching and learning quality in classroom, not just the process to measure what student have learned after the unit implementation.

2.10 Summary

The paucity of plant studies in biology education have often been mentioned for long time ago. Generally, many students have less interest and understanding in studying with plants. In response to alleviate these problems, the researchers need to provide much plant coverage in biology education in all educational levels. One promising strategy is to deal with developing a plant biology course that emphasizes a new plant biology topic offering the understanding of plants in the new perspective relevant to student's experience.

This study is an attempt to alleviate the plant paucity problem. The study aimed to develop a novel learning unit to promote a new students' conceptual understanding of plant defense mechanisms as well as promoting students' awareness in plant study. According to a review of scientific educational research, a constructivist perspective in both individual and social view is one of the foremost perspectives in teaching and learning of science. An inquiry-based approach is one of the effective teaching and learning models that fits with the view of constructivist. Furthermore, this approach has been proved very effective to promote conceptual understanding in the science concepts. Therefore, the inquiry-based approach grounded with the constructivist perspective has been selected to frame this present study and in that it may give the meaningful learning and understanding of the plant science concept offered from this study.

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CHAPTER III
METHODOLOGY

Overview

This chapter presents research methodology of two parts of the research study, scientific part and educational part. The former part describes the materials and methods used to investigate the scientific study. The latter part deals with the application of the scientific study to develop hands-on experiment and the learning unit to promote student's conceptual understanding on plant defense responses. In addition, this part includes the theoretical basis used to frame this study, research methodology, participants, ethical concerns, and procedure for data collection and analysis.

Scientific part

This part comprises of materials and methods used for scientific study on the induction of disease resistance by yeast antagonist, *P. guilliermondii* strain R13, against anthracnose fungus, *C. capsici*, in chili. Various scientific methods used to investigate the evidences for induced resistance by yeast treatment are also included.

3.1 Materials

3.1.1 Chemicals

Chemicals used in this scientific study are listed in Table 3.1.

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Table 3.1 Lists of chemicals

Chemicals	Manufacturers
1. Culture media - Agar, beef extract, glucose, potato dextrose agar, yeast extract	- Difco Laboratories, USA
2. Enzymatic substrates - Colloidal chitin azure (CM-Chitin-RBV) - Larminarin, L-phenylalanine	- Loewe Biochemica, Germany - Sigma-Aldrich, USA
3. Chemical standards - Bovine serum albumin, chitinase, glucose, *trans*-cinnamic acid	- Sigma-Aldrich, USA
4. Buffer solutions - Boric acid, ethylene diaminetetraacetic acid, β-mercaptoethanol , polyvinyl polypyrrolidine, sodium acetate trihydrate, sodium tetra borate, phosphoric acid	- Merck & Co., Germany
5. Solvents - Acetone, chloroform, cyclohexane, methanol	- Merck & Co., Germany
6. Miscellaneous compounds - Dinitrosalicyclic acid, ethanol, hydrochloric acid, osmium tetroxide, potassium sodium tartrate.$6H_2O$, sodium chloride, sodium hydroxide, sulfuric acid, - Tween-80, vanillin	- Merck & Co., Germany - Sigma-Aldrich, USA

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3.1.2 Plant Material

Mature red chili fruits (*Capsicum annuum* L. var *acuminatum* Fingerh.) without physical injuries or infections were hand-harvested from an untreated field in Supanburi Province, Thailand, and then transported immediately to the laboratory. All selected fruits were uniformly sorted for size **(**1.8-2.0 × 14-15 cm**)** and color. Before use, the fruits were cleaned with 0.5% NaCl for 5 min and then washed in sterile-distilled water. After air drying, chili fruits were surface-sterilized by wiping them with 70% ethanol solution.

3.1.3 Yeast and Pathogen

The yeast *P. guilliermondii* strain R13 previously isolated from rambutan fruit (*Nephelium lappaceum* L.) in Thailand was obtained from the Institute for Innovative Learning, Mahidol University, Thailand. A pure culture was maintained at 4^{o}C on the slant medium of nutrient yeast dextrose agar (NYDA) (NYDA, 1 L distilled water containing 8 g beef extract, 5 g yeast extract, 10 g glucose, and 20 g agar). The active yeast cells were prepared by growing in 250-ml flask containing 50 ml of nutrient yeast dextrose broth (NYDB) (NYDB, NYDA without agar) with a loop of yeast inoculum. The liquid culture was incubated on a rotary shaker for 48 h at 28^{o}C. The cells were collected by centrifugation at 3000 x *g* for 20 min. Yeast cells were washed twice with sterile-distilled water and resuspended in sterile-distilled water. A haemacytometer was used to adjust the yeast concentration in sterile-distilled water to 5x10^{8} cells ml^{-1}.

C. capsici DOAC 1511 was obtained from the Mycological Laboratory of the Department of Agriculture (DOA), Thailand. The fungal pathogen was maintained on potato dextrose agar (PDA, Difco Laboratories, USA) slants at 4^{o}C. The culture was grown on PDA plates at 28^{o}C for 7 days in the dark. Spores of *C. capsici* were prepared by removing the culture agar with a bacteriological loop and suspending it in 10 ml of sterile-distilled water containing 0.05% (v/v) Tween-80. The spore suspension was then filtered through four layers of sterilized cheesecloth and adjusted to 5x10^{4} spores ml^{-1}.

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3.2 Treatment with Antagonist Yeast

3.2.1 Effects of *P. guilliermondii* Strain R13 on Chili Anthracnose Lesions

Each chili fruit was gently wounded (one 2-mm-deep and 6-mm-wide wound per fruit) at the equator with a sterile cork borer. After wounding, a yeast suspension (30 µl at $5x10^8$ cells ml^{-1}) was inoculated into each wound and incubated at various time intervals (0, 24, 48, and 72 h). The same amount of sterile-distilled water was placed on each wound of the control group. After inoculation, all fruits were stored at 28°C and 95% relative humidity (RH) in the dark. Then, a second wound (one 1-mm-deep and 4-mm-wide wound per fruit) was made approximately 1 cm away from the initial wound and inoculated with 10 µl of a spore suspension of *C. capsici* ($5x10^4$ spores ml^{-1}). After air drying, the chili fruits were put on plastic trays and wrapped with polyethylene sleeves under 95% RH and placed in a ventilated cabinet (Memmert, type UM 600, Schwabach, Germany) for 5 days at 28°C in the dark. The incidence of disease (the percentage of infected wounds that exhibited anthracnose symptoms) and the lesion diameter (diameter of rotted tissue were determined after 5 days of incubation. Lesion diameter was measured as the average length of the lesion along two perpendicular axes. To study the development of resistance at different distances from the yeast-treated wounds, similar experiments at 24 h after yeast treatment were performed. Afterward, the fungal-treated wounds were made at 1, 2, and 4 cm away from the initial yeast-treated sites and all treated fruits were incubated for 5 days at 28°C and 95% RH in the dark. The incidence of disease and the lesion diameter were also determined after incubation for 5 days. The experiment was replicated three times (trial 1-3), and each treatment in each trial was represented by 30 replicate fruits.

3.2.2 Effects of *P. guilliermondii* Strain R13 on Phytoalexin Induction

Phytoalexins are the low molecular weight antimicrobial compounds produced by plants as a response to infection or stress. In nature, phytoalexins are produced at or nearby areas of infection and their accumulation is associated with disease resistance in plants (Kuc', 1995). Different plant families produce different

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kinds of phytoalexins. Capsidiol, sesquiterpene with structure shown in Figure 3.1, is a major phytoalexin in *Solanaceae* family which *C. annuum* belongs to.

The effect of the yeast on capsidiol phytoalexin accumulation in wounded chili fruits was studied. Each chili fruit was wounded (one wound per fruit, 2-mm deep and 6-mm wide) with a sterile cork borer. Then, 30 µl of yeast suspension was applied to each wound. Fruits treated with sterile-distilled water served as controls. After 0, 12, 24, 48, and 72 h of elicitation and incubation all treated fruits at 28^{o}C (95% RH) in the dark, tissue samples from necrotic sites (yeast inoculated wounds) were removed with a sterile cork borer (10-mm diameter). Capsidiol was extracted from chili tissues with $CHCl_3$:MeOH (2:1, v/v) according to the procedure developed by Egea, Garcia-Perez, & Candela (1996) with slight modification. 3 g of fresh tissue was homogenized with a mortar and pestle and liquid N_2. The extract was washed twice with $CHCl_3$. Tissue debris was removed through three layers of cheesecloth, and the extract was evaporated to dryness in an evaporator (Roto-evaporator-R, Rotovac, Buchi, Switzerland). Before TLC analysis (see next paragraph), the residue was resuspended in 500 µl $CHCl_3$. The experiment was performed three times (trial 1–3), and each treatment in each trial was represented by 30 replicate fruits.

Capsidiol was quantitatively assayed by TLC densitometric analysis on a pre-coated 10 cm × 10 cm silica gel plate (0.25-mm thick, Kieselgel 60F254, Merck, Germany). The extract was applied to the plate with a CAMAG Linomat 5 applicator (Lot, Belgium). The TLC plate was then developed in a chamber tank (CAMAG) saturated overnight at room temperature using a freshly prepared cyclohexane:acetone (1:1, v/v) solvent system. The migration distance of the solvent was about 8 cm, and the development of the TLC plate required about 20 min. After the plate was air-dried for 5 min at room temperature, the plate was dipped into the vanillin reagent (prepared by adding 1.4 g vanillin in 40 ml MeOH containing 250 µl of concentrated H_2SO_4) and was then heated for 3 min at 110^{o}C. The capsidiol band developed a dark blue color with an Rf of 0.55. After development, the plate was air-dried, and the sample and standard were scanned vertically at 200-700 nm with a CAMAG TLC-Scanner 3 and winCATS software (CAMAG). The area of each sample capsidiol peak was then quantified to give the capsidiol content based on a capsidiol standard curve. Authentic

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capsidiol was kindly provided by Dr. Joseph Chappell, Department of Plant and Soil Sciences, Kentucky University, USA.

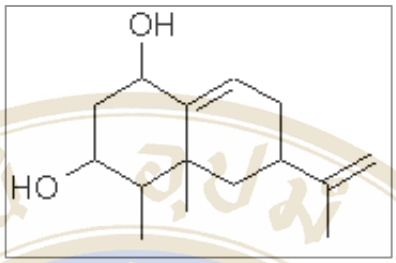

Figure 3.1 Chemical structure of capsidiol

The toxicity of capsidol in the extracts of chili tissue was also tested against the anthracnose pathogen. To investigate the fungitoxicity test, the chili extract was separated using the TLC process mentioned above. When the plate was completed in the developing phase, the air dried plate was oversprayed with a dense suspension of spores of *C. capsici* ($5x10^4$ spores ml^{-1}). The plate was then incubated in a moist chamber which was prepared by placing sterile tissue paper or filter paper on the bottom part of a container with a lid and moistened with sterile-distilled water. The chamber was kept at 28°C (95% RH) for 5 days. During this time, the plate was monitored to determine mycelium growth of the fungus.

3.2.3 Effects of *P. guilliermondii* Strain R13 on Defense-related Enzyme Induction

3.2.3.1 Enzyme Extract

Each chili fruit was wounded and treated with the yeast and sterile-distilled water according to the procedure in section 3.3.1. After treatment, chili fruits were kept at 28°C in the dark under 95% RH. Then, chili tissues were collected at 0, 24, 48, and 72 h, and the tissues from each fruit were removed with a sterile cork borer (6-mm diameter) at a location 1 cm from the edge of the yeast- and water-treated wounds. Enzymes were extracted at 4°C using the method of Cao, Zeng, & Jiang (2006) with slight modification (see the next paragraph). Treatments consisted of three trials, and there were 30 fruits per treatment per trial.

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For extraction of chitinase (CHI) and β-1,3-glucanase, 5 g of the sampled chili tissue was sliced and then ground with a chilled mortar and pestle and 5 ml of 100 mM sodium acetate buffer (pH 5.2) containing 5 mM β-mercaptoethanol and 1 mM ethylene diaminetetraacetic acid (EDTA). The homogenate was centrifuged at 15,000 x *g* at 4°C for 20 min, and the supernatant was then collected for the enzymatic assay. For extraction_of PAL, 5 g of the sampled tissue was ground with a chilled mortar and pestle and 5 ml of 50 mM sodium borate buffer (pH 8.8) containing 5mM β-mercaptoethanol and 2mM EDTA and 4% (w/v) polyvinyl polypyrrolidone. After homogenization, the homogenate was centrifuged as described for CHI and β-1,3-glucanase. The soluble protein content was assayed according to the method of Bradford (1976) using bovine serum albumin (A9647, Sigma-Aldrich Co., USA) as the standard. Standard curve of protein is shown in Appendix I.

3.2.3.2 Enzyme Assay

CHI activity was determined by the method of Wirth and Wolf (1990) with some modifications using a carboxymethylchitin-ramazol brilliant violet solution as substrate (CM-Chitin-RBV 2 mg/ml, Loewe Biochemica, Germany). A 100-µl portion of the crude enzyme solution and 200 µl of CM-Chitin-RBV solution plus 100 µl of 100 mM sodium acetate buffer (pH 5.2) were mixed and incubated for 2 h at 37°C. After incubation, the reaction was terminated by adding 100 µl of 1.0 M HCl. The reaction mixture was cooled on ice for 10 min and then centrifuged at 15,000 x *g* for 5 min (J2-MC, Beckman Coulter, USA). The supernatant was measured spectrophotometrically at 550 nm in an automated microplate reader (Wallac 1420 VICTOR2, PerkinElmer, Finland), and CHI activity was calculated as described by Wirth and Wolf (1990). The CHI activity was measured as international units (U) per milligram of total protein based on a calibration curve of chitinase from *Streptomyces griseus* (Krainsky) Waksman & Henrici (C6137, Sigma). One unit was defined as the amount of enzyme required to catalyze the formation of 1 nmol product per minute. Standard curve of chitinase activity is shown in Appendix J.

β-1,3-glucanase activity was determined as the amount of reducing sugar released from larminarin. This was done by using glucose as the standard according to the method reported by Abeles and Forrence (1970) with slight modification. β-1,3-

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glucanase activity was assayed by incubating 100 µl of enzymatic extract plus 100 µl of 2% (w/v) larminarin (L9634, Sigma) at 40°C for 1 h. The reaction was stopped by adding 400 µl of dinitrosalicylic reagent (prepared by adding 300 ml of 4.5% NaOH to 880 ml of a solution containing 8.8 g of dinitrosalicyclic acid and 255 g of potassium sodium tartrate.$6H_2O$), and then heating the mixture in boiling water for 5 min. For the spectrophotometric measurement, the mixture was diluted 1:10 with distilled water, and absorbance was measured at 490 nm. β-1,3-glucanase activity was expressed as units per milligram of total protein, where one unit is defined as the enzyme activity that catalyzes the formation of 1 µmol glucose per hour. Standard curve of glucose is shown in Appendix K.

Phenylalanine ammonia-lyase (PAL) activity assay was determined based on the production of *trans*-cinnamic acid following the method of Yao and Tian et al. (2005) with slight modification. The reaction mixture of 1 ml of enzyme extract, 2 ml of 50 mM sodium borate buffer (pH 8.8), and 1 ml of 20 mM L-phenylalanine (107256, Merck) was incubated for 1 h at 37°C. The reaction was terminated by addition of 1 ml of 1 M HCl, and the mixture was centrifuged at 10,000 x *g* for 15 min. The absorbance of the supernatant was measured at 290 nm using an ultraviolet spectrophotometer (Beckman Coulter, DU© 800, USA). The amount of *trans*-cinnamic acid produced was determined based on its standard curve (C80857, Sigma). PAL specific activity was defined as nmol of *trans*-cinnamic acid per hour per mg of total protein. Standard curve of cinnamic acid is illustrated in Appendix L.

3.2.4 Scanning Electron Micrographs of Fungal Pathogen on Treated Chili Wounds

Scanning electron microscopy (SEM) was used to examine the germination and growth of *C. capsici* in surface wounds located 1 cm from wounds treated with *P. guilliermondii* strain R13 or sterile distilled water. A yeast suspension (30 µl at $5x10^8$ cells ml^{-1}) or sterile distilled water had been treated for 24 h and stored at 28°C (95% RH) before inoculation a spore suspension of fungal pathogen (10 µl at $5x10^4$ spores ml^{-1}), and SEM was done 24 and 48 h after the pathogen was inoculated and kept at 28°C (95% RH). The surface peel of *C. capsici*-inoculated wounds was excised by a sterile razor blade as slices with a total area of 5 mm^2 each, and

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approximately five peel sections from different fruits were excised in each treatment (five fruits per treatment). Excised tissues were immediately fixed with osmium tetroxide for 1 h. The samples were then dehydrated in a graded ethanol series (30, 50, 70, and 90%) for 10 min at each level. After dehydration, the tissues were dipped in 100% ethanol three times for 10 min each time. Samples were then critical-point dried with liquid CO_2 (Balzers Critical Point Dryer, SCD 020, Liechtenstein) and coated with gold-palladium (Balzers Ion Sputter, SCD 040, Liechtenstein). The samples were viewed using SEM (JEOL, JSM 5410LV, Japan) operating at 15 kV.

3.3 Statistical analysis

To test the effects of treatments and time of fruit inoculation, and distance from the yeast-treated wound on the induction of resistance (disease incidence and lesion development), the data were analyzed using two-way analysis of variance (ANOVA). Data for enzyme activity were evaluated by using the general linear model (GLM) procedure (one-way ANOVA). Mean differences at $p < 0.05$ were considered to be significant. Results were confirmed by three independent experiments. All statistical analyses were performed with SPSS (version 10.0 for Windows, SPSS Inc., Chicago, IL, USA).

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Educational Part

Regarding to the reasons mentioned in the introduction chapter about the need to develop a new learning unit to promote students' understanding on the plant defense responses concept, some findings and laboratory techniques from the scientific study of the induction of plant resistance by antagonistic yeast were used to design the hands-on activities included in the learning unit. Other inquiry-based activities were also included to incorporate in this new developed learning unit to promote student's learning on plant defense responses.

3.4 Theoretical Basis of the Present Study

Learning is an active process in which the new knowledge is constructed based on prior and acquired experience. To establish meaningful learning, students need to be involved in the process that they actively collect, organize, and process information (Frailich, Kesner, & Hofstein, 2008). Based on the idea of constructivism, this learning theory emphasizes the learner rather than the instructors. It views the learner as the one who takes responsibility for their own learning process and knowledge cannot be transmitted but must be constructed by the mental activity of the learner (Brooks & Brooks, 1993). Constructivism suggests that students use ideas already in their minds interact with their experience for developing the new knowledge. In addition, the knowledge construction also needs to involve both individual and social process (Driver et al., 1994). For many years, constructivist perspective has become a prominent position which provides a powerful theoretical framework for researchers in science education (Appleton, 1997; Baviskar, Hartle, & Whitney, 2009).

Due to the science education reform, many science educators have focused on the implementation of inquiry-based approach in the science classroom with the hope to enhance student achievement (NRC, 1996). The effectiveness of using inquiry-based approach in classroom has been well documented (Zion et al., 2004). For instance, teaching through inquiry provide many benefits for students' learning including enhancing students' conceptual understanding in science concepts and nature of science, improving science process skill, and promoting students' attitude toward science (Beaumont-Walters & Soyibo, 2001; DeBore, 2004; Krystyniak &

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Heikkinen, 2007). Inquiry teaching provides students to see and understand the scientific process in which the scientists use to answer questions about the natural world (DeBore, 2004). At the heart of inquiry approach which is consistent with the constructivist view emphasizes the idea that students construct their own understanding by taking an active role in learning process. Learning science based on inquiry approach involves students to ask their own question or the question maybe posted by the teacher, to design and conduct investigation, to gather, analyze data, and communicate their findings to other peers, and to draw the reasonable conclusion (Chang & Mao, 1999; NRC, 2000).

Related to these notions above, the researcher believed that the inquiry as teaching and learning strategy based on constructivist perspective was best challenged to engage students in meaningful inquiry and help them to construct the meaningful science concept of plant defense mechanisms as aimed in this research purposes. Therefore, the inquiry-based approach grounded with constructivist view was served as the theoretical framework of this study as presented in Figure 3.2. Based on the constructivist learning theory, this study believes that the students actively build up their own knowledge (individual process) through the interaction with others (social process) such as classmates and teacher rather than be passively received the knowledge. Furthermore, the new knowledge construction is developed through the connection of students' existing knowledge and believes, together with the new information gained from the new experience (as shown in the left part of Figure 3.2). An instructional process is learner-centered where student acts as an active learner and teacher acts as a facilitator. According to the inquiry-based approach of this study (see in the right part of Figure 3.2), the students were provided to learn with active firsthand experiences through a variety of guided-inquiry-based activities that enable them to make their own knowledge construction. Through the guided-inquiry instruction the inquiry questions were firstly posted by the teacher, however, students need to devise their own procedure to solve the problem. As mentioned by several researchers about the benefits of using the guided-inquiry teaching, these guided-activities can promote student instruction as a desired goal, can reduce student frustration due to achieving undesirable results and fear of the unknown, and can be a path to make transition from a structured inquiry level to a more open inquiry level

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(Germann, Haskins, & Auls, 1996; Trautmann, MaKinster, & Avery, 2004). Five key essential features of inquiry-based teaching and learning proposed by the NRC (2000) were employed to guide and sequence the teaching and learning process of the instruction including: (1) student engages in scientifically oriented questions, (2) students give priority to evidence, (3) students think critically about the relationship between the evidence and their explanation (4) students evaluate their explanations and analyze alternative explanations, and (5) students communicate their "new" understandings with their peers. Additionally, to mediate the success in learning, students were also encouraged to have social interaction in group working (student-student interaction), and discussion and express critical opinion with teacher (student-teacher interaction). In doing that, students can communicate, reflect, and justify each individual idea resulting in the construction of knowledge.

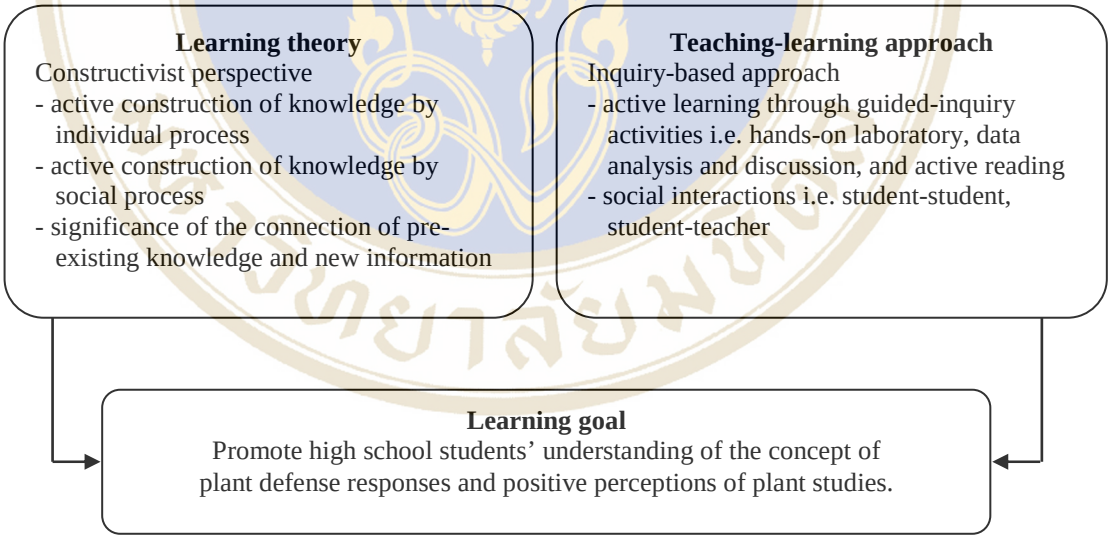

Figure 3.2 Theoretical Framework of this study

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3.5 Research Methodology

As a consequence of our preliminary investigation, we developed a new learning intervention called “the Fighting Plant Learning Unit (FPLU)” which employs a guided-inquiry approach as its mode of instruction. The focus of our study was to investigate the educational effectiveness of the FPLU in developing students’ conceptual understanding of plant defensive responses to biological invaders such as microorganisms and insect herbivores. We also determined whether the unit influenced student interest in studying about plants. In addition, this study investigated the effectiveness of teaching within a constructivist environment while using an inquiry-based teaching approach to present information related to plant defense responses.

The research design of this study involved two main stages described as follows:

1. Development of a learning unit based on an inquiry approach and constructivist perspective to promote students’ conceptual understanding of plant defense responses for high school students,
2. Implementation of the learning unit to high school students and investigation of the educational effectiveness of the learning unit on student achievement and their perception of plant study and the learning intervention.

The stages of the study and activities involved in each stage are summarized in Figure 3.3

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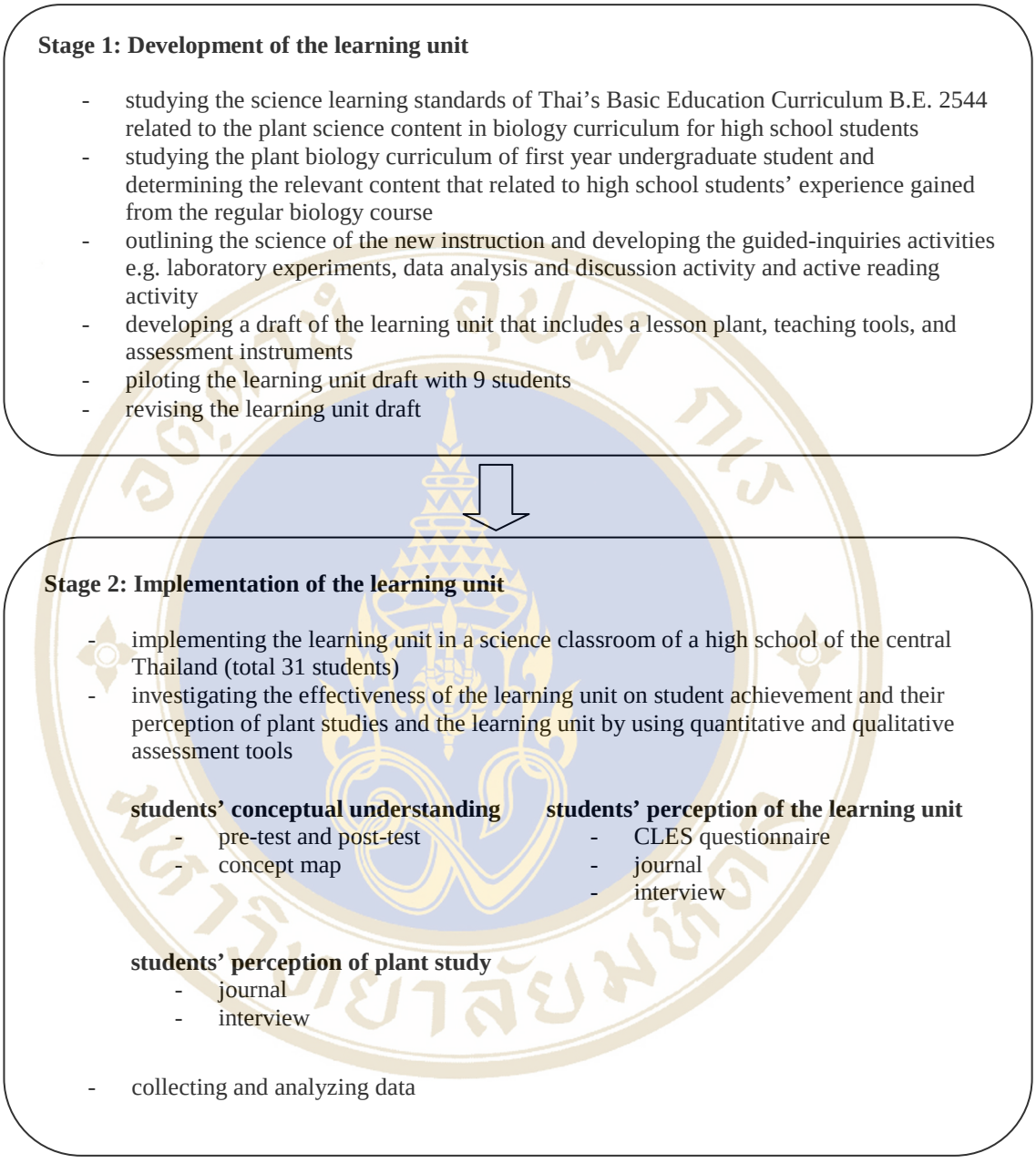

Figure 3.3 Research methodology of the study

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3.5.1 Development of Learning Unit for High School Students

3.5.1.1 Development of the Content of the Instruction

The FPLU was designed to serve the national science educational standard of sub-strand Living Things and Living Processes which emphasized the requirements for grade 9-12 students to learn about the behavioral responses of organisms including the response of plants to external stimuli in their environment (IPST, 2001, 2003). The unit's content is focused on how plants produce defensive responses against biological invaders including microorganisms and insect herbivores. The outline of content was firstly framed based on the main concepts which first year undergraduate student of science faculty required to study in the topic area of plant defensive response. The initial scope of content was evaluated and subsequently revised after discussion with one botany expert, two biology educators and three biology teachers. The revision sought to validate content, learning outcomes, to identify activities, and to confirm the appropriateness of the unit for grade level and school setting. Finally, the scope and appropriateness of the unit's content was based on the prerequisite knowledge that Thai high school students should acquire from the regular biology curriculum.

The main contents of instruction aim to promote students' fundamental understanding that plants are active organisms which can produce defensive responses against the invaders for the purpose of their survival like other organisms. Plants have unique ways to produce their own defensive responses include local and systemic defense responses against pathogens, and direct and indirect defense responses to insects produced by plants.

3.5.1.2 Development of Teaching and Learning Activities

3.5.1.2.1 Teaching and Learning Strategy

The teaching and learning strategy of the FPLU was developed based on the guiding principles of constructivist-based learning and the inquiry approach as described by the National Education Act of Thailand (ONEC, 1999) and the NRC (2000). Regarding to the teaching and learning strategy, each lesson of the FPLU recognizes the six pedagogical characteristics of constructivism proposed by Simpson (2001) and were incorporated into the development and implementation of the learning unit.

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1. Students' preconceptions were assessed and strategies were created to assist in the refinement of existing concepts. This process is based on the assumption that learners come to class with their own prior-knowledge and experience.
2. After addressing the students' preconceptions, they were provided with meaningful inquiry-based activities.
3. Social interaction is one main path to mediate successful learning, therefore in all lessons of the FPLU, students were encouraged to work collaboratively with friends (student-student) and also with the teacher (student-teacher).
4. Taking into account the individual differences within the classroom, students were given the opportunity to present their understanding through a diversity of assessment techniques such as discussion, presentation, testing, concept mapping and interviewing.
5. The teacher constantly monitored signs of difficulty in the students' learning and acted as a facilitator to help them overcome any problems they encountered.
6. The teacher provided individual feedback to students based on their individual understanding of the unit's concepts.

In addition, the researcher employed five essential features of classroom inquiry described by the NRC (2000, p.25) to guide and sequence the learning process in each lesson of the FPLU. These features promote a clearer and deeper understanding of the science concepts included in the learning unit.

1. Inquiry-based activities should start with a scientific question that is meaningful and relevant to the students and should assist them in devising an empirical investigation to help answer the question. The guided-inquiry questions are proposed by the teacher.
2. In the inquiry classroom, students should give priority to evidence, using it to develop and evaluate their explanations of the scientific phenomena. The empirical evidence is a result of their own observations and is obtained and analyzed with appropriate tools and techniques.

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3. The students should think critically about the relationship between the evidence and their explanation, and then use it to formulate their explanations. This is their way to relate what they observed and what they already know leading to the development of new knowledge.
4. The students should evaluate their explanations and analyze alternative explanations to ensure that their new understanding is consistent with currently accepted scientific knowledge.
5. The students should communicate their “new” understandings with their peers. This provides a mechanism for their peers to critique their understanding and a means for initiating new questions to investigate.

3.5.1.2.2 Teaching and Learning Activities

After completion of framing the content of the FPLU, the inquiry-based activities were developed to promote the conceptual understanding of the science concepts. Through the use of the FPLU, researcher designed that the students acquired new knowledge by actively investigating through a five sequential activities: Engagement, Experiment, Experimental data analysis and discussion, Active reading ,and Application. An interactive activity of hands-on experiment was designed based on the scientific research of the induction of disease resistance by the yeast *P. guilliermondii* R13 in chili fruits in part of the induction of phytoalexins by the natural yeast. The laboratory techniques were adapted to be suitable for high school students. In addition, chemicals and materials used in the experiment were safe and easily found in the local area. The overall draft of learning unit was assessed by three experts and three biology teachers, and revised according to their comments. Afterward, a pilot trial of the revised draft was conducted in a high school with 9 twelfth grade students.

The lists of activities and science contents derived from each activity are summarized in Table 3.2. Details of each activity revised completely after the pilot trial are described below (see Instruction of the FPLU).

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Table 3.2 Activities and content outlines of the FPLU

Activities	Content Outlines
1. Engagement	Plants are invaded by biological enemies e.g. microorganisms, insect-herbivores.
2. Laboratory experiment	Plant defense response to microorganisms - local defense response e.g. phenolic compound, phytoalexins accumulation
3. Experimental data analysis and discussion	Plant defense response to microorganisms - systemic defense response e.g. defense-related protein production
4. Active reading	Plant defense response to insect-herbivores - direct defense response e.g. enzyme inhibitor production, toxin production - indirect defense response e.g. plant volatile production
5. Application	Induced plant defense response is an eco-friendly agricultural strategy for plant protection.

Instruction of the Fighting Plant Learning Unit (FPLU)

Before conducting activities, students were provided essential skills needed for them to explore activities: laboratory skills such as thin layer chromatography technique (TLC), aseptic techniques, and construction of concept map. During the FPLU sessions, each group of four to five students participated in five sequential activities: Engagement, Experiment, Experimental data analysis and Discussion, Active Reading and Application, as indicated below:

Engagement. Instructions started by the teacher asking the students the questions: “In our environment, what are the biological invaders of plants” and “Can plants fight back?” together with observing live specimens of various fruits and vegetables which were infected by fungal pathogens. In addition, they were shown pictures and a video of plants damaged by plant parasites and insects. Then, students were asked to discuss and provide their ideas about plant responses. Those students

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who thought that plants could defend themselves were asked to share how they think plants might defend themselves from specific invaders.

Laboratory experiment. After discussion in engagement stage, the teacher posed the question: How can plants defend themselves against pathogens? Then, the students were challenged to conduct a guided investigation for the answer. Before performing the experiment, each group brainstormed how to conduct their own experiment based on a guided-protocol (see Appendix A) and materials provided by the teacher. The students were also asked to present their own experimental design to their classmates.

The experiment aimed to promote students' understanding of plant defense mechanism against microorganisms by producing chemical compounds in attacked area. In this experiment, red chili fruits (*Capsicum annuum L.*) were used as a familiar plant model and the yeast *Saccharomyces cerevisiae* was used as a microbial parasite. In this activity, Thin Layer Chromatography (TLC) is the recommended technique to separate chemical compounds produced by chilies due to its simple and inexpensive procedure, and requires minimal time to get results. When the chilies of the test group were inoculated with yeast and allowed to incubate for a few days, the students could observe the physical changes of dark brown necrotic wounds (see Figure 3.4A) which are a result of the oxidation of a phenolic compound in response to infection or injury (Nicholson & Hammerschmidt, 1992). Whereas, the control chilies that inoculated by sterile distilled-water were observed very little or no changes in the wounds (see Figure 3.4B). Then, the chili wounds were removed and extracted with a solvent. The extract was separated by the TLC technique to investigate a chemical defense substance (the blue spot as shown in Figure 3.5A) and its effect as an antimicrobial agent (inhibition zone as shown in Figure 3.5B). Through this activity, the students observed the production of the chemical compounds, namely phytoalexins, as one type of the local defense mechanisms that plants produce against pathogenic invaders.

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Figure 3.4 Physical changes on the chili wounded surface of the yeast-inoculated wound of the test group (A) and the water-inoculated wound of the control group (B).

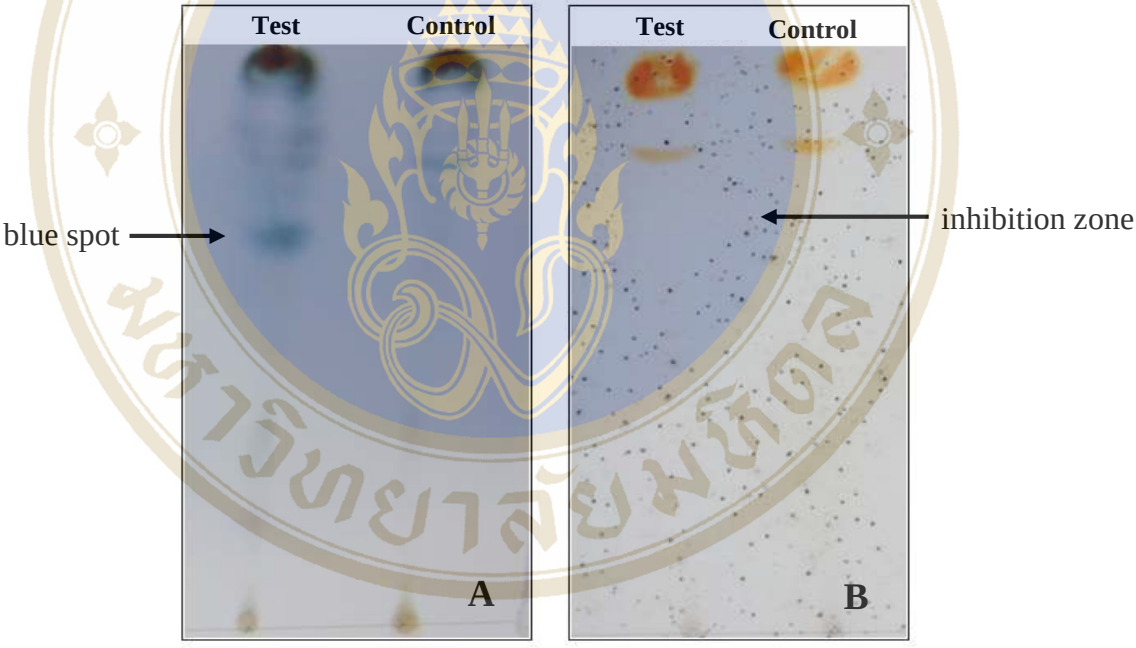

Figure 3.5 Two developed TLC plates for the investigation of chili defensive substance. (A) The plate was tested on phytoalexin detection of the chili extract of the yeast-inoculated chili (test group) and the water-inoculated chili (control group). (B) The plate was tested on antifungal activity of phytoalexin produced by chili. The zone with no fungal growth is due to an inhibitory effect of chili phytoalexin.

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Experimental data analysis and discussion. An experiments in which plant scientists investigated wound lesions on plants showing the indirect evidence of a systemic defense response of plants against pathogens was used to challenge student's thinking about the defensive response of plant. In this activity, the researcher cited a scientific study reported by Ghaouth, Wilson, & Wisniewski (2003) which was an investigation using apples instead of chili fruit. Students were provided the activity sheet (see Appendix B) to work in group and follow the instruction adapted from the study of Ghaouth, et al.

At the first step of the experiment, Ghaouth, et al., divided the apple fruits into two parts for the test group and the other control group. Each fruit was given a superficial wound by a small razor blade. Then, a suspension of antagonistic yeast, *Candida saitoana*, was placed into each wound of the test group, whereas sterile-distilled water was placed on each wound of the control group. After incubation at various time intervals (0-72 h), a second wound was made in each sample approximately 1 cm away from the initial wound. Then, a fungal spore suspension was inoculated into the second wound of all of the fruit and incubated for 7 days. After the researcher presented the procedure of the experiment to this step, the students were asked to predict what they think the second wound might look like after 7 days of incubation. Each group of students were then provided with the experimental results of the Ghaouth, Wilson, & Wisniewski (see Appendix B) showing that the second wound of all of the fruit was infected and appeared as expanded necrosis by the fungal infection. The results also showed that an average lesion diameter of the test group was smaller than that of the control group.

In this activity, the students were not only provided to critically analyze the study of Ghaouth, Wilson, & Wisniewski but also they were given the information which this present study investigated the induction of systemic defense response in chili fruit. The procedure and the experimental result were suggested to the students under the name of Nantawanit et al. study as presented in Appendix B.

Groups of students were then asked to think of possible explanations for the results of the Ghaouth et al. and Nantawanit et al., and then share their ideas with their classmates. From their discussion, the students learned that challenging plants with microorganisms could activate the plant defense responses. These kinds of

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defensive responses can be expressed in the plant tissue away from the site of infection called systemic resistance. In addition, this plant response can result in the activation of pathogenesis-related protein (e.g. CHI, β-1,3-glucanase) production throughout the whole plant's tissues (van Loon, 1997).

Active reading. In this segment of the module, the students were asked to summarize their current knowledge of plant defense responses to microorganisms. Subsequently, the students were asked to share their ideas of how they think plants might fight against other biological invasions such as insect herbivores. Then, each group of students was provided with a different scientific article or a scientific news release discussing "plant communication". They were asked to read the article and the news release, discuss it in their groups and present the main ideas from their reading to explain how they thought plant communication is related to the topic of plant defense. Next, the teacher provided additional learning resources such as scientific websites and textbooks and allowed the students to obtain more information about plant defenses to insect herbivores. From the activity, students learned about both types of defense mechanisms including the direct defense response and indirect defense response against the insects. To resist the insect attack, the direct defense responses involve the production of proteins to pest digestion and toxic substances. The indirect defense responses involve the recruitment of natural enemies of pests. In this latter case, damaged plants by insect attack emit the volatile chemicals that attract the natural enemies to be the plant bodyguard. In addition, at the same time, the volatile chemicals also work as the warning signals communicate to the plant neighbors to activate their defense and be ready for the attack. The phenomenon that plants can release and detect chemicals from each other, may be called as "plant communication" (Dicke, Agrawal, & Bruin, 2003). Lastly, each group of the students completed this segment by drawing a concept map of their understanding of how plants defend themselves against insects and then presented their work to class.

Application. The teaching sequence ended by challenging the students to think about the implications of their knowledge of plant defense mechanisms and apply this new knowledge to an authentic real world context. The students were asked

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to discuss problems facing agricultural production, including pathogen infection and insect attacks. They were asked to devise various strategies to protect plants from these invasions and discuss the advantages and disadvantages of each strategy. Finally, they were challenged to discuss this question: How can your knowledge of plant defense responses be applied to eco-friendly agricultural practices? After discussion, students were challenged to derive the conclusion that activating the production of various plant defense responses by application of suitable elicitors such as some strain of microorganisms can be used as an alternative strategy for plant protection (Kuc', 2000; Stout, Zehnder, & Baur, 2002).

3.5.2 Implementation of Learning Unit for High School Students

3.5.2.1 Participants

The FPLU based on inquiry approach was implemented in an inner city science school in the central Thailand and carried out in 2008 during the second semester. The participants were 31 science students (17-18 years of age) from a grade twelfth classroom. Among the participants, 22 were girls and 9 were boys. Prior to this study, all students had taken the topic of plant responses to the environment in their biology class following the Thai high school science standards taught by a biology teacher who has experience in teaching this topic for 4 years. In this science school, students typically enrolled in plant biology courses in the twelfth grade and this study had implemented after the regular biology course of plant topic had taught completely.

3.5.2.2 Activities

The instruction of the FPLU required the students to be active learners, while the teacher's role is more of a facilitator who provides materials, equipment and guidance so the students can effectively complete each lesson. The students were encouraged to think, inquire, apply and integrate their knowledge with real life problems, and work collaboratively with one another. A cumulative time of the learning activities involved 6 classroom sessions. The students were organized into 6 groups of five to six students. The course was scheduled as a 120 minute period per session. The steps of class activities and time schedule are summarized in Table 3.3.

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Table 3.3 Teaching Plan of the FPLU

Class Period	Content Outline	Class Activities	Time (min)
1	Plant biological invaders in environment - Microorganisms - Insect-herbivores	Engagement: 1) Students are engaged by giving problems to discuss together with observe live specimens of fresh fruits infected with fungal pathogens and various pictures and a video of damaged plants by insect attack. Posted questions are: - In the environment, what are the biological invaders of plants? - When plants are invaded by the invaders, can plants fight back? how and why?	15
	Experiment for studying plant defense response to microorganisms - Inoculation test microorganism to plant sample (red chili fruit)	Investigation: 2) Students investigate fresh chilies with anthracnose disease and discuss the occurrence of disease and how chilies respond to pathogens. Then, students are given the opportunity to perform an experiment.	10
		3) Teacher provides the experimental materials, equipment and brief protocol, and demonstrates how to perform the experiment to study plant defense responses to microorganisms.	15
		4) Students work in groups and conduct their own designed experiment to test their predictions.	35
		5) Each group of students inoculates test microbes to chili fruits. Afterward, the chilies are incubated for two days	45
2	(Continue to perform the experiment) Experiment for studying plant defense response to microorganisms (Local defense response) - Chili extraction	Investigation: 6) Students continue to plan their experiment to extract the incubated chilies.	20
		7) Students extract chilies and the extract is placed in a fume hood at room temperature for two days.	100
3	(Continue to perform the experiment) Experiment for studying plant defense response to microorganisms (Local defense response) - Chili extract separation by thin layer chromatography (TLC) - Chili extract detection by using vanillin reagent - Chili extract detection by spraying fungal spore suspension	Investigation: 8) Students separate the extract by TLC, then spray vanillin reagent to detect separated compounds. They will finally observe the results and collect data.	60
		9) Students separate the extract by TLC, and then spray fungal spore suspension to detect separated compounds. (After class for 3 days, they will then observe the results from the experiment.)	60
		10) Each group collects and analyses the data, and present results to classmate in the next period.	assignment

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Class Period	Content Outline	Class Activities	Time (min)
4	Plant defense response to microorganisms - Local defense response e.g. phenolic compound, phytoalexins accumulation - Systemic defense response e.g. defense-related protein production	11) Explanation: Each group presents the results to the class. Students discuss and share ideas to the class, and then draw conclusions. 12) Teacher demonstrates the scientific experiment for studying plant systemic defense responses to microorganisms. 13) Investigation: Each group is asked to predict the results of the experiment and then provided the experimental data to analyze, discuss and form conclusions. Then students present their ideas to the class. 14) Explanation: Class discusses, shares ideas and forms conclusions on plant defense response to microorganisms	40 10 30 40
5	Plant defense response to insect-herbivores - Direct defense response e.g. enzyme inhibitor production, toxin production - Indirect defense response e.g. plant volatile production	15) Engagement: teacher shows a video of an insect attack on plants and let students to discuss: Can plants fight back to insect herbivores, how and why? Investigation: 16) To find the answer, each group of students is provided with a different scientific article. Then, the students work collaboratively with groups to discuss and present the main idea from the articles. 17) Students are provided more learning resources including scientific websites, news releases and text books to inquire and extend their knowledge about plants defense against insect herbivores. Each group is given an assignment to discuss and derive the conclusion, and then draw a concept map of their understanding of plant defense responses to insects. They will present their concept map to the class in the next period.	10 90 20 and assignment
6	Plant defense responses to insect-herbivores - Direct defense response - Indirect defense response Eco-friendly agricultural strategy: induce plant defense response for plant protection	18) Explanation: Each group shares their inquired knowledge to classmate and discusses with the class to form a conclusion about plant defense responses to insect herbivores. 19) Teacher proposes the problem related to chemical contamination in the environment when using pesticide in agriculture and lets the students discuss scientifically about an eco-friendly alternative strategy for plant protection. 20) Application: Students are given a question to challenge their idea to apply gained knowledge to solve an everyday problem: How can your knowledge of plant defense responses be applied to eco-friendly agricultural practices? Each group brainstorms ideas and shares ideas to class. Then, the class collectively forms a conclusion.	45 15 55

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To investigate of the effect of the FPLU on student achievement and their perception, diverse assessment tools were used for data collection and analysis. Before the intervention began, students were interviewed to assess their perception of plants and plant study from their previous experiences. Students' prior knowledge and conceptual understanding of plant defense responses to biological invaders was examined by administration of a pre-test and interviews. The pre-interviewing was used to investigate students' perception of plants and plant study. The CLES questionnaire was also administered to investigate students' perception of the classroom environment based on a constructivist view of learning prior to start the unit.

At the end of the unit, the students' learning outcomes were evaluated to determine the effectiveness of the FPLU. The post-test and concept map drawings were used to obtain students' conceptual understanding gained from the unit, and the CLES questionnaire was used to assess students' perception of the FPLU learning environment. Semi-structured interviews were used to provide in-depth data of students' perceptions of plants and studying about plants as well as their perception of the effectiveness of the FPLU in relation to the teaching and learning strategies that were incorporated into the unit. In addition, throughout the FPLU implementation, writing journals were also used as the means to reflect students' perception of the plant study and the instruction.

3.6 Ethics

The ethical concerns have also become a great consideration in this research study to respect the right of all participants as well as protect the participants to psychological and physical discomfort, harm and danger that may arise during the research. These concerns were addressed all stages throughout the research study

At first, a formal letter was sent to the school principal to ask a permission to conduct this study in the target school. The letter detailed about the research goal and objective, group of participant and the research duration, and the research methodologies. When the research was allowed to implement in school, the teacher and students who volunteer to participate in the study were informed about the information of this study consisting of the goal and purpose of this study, and the

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duration and procedure of the research. Regarding to the data collection and analysis process, the participants were also informed that these process will be taken as anonymity and confidentiality which will not be disclosed in any way. Additionally, the participants were allowed to ask question in any aspect of the research study. They were also informed their right to withdraw from participating in the study at any time. Then, participants were asked to sign the consent form for agreement to participate in this study. Other ethical concerns deal with the fundamental responsibility to ensure that all participants are protected from any risk that may arise from the learning activities. Therefore, all learning activities and materials used in each activity were carefully developed based on the safety concerns for schools. Furthermore, they were warned about the risk that may occur in any activity and they were asked to wear and use any suitable safeguard protection.

3.7 Procedures Used for Data Collection and Data Analysis

This study used a mixed-method approach, combining quantitative and qualitative data collection to analyze the results of the unit. Methods used in the intervention include a conceptual understanding test, questionnaire, written documents such as concept map and journal writing, semi-structured interviews, and classroom observations. The data were collected before, during and after implementation of the unit. The details of each method are then described.

3.7.1 Conceptual Understanding Test

In order to assess the development of students' understanding of the plant defense responses, the conceptual test was used as a quantitative tool of this study. The two-tier format of the test was selected to develop the conceptual test of plant defense concepts. This kind of two-tier test allows researchers to investigate both correct/incorrect students' ideas and their reasons behind these ideas (Haslam & Treagust, 1987). This instrument contained 25 two-tiered multiple choice questions (see Appendix C) which surveyed information about plant defense responses to microorganisms and insects. The conceptual test was developed by the authors using the procedures defined by Treagust (1988) to identify students' understanding and alternative conceptions in a specific content area. Each item of the conceptual test

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included 2 parts. The first part of each item contained a statement of content that allowed students to answer either true or false. The second part consisted of a set of four possible multiple choice reasons for their answer to the first part. The incorrect reasons of the items were based on students' alternative view of plant and its characteristic identified from the preliminary survey of 148 high school students in the central Thailand as well as the reports from the literatures (Raven et al., 2005; Stamp, 2004, 2005). The content validity of the test was established by a group of three experts of botanical science, biology education, and biology curriculum development to assure that the test was correct and properly constructed for grade 12 students. The test was then piloted with 132 grade twelfth students. The Cronbach's alpha value of the test was 0.71.

At the beginning of the study, the conceptual test was administered to all students to assess their conceptual understanding about the mechanisms of plant defenses. A week after completion of the teaching intervention, the same conceptual test was also used as the post-test for all students. The time allowed for the test was about 50 minutes

In terms of data analysis, the SPSS software package (version 10.0 for Windows, SPSS Inc., Chicago, Illinois, USA) was used to evaluate the quantitative data. The pre-conceptual test and post-conceptual test gains were analyzed using paired *t*-test analysis ($p < 0.05$). The answer to each item in the conceptual test was determined to be a right only when a student responded correctly to both parts of the item. (The maximum test score is 25 points.)

3.7.2 Written document

Written document can be used as another source of data in the research study. The use of various written documents provides benefits with little or no reciprocity between the researcher and participant. Furthermore, these documents are tangible representations that illustrate people's experience, knowledge, actions, and values (McMillan & Schumacher, 2006; Merriam, 1988). In this study, two types of written document including concept map and journal were used to obtain the data.

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4.7.2.1 Concept map

As cited by Novak, (2002), concept map is one of the effective means to evaluate students' understanding of the science concepts. In several researches, it has been used as an evaluation tool to measure the knowledge construction of the individual learner (Kinchin, Hay, & Adam, 2000; Novak, 1990). To construct the concept map, it is needed to write the concepts and make the connection between the two concepts by placing a line labeled with an appropriate linking term. Importantly, the link between the concepts needs to be appropriate to explain the relation of the two concepts.

To further evaluate the conceptual understanding of the students in the plant defense response concepts, concept map construction is the second mean used in the study. One week before the FPLU started, the students were introduced how to write the concept map and they were also asked to practice the concept map construction. Only after the unit intrevention, the students were asked to draw the individual concept map. The time allowed for construction the concept map was about 40 minutes.

The holistic scoring rubrics adapted from Moni, Beswick, & Moni (2004) as presented in Table 3.4, was applied for concept mapping analysis. The rubric was validated and marked on three probing criteria including content, logic and understanding, and presentation. Maximum score of each criterion is 5 point and the total score is 15 point. The expected minimum and maximum total scores from the three concept map elements were 1 and 15 points. A maximum score of a five points for each element are demonstrated as follows: all relevant concepts are correct with multiple connections; understanding of facts and concepts are clearly demonstrated by correct links; and the concept map is neat, clear, and legible, has easy to follow links and has no spelling errors.

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Table 3.4 Holistic concept mapping scoring rubric (adapted from Moni, Beswick, & Moni, 2004)

Score	Content	Logic and Understanding	Presentation
5	All relevant concepts (14) of plant responses to biological factors are correct with multiple connections.	Understanding of facts and concepts of plant responses to biological factors is clearly demonstrated by correct links.	Concept map is neat, clear, and legible, has easy-to-follow links and has no spelling errors.
4	Most relevant concepts (10-13) of plant responses to biological factors are correct with multiple connections.	Understanding of facts and concepts of plant responses to biological factors is demonstrated by a few error links.	Concept map is neat, clear, and legible, has easy-to-follow links and has some spelling errors.
3	Few relevant concepts (6-9) of plant responses to biological factors are correct with two or more connections.	Understanding of facts and concepts of plant responses to biological factors is demonstrated but with some incorrect links.	Concept map is neat, legible but with some links difficult to follow and has some spelling errors.
2	Few relevant concepts (3-5) of plant responses to biological factors are correct with no connection.	Poor understanding of facts and concepts of plant responses to biological factors with significant errors.	Concept map is untidy with links difficult to follow and has some spelling errors.
1	1-2 relevant concepts are linked via the linking words.		

3.7.2.2 Journal

The journal writing is an assessment tool obtaining a source of qualitative data of students' perception. In this study, students were asked to write freely the short journal to express their personal perspective toward the plant study, and their preference toward the teaching and learning activities of the learning unit. At the end of each session, students were asked to use the extra time out of class to complete the journal according to following three questions.

1. What are the similarities and the differences between the plant activities in this session and previous experiences in the biology classroom?
2. From the activities in this session, did you acquire knowledge of plants that did not match your prior experience? What was it?
3. Do you enjoy participating in the activities of this session? Why?

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According to data analysis, the data obtained from the journal were coded using technique described by Strauss & Corbin (1998). The coding was used to identify the participants' responses, then, these data were labeled and categorized to form the meaningful group of data. The results of journal writing were used corroboratively with the interview data.

3.7.3 CLES Questionnaire

A questionnaire is one of the quantitative assessment means used to obtain the information of the individual perception and intention. Based on the present study, the constructivist perspective was used to frame the FPLU instruction. The Constructivist Learning Environment Survey (CLES) is a questionnaire used for measuring students' perception to the learning environment which they perceive the particular classroom environment is consistent to the constructivist epistemology. Originally, the CLES questionnaire was developed by Taylor & Fraser (1991). In different areas, several research studies have been employed the CLES questionnaire as a mean to investigate student's perception of classroom environment whether it is consistent with a constructivist view for teaching and learning. Also, the CLES questionnaire has been validated and the results revealed good validity and internal consistency reliability (Aldridge & Fraser, 2000; Fraser, 2002; Kim, Fisher, & Fraser, 1999; Taylor, Fraser, & Fisher, 1997).

To assess the students' perception of their learning environment of the FPLU, the version of CLES questionnaire developed by Salish I Research Project (1997) and validated in Thailand by Jittam (2008) and Jittam et al. (2008) was selected to use as an assessment tool. The Thai version of the CLES adapted from Jittam (2008) was reviewed and checked by three specialists to assess the consistency of wording used in the questionnaire (English and Thai version). The CLES questionnaire consists of 42 items and has five six-item scales, namely, Personal Relevance (PR), Scientific Uncertainty (SU), Critical Voice (CV), Shared Control (SC), Student Negotiation (SN), and Attitude (AT). Each CLES item consists of a five-response Likert scale with categories ranging 'almost always', 'often', 'sometimes', 'seldom', and 'almost never'. The items in each scale include both

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positive and negative statements. Table 3.5 illustrates the scale, sample questions, and the number of items of CLES questionnaire.

Table 3.5 Scale, sample questions, and number of items for the CLES questionnaire (Salish I Research Project, 1997)

Scale Name	Description	Sample Item	No. of Items*
Personal Relevance	Relevance of learning to students' lives	In this class, I get a better understanding of the world outside school.	(+) 1, 7, 13, 19, 25 (-) 30, 37
Scientific Uncertainty	Provision status of scientific knowledge	In this class, I learn that scientific view can change over time.	(+) 2, 8, 14, 20, 26 (-) 31, 38
Critical Voice	Legitimacy of expressing a critical opinion	In this class, it's OK for me to ask the teacher "Why do I have to learn this?"	(+) 3, 9, 15, 21, 27,32 (-) 39
Shared Control	Participation in planning, conducting and assessing of learning	In this class, I help the teacher to plan what I'm going to learn.	(+) 4, 10, 16, 22, 33, 36, 40
Student Negotiation	Involvement with other students in assessing viability of new ideas	In this class, I explain my ideas to other students.	(+) 5, 11, 17, 23, 28, 34, 41
Attitude	Enjoyment and interest in class	In this class, I look forward to the learning activities.	(+) 6, 12, 18, 24, (-) 29, 35, 42

* (+) positive item statement (-) negative item statement

The Thai version of the CLES questionnaire was administered before (pre-CLES questionnaire as presented in Appendix D) and after the FPLU (post-CLES questionnaire as presented in Appendix E) was implemented. The pre-CLES questionnaire emphasized students to focus on the prior experience of their biology classroom. Whereas, the post-CLES questionnaire asked students to focus on the FPLU implementation. The 42 item statements of both pre-CLES and post-CLES questionnaire are the same, but, at the end of the post-CLES question, the students were asked to express their opinions more toward the learning unit followed the 3 open-ended questions.

In analysis of the CLES questionnaire, the five-response Likert-scale in the positive item statements were scored with five points for an 'almost always' response

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to one point for an ‘almost never’ response, while the scoring procedure was reversed for the negative item statements. The numerical data of the six CLES scales were calculated by using statistics SPSS software version 10.0. Two main types of statistic analyses: the Cronbach’s alpha and *t*-test ($p < 0.05$) were used in this method. The Cronbach’s alpha reflected the reliability coefficients of the questionnaire. A paired *t*-test analysis was used to evaluate the significant difference between pre- and post-CLES questionnaire data.

3.7.4 Semi-Structured Interview

Interview is a research instrument provided more in-depth information (Cohen, Manion, & Morrison, 2007). In the present study, semi-structured interviews were used to gather in-depth data of students’ perception of plants, plant study, and the teaching and learning methodologies of the FPLU. Students were interviewed face-to face with a series of questions that can be modified the sequence, changed the wording and explained more to them. The interview questions had been validated by three experts and piloted with 9 students. The lists of interview questions are presented in Appendix F.

Before the class started, all students were interviewed individually regarding their perception of plants and their studies about plants, and their understanding about plant defense mechanisms. The students were not told about the purpose of the interview other than it being used to find out what they thought about studying biology. The interview was conducted one week before the start of the unit during free periods in a science classroom. Each student took about 20 minutes for the pre-interview. After implementing the learning unit, sixteen students were randomly selected (3 boys and 13 girls) to be interviewed to determine their perceptions of plants, studying about plants, as well as their perceptions of the FPLU. Each post-interview took 40 minutes and was conducted one week after the completion of the unit. All interview sessions were audio recorded and transcribed verbatim for data analysis. The broad categories of student responses were named, categorized, identified and interpreted as suggested by Strauss & Corbin (1998). The findings of from interview were used as triangulation to support the data from the journal writing and CLES questionnaire.

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CHAPTER IV
RESULTS

Overview

This chapter reports the results of the present study in both scientific aspect and educational aspect. The first part of result describes the effects of yeast antagonist *Pichia guilliermondii* strain R13 in induction of resistance in chili fruit based on several lines of evidences. The second part reveals the effects of the intervention of the inquiry-based learning unit of plant defense mechanisms on students' learning outcomes.

Scientific part

The results are regarding to the efficacy of *P. guilliermondii* strain R13 to induce resistance in chili tissue against *C. capsici*. The strain R13 treatment showed the potential in reduction of disease incidence and lesion diameter caused by anthracnose fungus. Additionally, several biochemical defensive responses including phytoalexin accumulation and defensive enzyme activities occurred after application of the yeast antagonist.

4.1 Efficacy of *P. guilliermondii* Strain R13 on the Lesion Development in Chili Fruit

The inoculation of antagonist *P. guilliermondii* strain R13 had a significant effect on the induction of resistance in chili fruit. The incidence of anthracnose and the diameter of anthracnose lesions were reduced in chili fruit when the wound inoculated with *C. capsici* was near a wound previously inoculated with *P. guilliermondii* strain R13 (Figures 4.1 and 4.2). The resistance was influenced by the amount of time of the yeast elicitation and by the distance between the *C. capsici*-infected area and the yeast-treated wound.

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The effect of the yeast treatment significantly increased with the time that yeast inoculation preceded pathogen inoculation as compared with the water control treatment (Figure 4.1, wounds were 1 cm apart). As illustrated in Figure 4.1A, percentage of disease incidence was reduced markedly with time of yeast treatment (0, 24, 48 and 72 h). At 72 h of incubation, the disease incidence was only 47% relative to the water-treated control. The yeast treatment was conducted at various times and was found to be effective in delaying pathogenic growth. Increasing time of yeast treatment reduced lesion development (Figure 4.1B). Lesion diameters of the treatment group at different times, 0, 24, 48 and 72 h, after yeast inoculation were 36.6%, 51.6% and 56.5%, respectively, significantly lower than that in the control group ($p < 0.05$). Figure 4.3 demonstrates smaller lesion diameters of fugal-infected wound adjacent to the yeast-treated site on yeast-treated chili (Figure 4.3B) compared to the control fruits (Figure 4.3A) (Chili was treated by yeast and water for 24-h prior to fungal inoculation.). Based on two-way ANOVA analysis, there was significant interaction between treatment and time of fruit inoculation on disease incidence ($F_{3, 16} = 103.05$, $p < 0.001$) and lesion diameter of chili wound ($F_{3, 16} = 339.53$, $p < 0.001$).

The level of inhibitory effect induced by *P. guilliermondii* also depended on the infected-distance of *C. capsici* from the yeast-treated site. The effect of yeast treatment decreased with greater distance between the yeast-treated wound and the *C. capsici*-infected wound (Figure 4.2, the yeast was treated 24 h before the pathogen inoculation). As shown in Figure 4.2A, the disease incidence in yeast-treated fruit was increased with the distance far from yeast-inoculated site at 1, 2 and 4 cm in approximately 51%, 61% and 77%, respectively, compared to the controls. Whereas, the disease incidence percentage of the control treatment fruit was higher than those measured in yeast-treated group in an average percentage of 94%. When *P. guilliermondii* was applied for 24 h and stored at 28^{o}C in the dark, the most effective distance in reducing *C. capsici* fruit rot was at the distance of 1 cm from *P. guilliermondii*-inoculated wound. At this tested distance, the percentage of the lesion diameter was 52.7% less than that in the control fruit. At a fungal infected-distance of 2 and 4 cm from the yeast-inoculated wound, the lesion development was also reduced 32.6% and 13.8%, respectively, compared with the controls as shown in Figure 4.2B. At all distances, however, yeast inoculation significantly reduced disease incidence

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and lesion diameter. We also found the significant interaction between treatment and distance from infected wound site on disease incidence ($F_{3, 16} = 43.38$, $p < 0.001$) and lesion diameter ($F_{3, 16} = 649.76$, $p < 0.001$).

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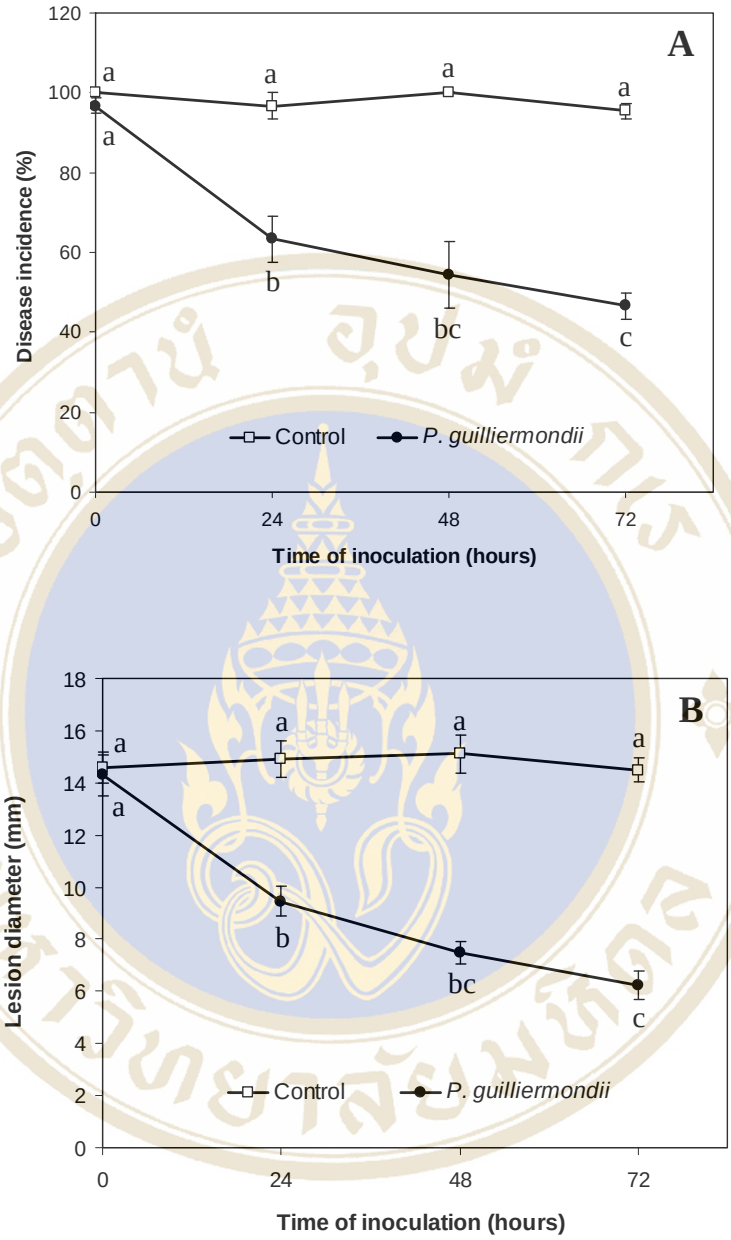

Figure 4.1 Effects of the duration of *Pichia guilliermondii* strain R13 treatment on disease incidence (A) and wound lesion development (B) caused by *Colletotrichum capsici* on chili fruits. The fruits were inoculated with the yeast and with sterile-distilled water (control) at 0, 24, 48, and 72 h before the fruits were inoculated with *C. capsici* at 1 cm from the edge of initial yeast-treated wound. Disease incidence (percentage of *C. capsici*-inoculated wounds with anthracnose symptoms) and lesion diameter were measured after 5 days at 28^{o}C and 95% RH. Each point is the mean (± SD) of three replicates (30 fruits per replicate). Means not sharing the same letter are significantly different ($p < 0.05$).

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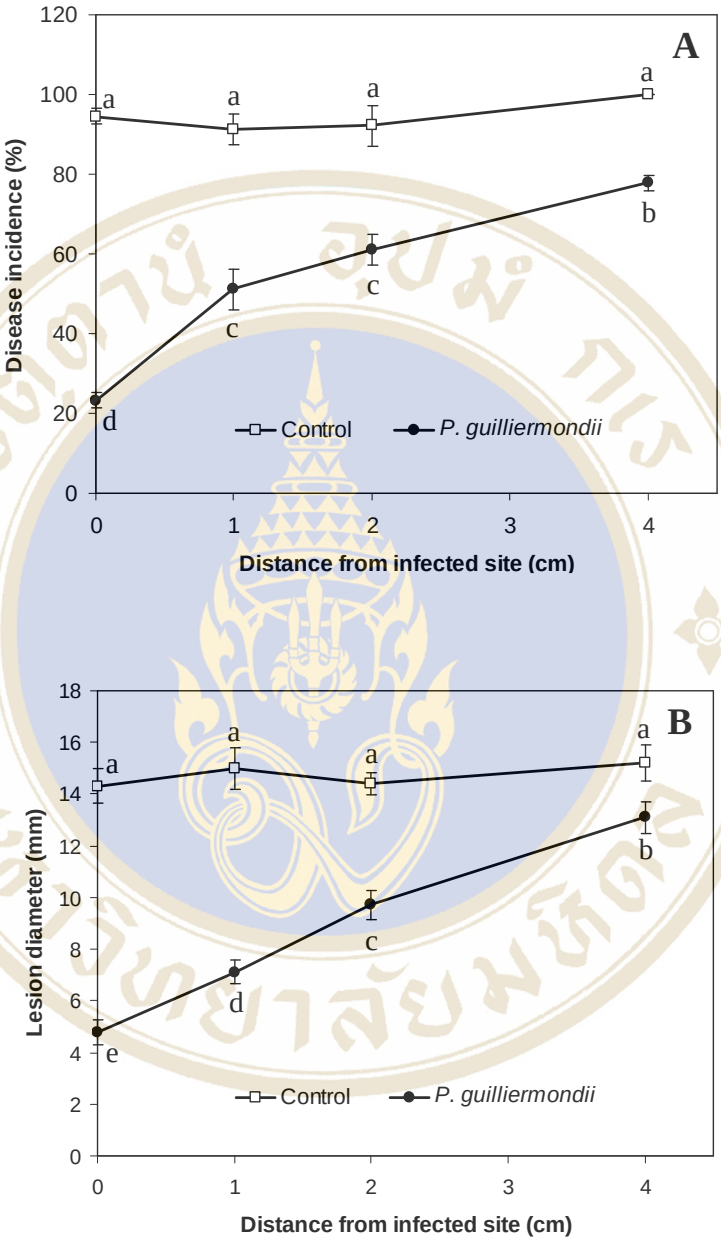

Figure 4.2 Effects of the distance between *Pichia guilliermondii* strain R13-treated wounds and with *Colletotrichum capsici* on disease incidence (A) and lesion diameter (B) on chili fruits. After yeast treatment for 24 h, wounds were made 1, 2 and 4 cm distant from the yeast-treated sites. The new wounds were then inoculated with *C. capsici*, and the fruits were stored at 28°C (95% RH) for 5 days before disease incidence and lesion diameter were measured. Each point is the mean (± SD) of three replicates (30 fruits per replicate). Means not sharing the same letter are significantly different ($p < 0.05$).

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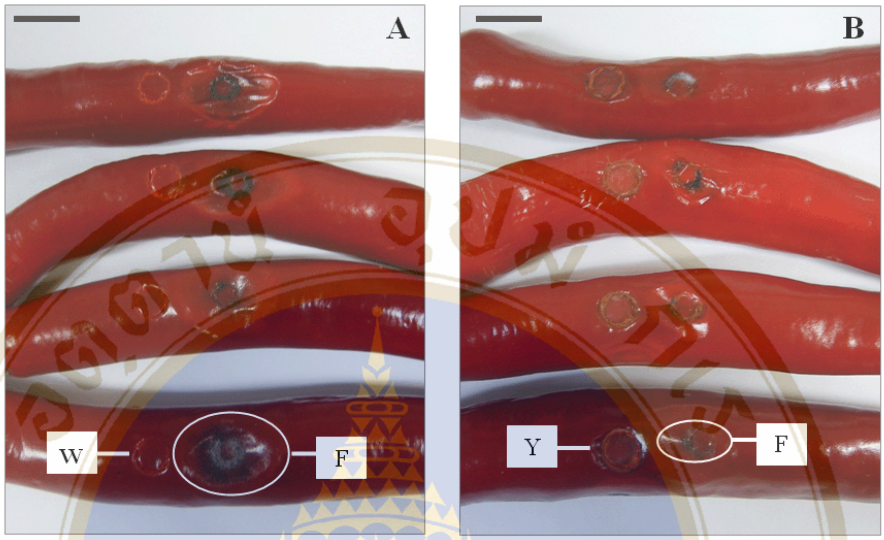

Figure 4.3 Effects of *Pichia guilliermondii* strain R13 on lesion development caused by *Colletotrichum capsici* on chili fruits (B) compared to the control (A). Chilies were wounded and treated by water (W) and yeast (Y) for 24 h prior to fungal inoculation (F) to the wound site that made 1 cm distant from the initial wounds (W and Y). Bar = 1 cm, W = water-treated wound, Y = yeast-treated wound, F = fungal-inoculated wound.

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4.2 Phytoalexin Induction by *P. guilliermondii* Strain R13

The chili fruits treated with *P. guilliermondii* strain R13 and sterile water at different incubation times (0, 12, 24, 48 and 72 h) were performed to investigate the induction of capsidiol phytoalexin. Afterward, chili tissue was extracted from necrotic wounds and the extract was analyzed using TLC technique. The separation of major phytoalexin in chili, capsidiol, was well defined with blue spots at an Rf of 0.55 identified with authentic capsidiol (Figures 4.4). As shown in Figure 4.4, the separation of chili extract from the yeast-treated tissue illustrated the increase of blue spot color of capsidiol started within 12 h after treatment up to 72 h of time course. No blue spots were observed in any of the extract of the water-treated control. Quantitative analysis of capsidiol production was done by TLC densitometric analysis. Each band intensity was scanned (200-700 nm) and the maximum absorbance of the blue spot color from capsidiol reacting with vanillin reagent was 600 nm. The maximum absorbance of the blue spot of standard capsidiol at 600 nm is shown in Figure 4.5. From the band intensity analysis, samples and standard zone areas were measured. The area in absorbance unit (AU) of each sample capsidiol peak (see Appendix G) was quantified to give capsidiol content based on a capsidiol standard curve (see Appendix H) calculated from winCATS program. Based on the analysis, capsidiol accumulation in chili tissue was produced in very low level (10.1 μg g^{-1} fresh weight) after 12 h of *P. guilliermondii* inoculation. However, the phytoalexin production was then sharply increased to 32.5, 38.6 and 58.6 μg g^{-1} fresh weight after the yeast treatment at 24, 48 and 72 h, respectively (Figure 4.6).

The capsidiol extracted from chili tissue was also tested its fungitoxicity against the fungus *C. capsici*. After spraying the plate with *C. capsici* spores and incubating the plate for 5 days, the separated TLC plate yeast-treated samples showed fungal aerial mycelia in all areas except for one spot. The zones of inhibition of mycelial growth were observed approximately at an Rf of 0.55 (Figure 4.7). Size of inhibition zone increased with yeast treatment time course starting from 12 h after treatment to 72 h. In contrast, no inhibition zone was observed in the control samples.

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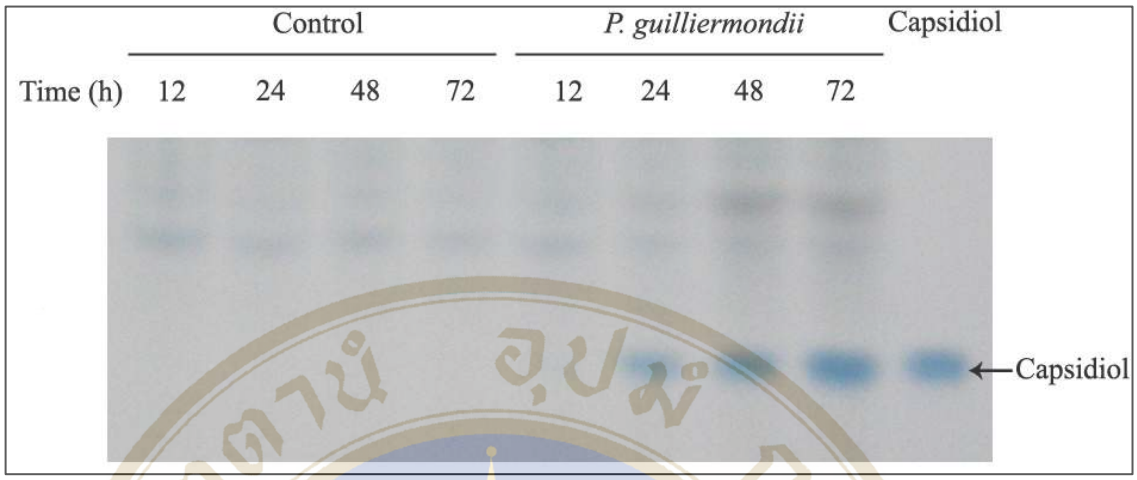

Figure 4.4 TLC detection of capsidiol in chili fruit tissue that had been treated with water (control) or with *Pichia guilliermondii* strain R13. Blue spots of capsidiol reacting to vanillin reagent were appeared at an Rf 0.55. Time represents the hours after treatment (12, 24, 48 and 72 h). The arrow on the right indicates the blue band produced by a capsidiol standard.

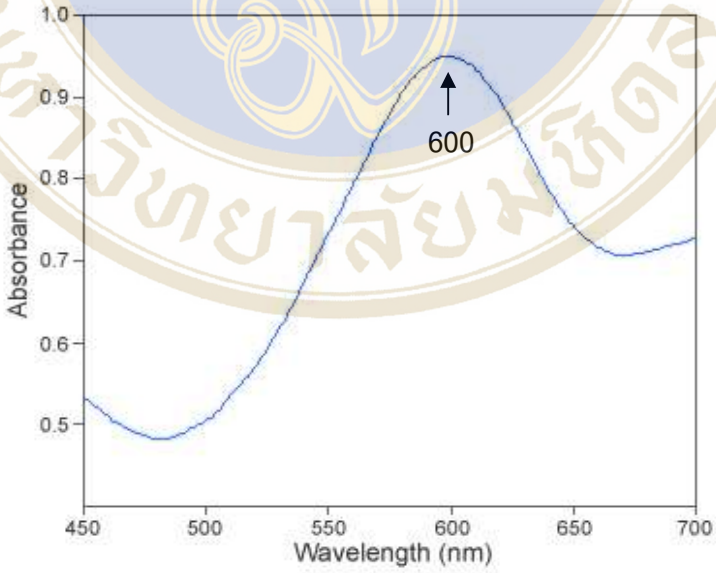

Figure 4.5 Wavelength scanning of authentic capsidiol band formed by color reaction to vanillin reagent. Maximum absorbance at 600 nm is indicated with the arrow.

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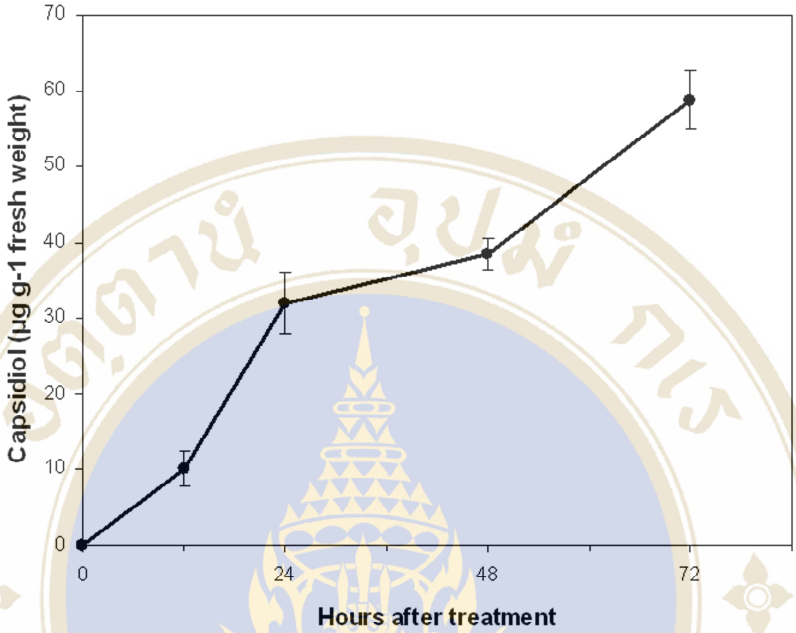

Figure 4.6 Accumulation of capsidiol in chili fruit tissue after treatment with *Pichia guilliermondii* strain R13. Phytoalexin capsidiol was measured quantitatively by TLC densitometric analysis. Values are means (± SD) of three independent experiments.

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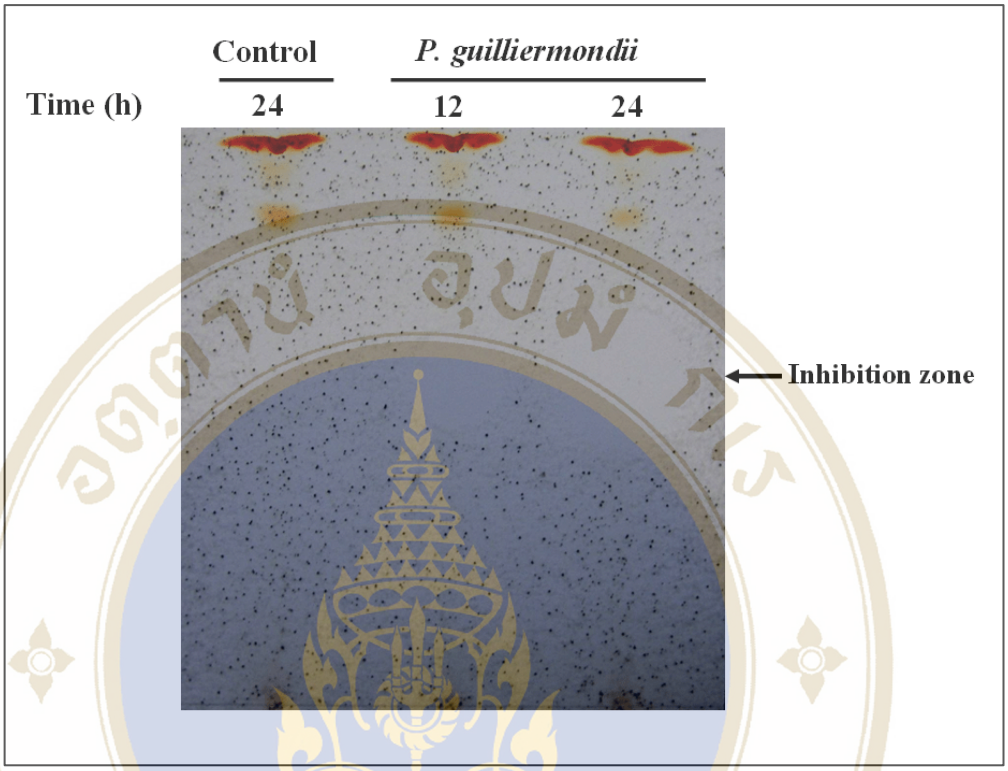

Figure 4.7 Fungitoxicity test of capsidiol in chili fruit tissue that had been treated with water (control) or with *Pichia guilliermondii* strain R13. Clean spot with no aerial mycelial growth is the inhibition zone produced on TLC plate by chili extract oversprayed with conidia of *C. capsici* and incubated for 5 days. Time represents the hours after chilies were treated by yeast antagonist and water. No inhibition zone was observed in control samples.

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4.3 Effect of *P. guilliermondii* Strain R13 on Defense-Related Enzyme Induction

PAL, CHI and β-1,3-glucanase are inducible enzyme that involved in plant defense system against pathogens. The amount of time required to induce enzymes in healthy chili fruit tissue near the wound-treated with the *P. guillermondii* strain R13 was investigated and compared with control fruit. The application of the yeast *P. guilliermondii* could markedly induce enzymes PAL, CHI and β-1,3-glucanase over the same time period relative to the controls (Figures 4.8, 4.9, and 4.10). The results in Figures 4.8, 4.9 and 4.10 illustrate that their activities increased gradually with elicitation time starting from 24 h up to 72 h.

In comparison to the controls, PAL activities in the yeast-treated fruit were 40.9%, 36.4% and 33.4% significantly higher than the controls after the treatment time of 24 h, 48 h and 72 h, respectively (Figure 4.8). Additionally, a lower level of enzyme activity in control group was observed and showed slight increase in PAL activity throughout the incubation times. The change in CHI and β-1,3-glucanase kinetics illustrated similar pattern as found in the PAL activity. Both were continuously increased throughout the elicitation period (Figures 4.9 and 4.10). With prolonged incubation times of 24 h, 48 h and 72 h after the yeast treatment, significantly higher activity of CHI (34.4%, 35.5% and 39.1%) was found compared to those of the controls (Figure 4.9). Meanwhile, the β-1,3-glucanase activity in yeast-treated fruit were, respectively, 53.9%, 57.8% and 58.3% significantly higher relative to the controls (Figure 4.10). In addition, as compared to the controls, kinetics of CHI and β-1,3-glucanase of water-treated fruit did not increase with elicitation times.

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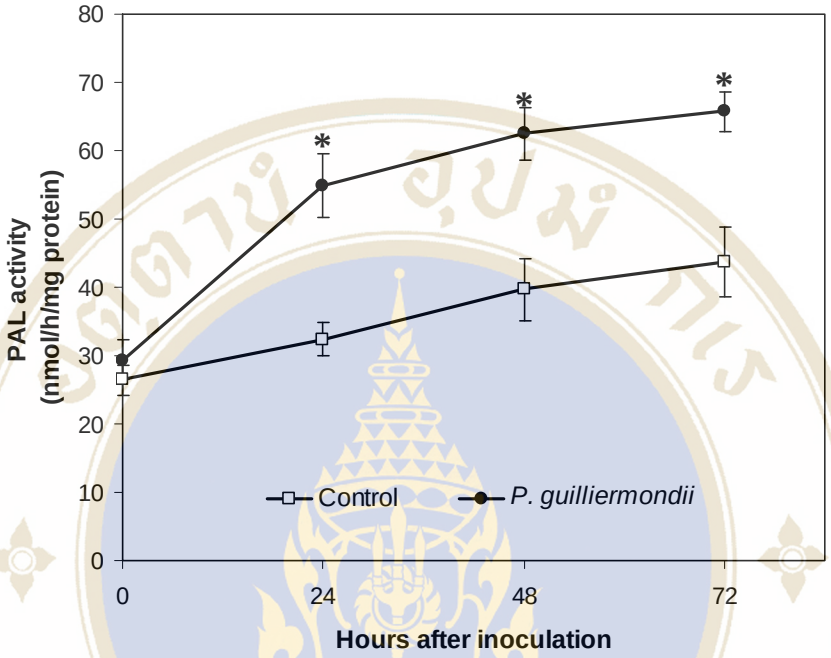

Figure 4.8 Effects of *Pichia guilliermondii* strain R13 and sterile water (control) on activity of the phenylalanine ammonia-lyase (PAL) in chili tissue near the yeast-treated site. The fruit was kept at 28°C and 95% RH for 0, 24, 48 and 72 h before crude extracts were obtained and analyzed. The values are means (± SD) of three experiments. Asterisks indicate significant differences ($p < 0.05$) between experimental treatment and control at the same time.

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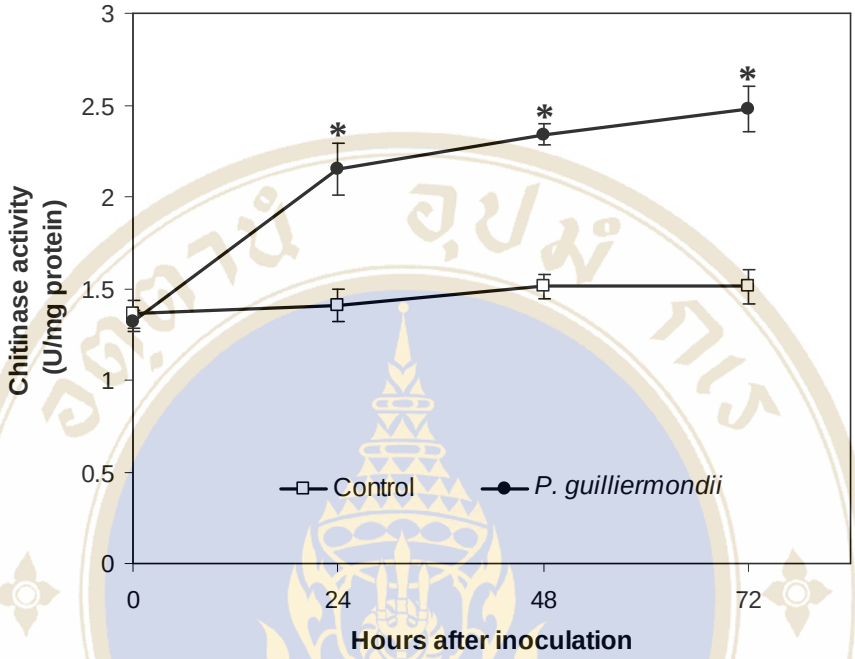

Figure 4.9 Effects of *Pichia guilliermondii* strain R13 and sterile water (control) on activity of the chitinase (CHI) in chili tissue near the yeast-treated site. The fruit was kept at 28°C and 95% RH for 0, 24, 48 and 72 h before crude extracts were obtained and analyzed. The values are means (± SD) of three experiments. Asterisks indicate significant differences ($p < 0.05$) between experimental treatment and control at the same time.

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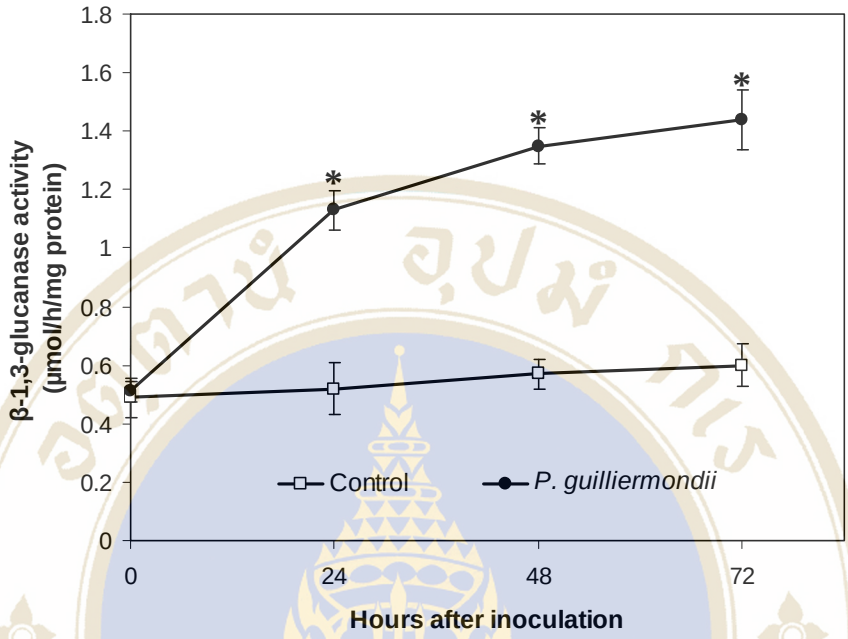

Figure 4.10 Effects of *Pichia guilliermondii* strain R13 and sterile water (control) on activity of the β-1,3-glucanase in chili tissue near the yeast-treated site. The fruit was kept at 28°C and 95% RH for 0, 24, 48 and 72 h before crude extracts were obtained and analyzed. The values are means (± SD) of three independent experiments. Asterisks indicate significant differences ($p < 0.05$) between experimental treatment and control at the same time.

4.4 Scanning Electron Microscope (SEM) of *C. capsici* in Wounds

In control chili tissue inoculated with *C. capsici*, results from SEM indicated that the morphology and growth of *C. capsici* were normal (Figures. 4.11A, 4.11C, and 4.11E). In contrast, *C. capsici* growth and morphology were abnormal in chili tissue previously treated with strain R13 (Figures. 4.11B, 4.11D, and 4.11F). At 48 h after yeast treatment, *C. capsici* spores were irregular (Figure 4.11B) compared with the normal falcate-shaped spore in the control section (Figure 4.11A). At 72 h, the fungal hyphae near the yeast-treated wound were shriveled but also were swollen at some points (Figures. 4.11D and 4.11F) relative to the uniform hyphae in the control (Figures 4.11C and 4.11E). In addition, few mycelia of *C. capsici* were

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observed at infected sites near the yeast-treated wound (Figure 4.11F) while abundant mycelia were observed adjacent to the water-treated site (Figure 4.11E).

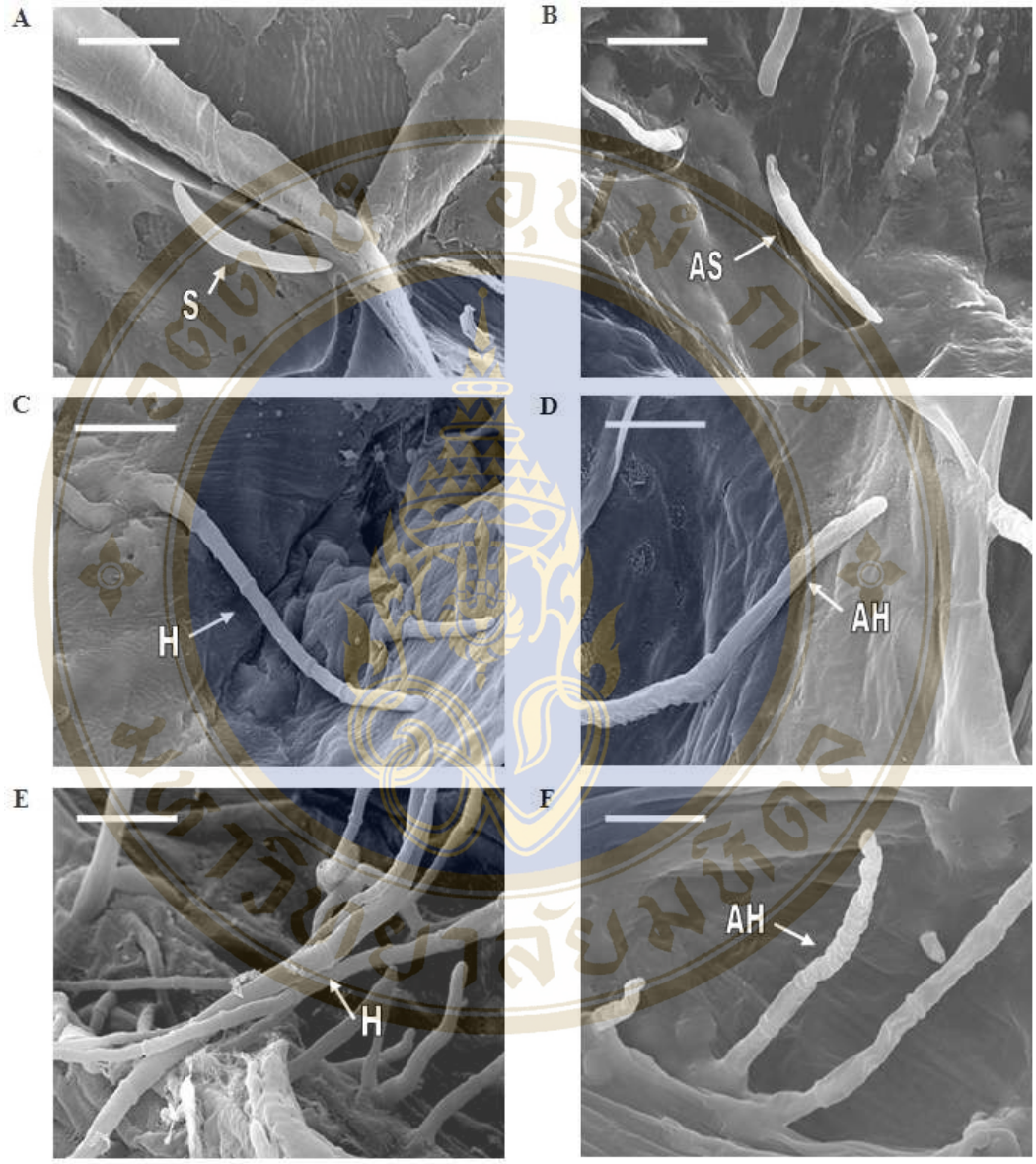

Figure 4.11 Scanning electron micrographs of *Colletotrichum capsici* spores and hyphae in infected sites adjacent to *Pichia guilliermondii* strain R13-treated wounds (B, D, F) or adjacent to water-treated wounds (A, C, E). A and B: Fungal-infected wound surface 48 h after yeast treatment and water control treatment, magnification × 2,000. C, D, E, and F: Infected wound surface 72 h after yeast treatment and water control treatment, magnification × 2,000. Bar = 10 μm. S = spore, AS = abnormal spore, H = hyphae and AH = abnormal hyphae.

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Educational part

In this section, educational findings are presented in two main parts. The first part deals with the findings before implementation of the learning unit FPLU. It intervened students' prior conceptual understanding on the concept of plant defense mechanisms and students' perception of plants and studying about plants. The second part presents the findings after FPLU implementation regarding the promotion of students' conceptual understanding and their perception of plants and plant study. The results also show students' perceptions of the teaching and learning strategies used in the FPLU as well as their views of the classroom environment created during the implementation of the unit.

4.5 Findings before FPLU Intervention

4.5.1 Students' Perception of Plants and Their Plant Study Experiences

The pre-interview sessions revealed what students perceived of plants as compared to animals. We asked the students to describe the similarities and differences between plants and animals. The results showed that most of students viewed plants and animals similarly in terms of being living organisms and possess characteristics of living organisms such as growth, nutritional requirement, respiration, and reproduction. Few students also indicated that plants and animals are capable of moving and responding to stimuli. The differences cited between these two organisms were their roles in nature (producer and consumer), their characteristics (movement and behavioral response), and their physical and biological properties (cells, organs, sensory system, sense of consciousness, etc.).

Pre-interview data showed that 71 % of the students had a preference for learning with animals. 29% of the students, who all are females, expressed positive feelings for studying about plants, due to their physical appearance (shape and color), diversity in the environment, and their clean appearance and safe handling. Meanwhile, 71% of the students indicated a similar trend in reasoning resulting in higher attention to animals; e.g. their ability to move, make sounds, and importantly, react to them (see Table 4.1). In addition, animals have physical and biological properties similar to humans. It was clear that they viewed their study of animals as

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being relevant to their everyday lives. Some students' responses showed their perceptions of plants as being passive and inferior to animals and humans. Some of these responses are illustrated as follows:

> *Author*: So, what make you prefer to study with animals?
> *Student*: Well, they can move and give response to me in many ways like run away, make many kinds of noises…um…obviously, animals are also more like us… have many human-like organs …so we can relate to them and that makes it easy to understand.
> *Author*: Ok, how about plants, why don't you prefer to learn with them?
> *Student*: I think plants are too static…and have no response to anything…they look the same boring.

Table 4.1 Students' perception of studying about plants compared to animals

Pre-interview questions	**Percentage of students' preference of plant and animal study (n = 31)**		**Some samples of students' answer**
	Plant study	**Animal study**	
Between plants and animals, what kinds of organism do you like to study in biology class? And why do you prefer to study with that organism?	29	71	*Students' preference on **plant study**:* - Plants have many kinds of species that we can work with. They are easy to find in environment or even buy from the market. - I love the green color of plants and also the various colorful shades of flowers. Study with plants helps to create more lively classroom. - I'm afraid to work with animals. I don't want to kill them. Working with plants makes me feel better and safe..um also plants don't have bad smell like animals. *Students' preference on **animal study**:* - We can plays with animals..they are cute. Animals give responses to us such as running away and making sounds to threaten. - Animals have many organ systems which are more like us [human], so we can compare what we have learned from animals to ourselves. - Animal experiments are interesting. Once, I had chance to do the frog dissection by myself…it was so exciting.

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When the interview questions were stressed on students' prior experiences in their biology classroom through plants and animal activities, most of the students explained that many animal activities captured their attention rather than plant activities. An individual student could give several examples of animal activities which they experienced mostly through the hands-on activities such as frog and worm and pig's heart dissection, behavioral responses of planaria, hydra observations, butterfly and frog life cycle study, animal movement, etc. It appeared that most students viewed activities that used animals as experiences that aroused their interest. In contrast, many students could not explain much about their past plant activities. Students mentioned that many plant lessons were provided to them by mainly giving a lecture or demonstration without providing a firsthand investigation. Moreover, most activities were less attractive in the materials and techniques used.

4.5.2 Students' Conceptual Understanding of Plant Defense Responses

Regarding the interviews about students' conceptual understanding before the FPLU implementation, most of the students did not understand that plants are able to produce their own defense responses to biological invaders for the purpose of their survival like other organisms. From the pre-interview data, 79% of the students thought plants were unable to defend themselves against pathogens, and 65% of them thought plants were unable to defend themselves against insects (see Table 4.2). They thought that plants were defenseless because they appear static, passive and lack the physical and biological properties of animals. The interviews indicated that most students were unfamiliar with the term "plant immunity" and they did not know that plants have their own way of fighting diseases unlike animal immune systems. Some students thought that because plants lack a blood and lymphoid system they also lack fluid-based mechanisms to defend themselves against pathogen invaders. However, some students had prior knowledge of volatile oils or chemical compounds produced by plants which are used to repel or kill biting insects. Also, their local community has launched a project which uses bitter plant extract to control the pests. Due to this local project, the students thought plants might have some chemicals to resist the pests.

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Table 4.2 Students' understanding on the defensive responses of plants against pathogens and insect-herbivores

Pre-interview questions	Percentage of students' answer to the question (n = 31)		Some samples of students' understanding
	Yes	No	
Can plants defend themselves against pathogens?	21	79	Alternative conception: *Plants cannot defend themselves against pathogens because (most common incorrect explanation):* - Plants have no blood system, so they don't have any antibodies and white blood cells to work against the pathogens. - Plants are at the low evolutionary level, they don't have many complex organs or systems like animals…so they may not have the developed system like the immune system. - Plants are just the static organisms. I never have seen that they can react to anything. If they can defend themselves, why we still see the infected wound on the plants.
Can plants defend themselves against insect-herbivores?	35	65	Alternative conception: *Plants cannot defend themselves against insect-herbivores because (most common incorrect explanation):* - Plants are unable to runaway and have no any organs like hands or fingers to push away the insects. I think they are just the passive organisms. - Plants have no brain, no blood or other system developed as animals. - Plants are just foods for animals. I have only seen that plants always sit still when they were eaten by the insects.

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4.6 The Effectiveness of the FPLU Intervention

4.6.1 Students' Conceptual Understanding of Plant Defense Responses

After using the FPLU, the data revealed that all participants developed a conceptual understanding of how plants respond to biological invaders. Regarding to the conceptual test, a *t*-test comparison of the pre- and post-two-tier multiple choice test scores yielded a significant difference ($p < 0.05$) (Table 4.3).

Students' concept maps illustrated that students were able to construct the concept map showing the link between the desirable main ideas as presented in the FPLU. According to the scoring of the rubric assessment tool adapted from Moni Beswick, & Moni (2004), the average score and qualities of the students' concept maps (12.36 out of 15.0) were at the high end of the scoring continuum and scores ranged from 9 to 15 points (see Table 4.4). Most students developed concept maps that showed relevant concepts of plant defense responses to biological invaders with appropriate multiple connections. Some students demonstrated an understanding of the complexity of knowledge that was presented in the FPLU. For example, key concepts and specific types of plant defense responses to microorganisms and insects were clearly highlighted with sub-links that were related to the basic needs of living organisms (as shown in the word "survival"), organism interaction (plant-plant interaction, plant-microbe interaction and plant-insect interaction), and the behavior of organisms in terms of their responses to internal and external stimuli which they have learned from the regular biology course (Figure 4.12).

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Table 4.3 Pre-test and post-test score on conceptual understanding of plant defense responses

Scale	Mean		Mean Difference	Standard deviation		*t*
	Pre-test	Post-test		Pre-test	Post-test	
Conceptual understanding (max. 25 points)	8.97	20.93	11.96	2.75	2.18	16.48*

* $p < 0.05$ (n=31)

Table 4.4 Evaluation of students' concept map by using scoring rubric adapt from Moni, Beswick, & Moni (2004)

Criteria	Score/Levels* (Mean ±SD)
1) **Content**: all relevant concepts of plant defense responses to biological invaders are correct with multiple connections (max.5 points).	4.26±0.58
2) **Logic and understanding**: understanding of facts and concepts of plant defense responses to biological invaders is clearly demonstrated by correct links (max.5 points).	4.13±0.56
3) **Presentation:** concept map is neat, clear, and legible, has easy to follow links and has no spelling errors (max.5 points).	3.97±0.48
Total score (max.15 points)	12.36±1.20

* The level of concept mapping quality

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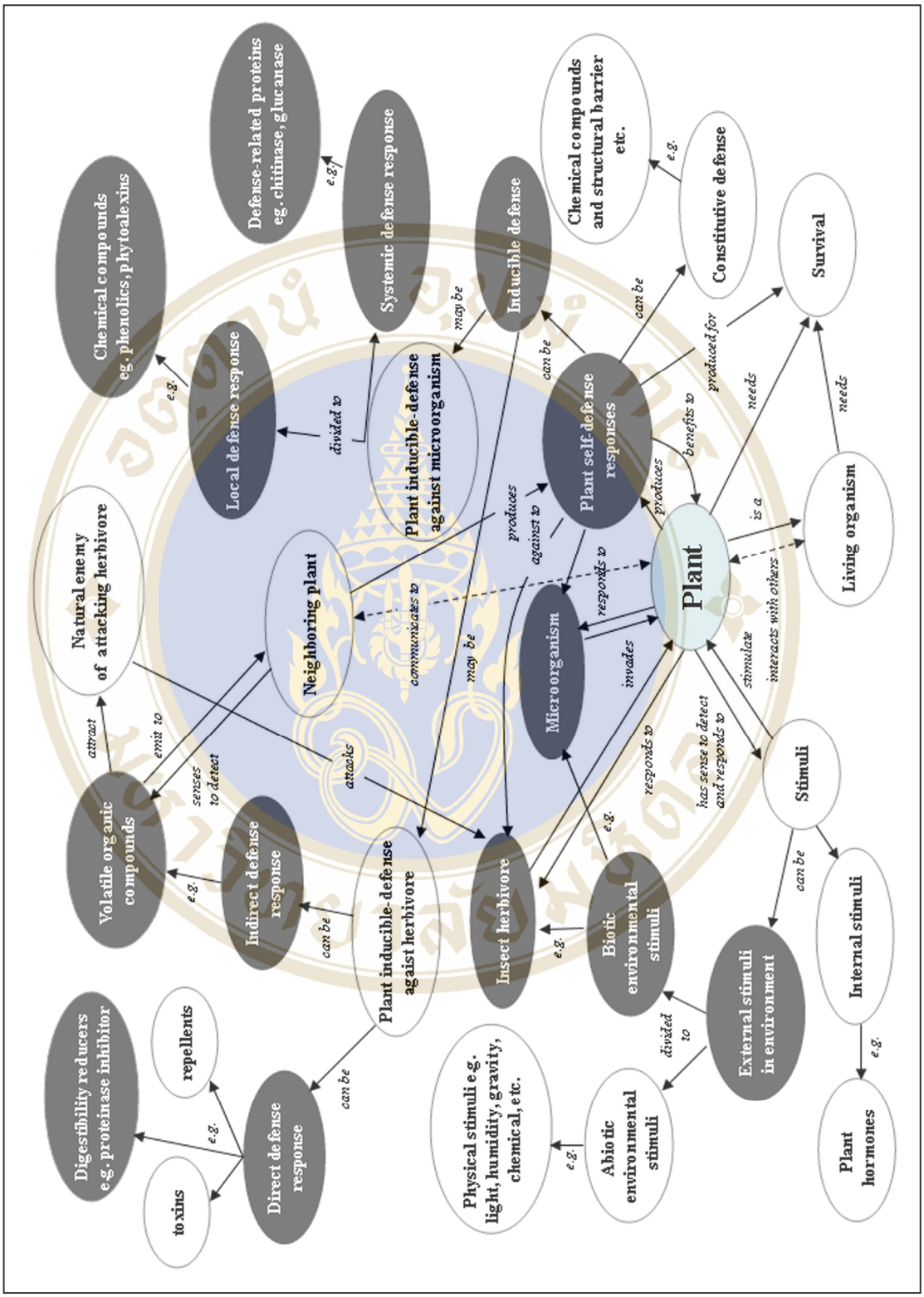

Figure 4.12 An example of a student's concept map on plant defense responses to biological stimuli. Concepts with grey background are key concepts of the unit. Additional concepts are presented with white background.

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4.6.2 Students' Perception of Plants and Plant study

Regarding the FPLU intervention, students have altered their perception of plants and the study of plants. The post-interview sought to answer the following question: Did the students understanding about plants improve after completion the unit? From the interview data and journal writing data, they have gained a new understanding of plant defense responses as described before in the section 4.6.1. They were able to explain that plants are active organisms which can respond to stimuli in their own ways for the purpose of survival like animals. Many students reported a greater appreciation of plant study from a new perspective in learning with plants. For example, some students indicated that they no longer saw plants as passive uninteresting organisms. In addition, some students said they now realized it was not enough to identify or judge something by only looking at its physical appearance. They understood the need for further investigation. Some examples of students' statement are as follows:

> At first, I believed that plants are just helpless organisms. After the experiment, I was so surprised when I clearly saw the clear zone on the TLC plate. It is unbelievable that plants can fight back.
>
> (Source: Journal)

> It's amazing that plants have several interesting ways to resist pathogens…like I just knew from my reading that plant cells have suicide mechanism to restrict the further growth of pathogen…there may be other secret stories of plant that we don't know yet.
>
> (Source: Interview)

> Even though we always see them [plants] sit still…it doesn't mean that they [plants] are being like what we are seeing. I just realized about that…we need to prove it first by scientific investigation.
>
> (Source: Interview)

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The findings also revealed that students saw some similarities between the plant activities in the past and the ones experienced in the FPLU which involved opportunities for them to perform a simple hands-on experiment using a local plant model and working in group. However, there were many differences in response from students regarding the experimental techniques, chemicals and equipments that they never experienced before, the guiding instruction which allowed them to use their ideas before conducting their own investigation, and the active reading activities through scientific papers which were published in the international journals.

In addition, before the unit implementation, most students felt that learning with plants was not as interesting as learning with animals. However, the activities in the learning unit were an influential factor in motivating them to learn about plants. Through the interviews as well as the reflection in the journals, most students expressed their higher interest in learning with plants compared to their prior experiences. Students cited several factors which raised their interest and captured their attention in plant study. Some of these factors include learning new things about plants, learning through hands-on activities, using interesting but simple experimental techniques, and being able to recognize the relevance of plant knowledge to their personal life. Some students had these to express:

> Actually, I think plant study is so boring. But this time [participating in the FPLU] is quiet different because I had a chance to conduct the experiment using many laboratory techniques I never experienced before. Also, it was very fun to work with the red chilies.
>
> (Source: Journal)

> This plant science class [the FPLU] is more interesting than my prior experience because I know that the thing I have learned is related to my life…such as if I were an agriculturist, I can use technique to stimulate plants' immunity…in stead of using chemicals…it's safe for us and environment.
>
> (Source: Interview)

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> I think plant science lesson would be more interesting when that class gives us to learn about the plant topic we never knew before or the secret of plants that just be revealed from the scientists…like in this class [the FPLU]…I just learned that plants can give warning to friends [neighboring plants] though.
>
> (Source: Interview)

4.6.3 Students' Perception of the FPLU

4.6.3.1 Teaching and Learning Activities

To assess students' perception of the learning unit regarding to the teaching and learning strategy implemented in the unit, three main instruments including; interview, journal, and CLES questionnaire, were employed and co-analyzed. Based on the interviews and journal writing, students reflected that, this type of learning differs from the regular method used in biology classrooms that they had been experienced and in that method was mainly stressed on giving lecture. Students apparently agreed that the FPLU provided little time for lecture and no any of handout containing the content knowledge was given to them. Differently, the learning unit mainly provided more inquiry-based activities for active experiences and encouraged to work in group for construction their own knowledge of plant defenses.

From the interview data, when the questions focused on how the students developed their conceptual understanding of plant defense responses, students explained that the guided inquiry-based activities provided an opportunity for them to refine their understanding of this science concept. Students indicated that the scientific investigations through hands-on experiences, the evaluation and explanation of their laboratory findings, the active readings, and the critical discussions with their peers and teacher [researcher] all contributed to a better understanding of plant defense mechanisms. Even though, they received a prescribed protocol from the teacher [researcher], they still needed to design and select their own method of investigation throughout the guided activities. A few additional comments from the interview were:

> I learned mostly from doing activities together with my friends...teacher didn't give much lecture like other classes. She [researcher] asked us to think and do activities by ourselves first.

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> I have read paper and shared ideas with my group and others …when other groups presented [their work] and we discussed each other…well…from this I got ideas for my concept map.

Through the interview and the journals, students indicated that most of them had a preference to learn through doing activities rather than passively receiving information. In addition, before the module implementation, most students felt that learning with plants was not as interesting as learning with animals. However, the activities in the module motivated them to learn about plants. They enjoyed performing the laboratory investigations by using a familiar plant as a learning model. Even though, the students had never experienced most of the techniques used for investigation, such as TLC and the induced plant resistance technique, they thought these strategies were interesting and highly motivating. However, practicing new techniques and equipment prior to conducting real investigation was needed.

The findings also showed that students liked the scientific readings which provided them with information to analyze, interpret and discuss scientific ideas. Even though most of them encountered some reading difficulty, they responded that working in groups and through teacher [researcher] facilitation help them to access the information from the readings. Moreover, the students said the scientific concepts learned from the FPLU were new and interesting, applicable to everyday life. It also encouraged them to think of other learning module by using plants to learn. Here are some quotes from students that were interviewed:

> I prefer learning by doing activity than listening to the lecture…I like to do experiment…science classes should be taught like this.

> It's fun to do the experiment with chilies…like we were cooking in the lab…but use the scientific equipment that I never used …I also would like to conduct the experiment with other fruits such as guava, tomato, apple and many else.

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> It's good to read scientific papers because I just knew that plants have many ways to defend against the insects…I was so amazing that plant can give warning to other plants…science helps us to know many secret things in the world.
>
> Reading English article was quiet difficult for me…well, it took up a long time…when shared idea with my friend and also teacher…it helped understanding…and I felt…I was not alone.

4.6.3.2 Constructivist Learning Environment

To measure students' perceptions of a constructivist learning environment in the FPLU, the CLES questionnaire was used as the assessment instrument. The internal consistency of each CLES scale from the CLES questionnaire was estimated by using the Conbrach's alpha coefficient with individual students as a unit of analysis. The Conbrach's alpha values of all 42 items of the pre- and post-CLES questionnaire were 0.69 and 0.71, respectively. Table 4.5 shows that the alpha reliability ranged from 0.54 to 0.80 for the pre-CLES questionnaire and from 0.56 to 0.80 for the post-CLES. To evaluate the students' perception of the classroom environment in which the FPLU was implemented, the pre-test and post-test mean scores for the CLES scales were compared using a *t*-test analysis. The results showed that post-test scores were significantly higher than the pre-test scores in all six of the CLES scales at 0.05 level as presented in Table 4.5 and Figure 4.13. The difference between the pre-test and post-test scores were respectively 1.22, 0.68, 0.65, 0.63, 0.45, and 0.26 for *Shared Control (SC), Attitude (AT), Critical Voice (CV), Personal Relevance (PR), Student Negotiation (SN) and Scientific Uncertainty (SU).*

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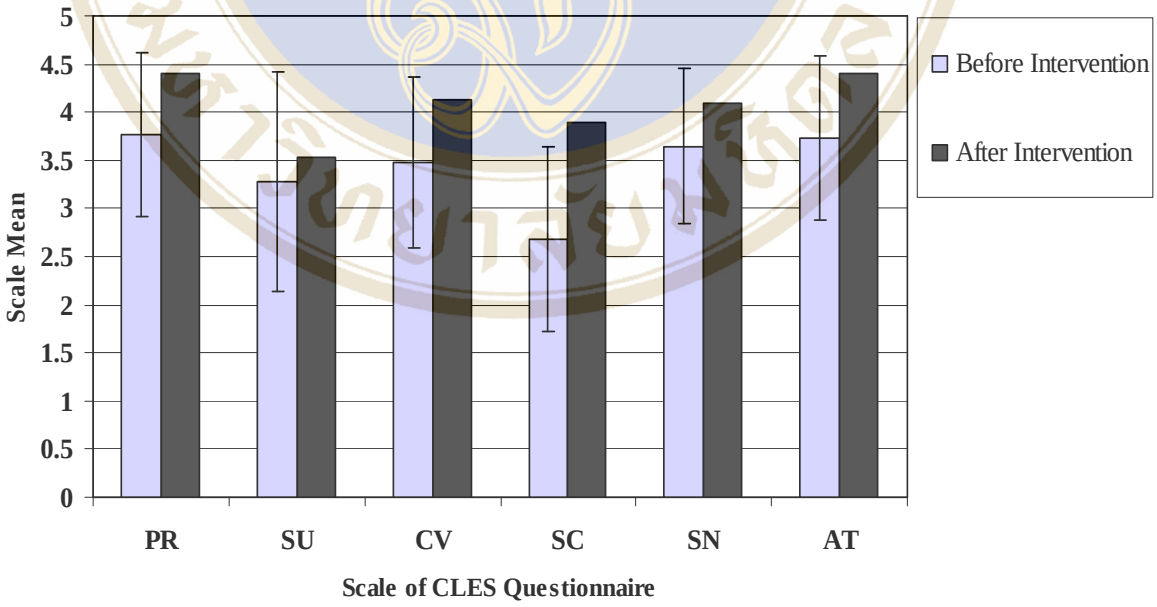
Table 4.5 Results from CLES survey on students

Scale	Alpha reliability coefficient		Means		Mean Difference	Standard deviation		*t*
	Pre-test	Post-test	Pre-test	Post-test		Pre-test	Post-test	
Personal Relevance	0.72	0.80	3.77	4.40	0.63	0.85	0.59	9.90*
Scientific Uncertainty	0.54	0.56	3.28	3.54	0.26	1.14	1.13	3.79*
Critical Voice	0.62	0.61	3.48	4.13	0.65	0.89	0.65	9.22*
Shared Control	0.80	0.78	2.68	3.90	1.22	0.96	0.65	16.15*
Student Negotiation	0.69	0.73	3.65	4.10	0.45	0.80	0.61	7.36*
Attitude	0.74	0.78	3.73	4.41	0.68	0.85	0.61	11.45*

* $p < 0.05$ (n=31)



*** Significant difference ($p < 0.05$)**

Figure 4.13 Students' perception of learning environment before and after inquiry-based learning unit intervention as measured by using pre- and post-CLES questionnaire (n = 31).

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Findings from open-ended questions of post-CLES questionnaire revealed students' preference of learning environment of the FPLU. Based on the first open-ended question that asks whether students were pleased about the learning environment in the FPLU, all 31 students responded their preferences in this learning environment with different reasons. The reasons for their preferences as identified and grouped in relation to the characteristic of all CLES scales except the *Scientific Uncertainty* scale were summarized in Table 4.6. In conclusion, students considered the relevance of the content to real life, expressing critical opinions, classroom involvement, collaborative work, and interesting and enjoyable activity as positive factors in being taught with the FPLU. Most of the students felt that these factors provided a rich and lively classroom climate which increased their interest and focus on learning.

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Table 4.6 Reasons for students' preference in the learning environment of the FPLU

Learning Environment	Samples of students' reason	Number of responses
Personal Relevance	- I have learned about plant science that the gained knowledge can be applied to use in agriculture. I think it is very useful to learn about this. - The content is interesting and not too difficult. I can connect what I have learned with my everyday life.	3
Scientific Uncertainty	-	0
Critical Voice	- The teacher was so kind. She always allowed us to ask the questions and helped us to learn in many ways. - I felt free to tell the teacher about my difficulties in learning and she always helped me.	4
Shared Control	- I like the way that the teacher let us participate in the learning activity or asking our opinion whether the activities fit for us or the preferred learning strategy - I was relax in this class, because the teacher asked us many times what we would like to do or to learn or even the way of assessment	3
Student Negotiation	- The teacher let us work together in group in every session. I felt safe to have my friends who can help me to learn. - I like many activities especially when the teacher let every groups compared their opinion and gave suggestion to each other.	4
Attitude	- I actually like to do the experiment. It was so fun to use the chilies in the laboratory. - This unit provided many interesting activities in the class, not just only a lecture.	17

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CHAPTER V
DISCUSSION

Overview

This chapter presents the interpretation and discussion of the research findings. In the first section, it begins with the discussion on the results of the scientific study. Next, it is followed by the discussion on educational research findings. The recommendation for future research or limitation of the research study is also given at the end of each research aspect.

5.1 Scientific Aspect

In the past two decades, the mechanisms by which the biocontrol agent inhibits plant pathogens have been focused in many studies, however, a few works has been investigated in the area of using biological control to suppress postharvest diseases of fruit (Janisiewicz, Tworskoski, & Sharer, 2000). Understanding the mechanisms of action of antagonists provides many benefits for agriculture. For example, the identified useful traits could be upgraded by genetic tools for improving and developing the suitable and effective formulations of biocontrol agent as well as establishing the screening criteria of new potential biocontrol agents (Casteria et al., 2003; Druvefors, 2004).

Commonly, multiple mechanisms of action of antagonist microorganisms have been shown to be responsible for biocontrol system. Various observations through the modes of action by which the yeasts resist to pathogens have been reported (Bar-Shimon et al., 2004; Castoria et al., 2003; Chan & Tian, 2005; Janisiewicz, Tworkoski, & Sharer, 2000; Wisniewski et al., 1991; Zhao et al., 2008). Among several yeast antagonists, the antagonistic yeast strain *P. guilliermondii,* has been investigated broadly in the control of a number of postharvest diseases in fruits (Droby et al., 1989; McLaughlin et al., 1990; Paster et al., 1993; Rodov et al., 1994). Several studies of possible mechanism of actions of *P. guilliermondii* against fungal

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pathogens have been investigated involve the competition for nutrients (Droby et al., 1989), tenacious attachment along with secretion of cell wall degrading enzyme (Wisniewski et al., 1991) and induction of defense response (Rodov et al., 1994; Zhao et al., 2008).

Like other studies, as reported by Chanchaichaovivat, Panijpan, & Ruenwongsa (2008), they revealed that control of the anthracnose fungus, *C. capsici*, by the antagonistic yeast strain *P. guilliermondii* R13 in harvested chili involves several modes of action and these are attachment to hyphae, competition for nutrients, and secretion of fungal hydrolytic enzymes. They also suggested other additional modes of action which are possible to be included for the strain R13 biocontrol system such as the tolerance of yeast to oxidative stress and the induction of host resistance.

This present study focused on determining whether *P. guilliermondii* strain R13 induced resistance against *C. capsici* caused chili anthracnose. In this study, treatment of chili wounds with *P. guilliermondii* strain R13 was shown to induce a resistance against *C. capsici* that resistance was expressed in fruit tissues distant from the yeast-treated sites. Several lines of evidence resulting from our present study indicated that induced resistance might be a mechanism by which the yeast *P. guilliermondii* resists *C. capsici* in harvested chili fruit. This evidence included changes in enzyme activity (PAL, EC 4.3.1.24; CHI, EC 3.2.1.14; β-1,3-glucanase, EC 3.2.1.39) and phytoalexin capsidiol accumulation. All these might contribute to reducing disease incidence, delaying lesion development, and suppressing the growth of *C. capsici* on yeast-treated chili fruit.

Prior application of yeast *P. guilliermondii* strain R13 at one site caused resistance against subsequent inoculations of the fungus by *C. capsici* in chili tissue at another site clearly separated from it. Disease incidence and rot development of chili fruit after inoculation with the fungal pathogen were markedly reduced with increased treatment times relative to those of the water-treated controls. Another contributing factor to the level of resistance was the distance of the *C. capsici* infected site from the *P. guilliermondii*-inoculated site, in that the resistance was greatest when the two sites were close together. Analysis of interaction effect between treatment and time of fruit inoculation, and treatment and distance from infected site also revealed the significant interaction effect on the resistance level indicating that the factors investigated as well

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as their interactions significantly influenced the induction of resistance in chili fruit. These results correspond with those of Droby et al. (2002), who found that the development of induced resistance in harvested grapefruit after *Candida oleophila* Montrocher strain 182 application was related to the duration of yeast inoculation, the distance of pathogen-inoculated sites from the yeast-treated wounds, and the yeast concentration. In addition, the same pattern of the reduction in lesion diameter caused by fungal pathogen after yeast treatment seems to be appeared in the study of the induction of resistance by Ghaouth, Wilson, & Wisniewski (2003). In their study with fresh apple, the antagonist and the pathogen were applied in spatially separated wound. They observed that when applied the yeast *C. saitoana* on the apple wounds with 48 and 72 h prior to inoculation with *B. cinerea*, lesion development was reduced by more than 50% and 70%, respectively.

One of the most studied mechanisms of plant induced resistance is the rapid accumulation of antimicrobial secondary metabolites know as phytoalexins (Hammerschmidt, 1999). A numerous studies have been shown that phytoalexins were induced by various elicitors (Kuc′, 1995). Many studies reported the ability of yeast antagonists to elicit phytoalexins in various harvested fruits. For example, application of the yeast *P. guillier mondii* induced the accumulation of scoparone and scopoletin even in the absence of pathogens in citrus fruits e.g. lemon and orange (Rodov et al., 1994). In grapefruit peel tissue following treatment with *C. oleophila*, the phytoalexin scoparone, scopoletin and umbelliferone were increased as reported by Droby et al. (2002).

In this study, the accumulation of capsidiol phytoalexin was used as an indicator of the induction of plant defensive response. The results of our study demonstrated that the *P. guilliermondii* strain R13 induced the production of capsidiol in necrotic tissue. Capsidiol was first detected at 12 h and continued to increase for 72 h. The result from fungitoxicity test indicates the antifungal activity of capsidiol extracted from yeast-treated chilies against the growth of *C. capsici*.

Induction of phytoalexins has long been considered to play a primary role in plant defense against pathogens (Chavez-Moctezuma & Lozoya-Gloria, 1996; Kuc′, 1995). To provide the plant protection from fungal invasion, phytoalexin must be fungitoxicity and accumulate sufficiently rapidly to inhibitory contents (Stoessl,

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Stothers, & Ward, 1976). The sesquiterpenoid capsidiol is a major phytoalexin in chili pepper (*Capsicum annuum*) plants (Stoessl, Unwin, & Ward, 1972), and its production is elicited either by pathogen attacks or treatments with various abiotic and biotic factors (Chavez-Moctezuma & Lozoya-Gloria, 1996). Capsidiol is absent from healthy, however, it is produced by healthy tissue. Capsidiol is likely to accumulate at the site of rotted tissue after infection (Adikaram, Brown, & Swinburne., 1988). The role of capsidiol in the defense response of chili pepper against fungal pathogens has been shown in several studies, and its accumulation in fruit tissue may inhibit fungal infection (Adikaram, Brown, & Swinburne, 1982, 1988; Egea, Alcazra, & Candela, 1996; Molot et al., 1981; Ward et al., 1973). The correlation between capsidiol accumulation and induced resistance has also been studied by several researchers both in chili plant and fruit. For instance, in immature chili fruit, application of glucans from the hyphal walls of *Glomerella cingulata* in the wound surface for 24 h or longer accumulated levels of capsicannol phytoalexin sufficient to inhibit rot development caused by *B. cinerea* (Adikaram, Brown, & Swinburne, 1988). In pepper plant, experiments on induced resistance revealed that a second inoculation of the stems by *Phytophthora capsici* resulted in a small wound development and in capsidiol accumulation. The greater amount of capsidiol accumulation, the smaller necrosis lesion on pepper stem. Thus, this study confirms the direct involvement of capsidiol in fungal inhibition and its relation with disease resistance (Egea, Alcazra, & Candela, 1996). In addition, Ahmed, Sánchez, & Candela (2000) also reported that treatment of pepper seeds and roots with *Trichoderma harzianum* isolate 2413 resulted in a high level of capsidiol accumulation in the pepper plant stem, and they concluded that this accumulation might have slowed the decay development caused by *P. capsici*.

PAL, CHI, and β-1,3-glucanase are known to be involved in plant disease resistance (Anand et al., 2009; Droby et al., 2002, Yao & Tian, 2005a; Zhao et al., 2008). PAL (EC 4.3.1.24, formerly EC 4.3.1.5) is a key enzyme in the first stage of phenylpropanoid metabolism leading to the synthesis several compounds of lignin, phenols, phytoalexins, and other compounds involved in a localized plant resistance process (Hahlbrock & Scheel, 1989; Shadle et al., 2003). PAL plays its role in deamination the amino acid L-phenylalanine to form trans-cinnamic acid and ammonia (McInnis, Clemems, & Kermode, 2008). Many reports have shown the evidence for

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induction of plant defense responses after elicitor application by showing higher gene production or activity of PAL. Likewise, acibezolar-S-methyl treatment in harvested yali pear fruit reduced disease incidence and lesion area rotted by *P. expansum*. In addition, enhanced resistance by ASM in pear fruit was associated with enhancing activity of several main defensive enzymes including PAL (Cao, Jiang, & He, 2005). Pretreatment sweet cherry with salicylic acid and methy jasmonate significantly reduced disease lesion diameter caused by *Monilinia fructicola*. Treatment of the fruit by these two chemicals induced activity of such key defensive enzyme like PAL (Yao & Tian, 2005b). Yedidia et al. (2003) reported that *Trichoderma asperellum* - inoculated cucumber root was shown to systemically inhibit the proliferation of *Pseudomonas syringae* in leaves. This reduction coincided with transcript accumulation of mRNA of defense-related gene, PAL and hydroxyperoxide lyse gene. In tomato and hot pepper, Ramamoorthy, Raguchander, & Samiyappan (2002) observed the reduction in damping-off incidence, when applied talc-based formulation of *P. flouencens* to the test plants. Also, pretreatment of both tomato and hot pepper by resulted in the induction of defense-related enzymes (PAL, peroxidase, and polyphenol oxidase) and chemicals in plant which these may involve in resistance against *Phythium* invasion.

CHI (EC 3.2.1.14) and β-1,3-glucanase (EC 3.2.1.39) produced in many plant species have been intensively studied and identified as the PR-protein that functions in the plant defense response (van Loon, Bakker, & Pieterse, 1998). These two hydrolytic enzymes have direct antifungal activity attack a number of fungi (Bowles, 1990; Edreva, 2005; van Loon, 1997) and in that they decompose β-1,3 glucan and chitin which are the principal components of fungal cell wall (Ruiz-Duenas & Martínez, 1996). Combination of CHI and β-1,3-glucanase shows stronger antifungal activity than when each enzyme functions separately (Mauch, Maunch-Mani, & Boller, 1988). β-1,3-glucanase has also been reported to have an indirect effect by degrading the fungal cell wall and then releasing an oligosaccharide elicitor that activates plant defense mechanisms (Tian et al., 2007).

The association of CHI and β-1,3-glucanase in the induction of defense response by a variety of inducing agent including yeasts have been demonstrated by several researchers. Particularly, many laboratories have intensively focused on the

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study with *C. annuum* species in the induced disease resistance. As reported by Hwang et al. (1997), spraying pepper plants with DL-β-amino-n-butyric acid protected *Phytophthora capsici* and it also induced the synthesis and accumulation of several isoforms of CHI and β-1,3-glucanase in plant stem tissues. Application of talc-based formulation of *P. fluorescenes* on chili seed effectively reduced the disease incidence in chili plant. Further, the expression of various defense-related enzymes (PAL, peoxidase; polyphenol oxidase; CHI, β-1,3-glucanase) and chemicals was found to involve in the induction of systemic resistance against *C. capsici* infection (Ramamoorthy & Samiyappan, 2001). The same result appears in a report of Anand et al. (2009) who elucidated the role of azoxystrobin and *P. fluorescenes* in the induction of systemic resistance. They found that the activity of defensive enzymes including CHI and β-1,3-glucanase was increased in azoxystrobin and *P. fluorescenes*-treated chili plants.

In the present study, application of *P. guilliermondii* strain R13 in chili fruit substantially increased PAL activity to a greater extent than that in the controls. As mention earlier, the key biosynthetic enzyme like PAL involves in the activation of phytoalexin systhesis in plant (Hammerschmidt, 1999). Thus, we though observed the increased PAL activity in the yeast-treated chili was correlated with an increase level of capsidiol phytoalexin. Similarly, CHI and β-1,3-glucanase activities were enhanced in chili fruit treated with antagonistic yeast. The increase in PAL, CHI, and β-1,3-glucanase activity in the yeast-treated chili tissue correlated with the reduction of disease incidence and the reduced diameter of lesions caused by *C. capsici*. Our results agree with those of Yao & Tian (2005a), who found that prior treatment with *Cryptococcus laurentii* (Kuffer.) Skinner in harvested peach fruit resulted in smaller lesions caused by blue mold *Monilinia fructicola* and *Penicillium expansum* Link; in that study, the antagonistic yeast induced higher activity of PAL, CHI, β-1,3-glucanase, and peroxidase, and the authors concluded that induced resistance might be the major mechanism by which the yeast inhibited the fungal pathogens. Additionally, Zhao et al. (2008) documented that the yeast *Pichia guilliermondii* strain CNM 2.1801 decreased the infection rate and rot development caused by *Rhizopus nigricans* on

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tomato fruit; the increased activities of defensive enzymes such as PAL, CHI, and β-1,3-glucanase correlated with the onset of induced resistance.

SEM revealed morphological abnormalities of the spores and hyphae of *C. capsici* in pathogen-infected wounds near sites inoculated with *P. guilliermondii* strain R13. The SEM micrographs also indicated that mycelial growth of *C. capsici* was restricted by proximity to the yeast. Distorted spore and hyphal morphology, including irregular constrictions and swellings, is common in the presence of fungal inhibitory substances (Clemons & Sisler, 1971; Richmond, 1975). As noted, treating chili with *P. guilliermondii* strain R13 enhanced the production of defense-related enzymes like PAL, CHI, β-1,3-glucanase, and phytoalexin accumulation. Therefore, it is possible that the increased production of these compounds resulted in abnormal *C. capsici* morphology and inhibition of *C. capsici* infection and disease. This same hypothesis appeared to show in the report of Droby et al. (2002) who observed the restriction of hyphal growth and spore germination of *P. digitatum* in inoculated wound near the yeast *C. oleophila*-treated sites. Droby et al. concluded that the presence of induced defensive substances (PAL, CHI, β-1,3-glucanase, and phytoalexin) in grapefruit tissue is likely to be involved in the inhibition and subsequent suppression of *P. digitatum* infection.

As reported by Chanchaichaovivat, Panijpan, & Ruenwongsa (2008), *P. guilliermondii* strain R13 can inhibit *C. capsici* by directly attaching to the hyphae, by competing for nutrients, and by producing fungal hydrolytic enzymes. The current study shows that yeast treatment also induces the accumulation of phytoalexin and increases the activity of the defense-related enzymes PAL, CHI, and β-1,3-glucanase, and that induced resistance is another potential mechanism by which the yeast suppresses the fungus. Taken together, the two studies suggest that multiple mechanisms of action might explain how the antagonist *P. guilliermondii* strain R13 provides effective control of postharvest chili anthracnose (Chanchaichaovivat, Panijpan, & Ruenwongsa, 2007). Understanding the potential mode of action of antagonist yeast in control of postharvest fruit disease is important and in that it could help us to obtain guidance for using them as alternative application in the future. However, other areas in the induction host resistance or new possible mode of action still need to be further studied. For instance, Xu & Tian (2008) pointed out that

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numerous studies were stressed on investigating in defense-related enzymes in order to study the induction of resistance. Less research is demonstrated the evidence in the area of antioxidant proteins induced by microbial antagonists in postharvest fruit. Based on their study, they concluded that the induction of antioxidant defense response may be an important mechanism which yeast *P. membranaefaciens* mitigating pathogen-induced oxidative stress to postharvest sweet cherry and control the disease. In 2003, Castoria et al. (2003). focused on the fact of rising in oxidative stress in plant tissues as a result of both wounding and pathogen infection. This fact led them hypothesized that an effective antagonist, like yeast, should possess resistance to oxidative stress to ensure rapid colonization in wound. Form their research, they found that the two yeasts, *Cryptococcus laurentii* and *Rhodotorula glutinis*, exhibited rapid colonization in the apple wound together with the ability to resist to reactive oxygen species-generated oxidative stress. Thus, Castoria et al. conclude that resistance to oxidative stress might be a new possible mechanism of action of antagonistic yeast against wound pathogen.

Several reports have shown the potential of *P. guilliermondii* as the biocontrol agent in a wide range of commodities against various fungal pathogens (Droby et al., 1989; McLaughlin et al., 1990; Paster et al., 1993; Rodov et al., 1994). Regarding the safety concern of effective and safe microbial antagonist, *P. guilliermondii* has also been shown to be non-toxic yeast antagonist by many researchers (Arras, Nicolussi, & Ligios, 1999; Mari & Guizzardi, 1998; Winiewski et al., 1991). Like other strains, *P. guilliermondii* strain R13 have been shown to be effective biocontrol agent for postharvest chilli anthracnose, therefore its safety should also be concerned and elucidated. In addition, replications of the similar study with the large-scale samples and in the field are needed to determine how *P. guilliermondii* strain R13 can be used commercially for controlling post-harvest rots in chili.

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5.2 Educational Aspect

5.2.1 Implementation of the Inquiry-Based Learning Unit

The main finding of this study was the demonstrated success of implementing the FPLU in the biology classroom. The FPLU helped students further their understanding of plant defenses. In addition, the students not only have positive attitudes toward the FPLU, but also have positive perceptions of plants and studying about plants.

In the beginning of this study, most of the students expressed little interest in learning about plants compared to animals as a result of several reasons. These results are consistent with the reveals of many educators such as Tamir & Jungwirth (1974), Wandersee (1986), Wandersee & Schussler (1999), Tunnicliffe (2001) and Strgar (2007). This present finding illustrated that many students considered plants as the inferior organism and valued animal life higher than those of plants. As suggested by Wandersee & Schussler (1999), these conditions are the result of zoochauvinism and plant blindness which some of such symptoms are thinking that “plants are merely the backdrop for animal life, failing to see, notice or focus attention on plants in one’s daily life, misunderstanding what plants need to stay alive, and overlooking the importance of plants to one’s daily affairs”. Preventing plant blindness as well as increase interest and appreciation of plants is considered to be an important and necessary issue. A promising strategy to alleviate the problem is the challenge of reforming plant biology teaching and curriculum to have a greater emphasis on plants studying for students in all levels (Carter, 2004; Hershey, 1992; Hershey, 2005; Uno 1994).

Additionally, before the intervention by the FPLU, most of the students had no idea about the plant defense mechanisms against the biological invaders such as microorganisms and insects. This result is similar those cited by Raven et al. (2005), Stamp (2004, 2005) about students’ alternative conceptions of plant defenses. Raven et al. (2005) explained that students normally view plants as helpless and passive organisms in the face of environmental dangers as a result of their lack of mobility. Students usually possess the alternative ideas in that plants have no option of ‘uprooting and walking away’. Furthermore, they also reserve the concepts of predator and prey relationship for animals. Therefore, students do not understand that plants are

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living organisms that would react to the plant predators (herbivores and microorganisms) by producing self-defense strategies for the purpose of survival. Stamp (2004) also described that the knowledge of plants defend system was surprising to most people because they eat only domesticated plants which are edible, therefore this lead them lack of the experiences that many wild relatives are toxic. More importantly, during the last ten years, it was found that there was less coverage and little information about the plant defense concept published in the biology textbooks (Stamp, 2005). These reasons may contribute to the misinformation and now it has become a misconception of many people about the lack of defense response of plants. Misunderstanding of the basic idea of plant defenses makes it difficult for people to see the unique characteristic of plants as the active and viable organism in the environment. As stamp (2005) noted, if students have a misunderstanding in this concept, they may not appreciate and understand how we have had to work with the plant defenses leading to the consequences for pest control and future agriculture. Based on the idea explained by Raven et al. (2005), Stamp (2004, 2005) and taken together with the view of zoochauvinism and plant blindness, all factors might contribute to students' alternative conception on plant defense.

After the FPLU was implemented, the students refined their views of plant defense mechanisms. Post-test scores were significantly higher than those of the pre-test and the students' concept maps showed relevant concepts regarding plant defenses. In addition, post-interviews revealed that the students no longer viewed plants as passive and helpless organisms, but perceived them to have their own active and unique defense characteristics. The students explained that they altered their concepts of plants and constructed their understanding through hands-on experiences, collecting and analyzing data, reading and discussion in groups with their peers. These findings are similar to those of Hofstein & Lunetta (2004), Hofstein & Mamlok-Naaman (2007), Lazarowitz & Tamir (1994) and Tobin (1990) who pointed out the significant benefits of providing students' the opportunity to perform hands-on laboratory investigations. They indicated that hands-on experiences can promote students' understanding of complex concepts as well as enhance their interest and motivation. The effectiveness of conducting investigations and data analysis and opportunities for discussion is similar to that found by other studies (To-im &

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Ruenwongsa, 2009; Duit & Glynn, 1996; Gilbert & Priest, 1997) in that such activities help students generate reasonable explanations by thinking critically and sharing with their peers.

Additionally, the students indicated that the selected readings from various learning resources were also instrumental in their understanding of plant defense mechanisms. Our student perceptions derived from their readings are consistent with other educators. For example, Chiappetta (1997) and Osborne (2002) have suggested that active reading and information gathering from various resources such as articles in books, scientific magazines, or the internet could be designed as inquiry-based activities to promote students' learning.

The students in our study also perceived small group work and teacher facilitation as factors contributing to their understanding of plant defense mechanisms. These results are in accordance to the views of many educators. For example, Van Zee et al. (2005) pointed out that learning by students who work together in small groups allows them to collaborate in developing arguments and explanations with classmates, as well as refine their thinking with regards to specific concepts. With this benefit, group work is widely recognized as a promising teaching method that promotes students' learning through the process of socialization (Cohen, 1994).

At the heart of inquiry instruction, students are active learners that generate their own knowledge, however, they also need substantial facilitation from teachers (Bell, Smetana & Binns, 2005; Driver et al., 2004). As noted by Crawford (2000), this kind of guided-inquiry demands a significant level of teacher involvement and the teachers' role as that of a facilitator or a guide. Our findings indicate that the guided-inquiry format of the FPLU is effective in promoting the development of students' science concepts related to plant defenses. These findings correspond with the existing reports by several researchers such as those of Chang & Mao (1999), Farrell, Moog, & Spensor (1999), Lewis & Lewis (2008), NRC (2000), and Penner, Lehrer & Schauble (1998).

To focus on students' perception of plant study, at the end of the unit, we observed that the students expressed more interest and appreciation in learning with plants. Through post-interviews, journal and CLES questionnaire, students also revealed their positive feelings toward the FPLU. In this unit, the students were

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provided opportunities to see plants in a new light as an active organism different from their prior perceptions. By using various inquiry-based activities, students perceived plants as active creatures which can fight back to the invaders in diverse ways. These findings indicated that the use of appropriate teaching activities, methods, and materials as well as selecting appropriate content relevant to students' everyday experiences could influence students to change their perceptions about learning from plants. This finding is consistent with the suggestion of Bowker (2004), Hershey (1992), and Senchina (2008). More importantly, when shown a new perspective of plants, students' interest in learning with plants was aroused (Strgar, 2007).

Regarding the CLES questionnaire analysis, the results suggested that all the CLES scales possess acceptable internal consistency (George & Mallery, 2003) except the *Scientific Uncertainty* scale (0.54 of pre-test CLES and 0.56 of post-test CLES) which was similar to those reported by Kim, Oh, & Yang (1999) and Taylor, Fraser, & Fisher (1997). Several studies have found that the students generally do not have a good understanding of the tentative nature of scientific knowledge (Lederman, 1992; Lederman & O'Malley, 1990) which may possibly be a factor leading to the low reliability of the *Scientific Uncertainty* scale from the CLES survey. In addition, there were significant perceptual differences on all six scales between the pre-test and post-test CLES. The result suggested that the students perceived that the FPLU learning environment is more constructivist-oriented than the previous biology class. The most predominant difference occurred on the *Shared Control* scale. This finding revealed that students perceived more opportunities to have a shared role with the teacher, which included; participating in the classroom planning and conducting and assessing of the learning activities (Taylor, Fraser, & Fisher, 1997). In terms of *Personal Relevance*, students perceived that the learning exercises in the intervention relevant to their out-of-school experiences. In the scale of *Critical Voice*, they felt that it is legitimate and beneficial to express a critical opinion about teacher's pedagogical plans and methods regarding to the quality of their learning activities. Result of *Student Negotiation* scale indicated that students perceived more involvement in the mutual interaction to justify to others on building new knowledge. In terms of *Attitude scale*, the finding reflected that students seemed to enjoy the learning activities. Whereas, the results showed the smallest difference between the pre-test and post-test

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scores occurred on the *Scientific Uncertainty* scale indicating that students perceived low learning experiences related to the tentative nature of science knowledge from the unit. This result suggests that the FPLU should place more emphasize on providing opportunities for students to learn about the provisional status of scientific knowledge.

To assess the overall perception of the FPLU through the interviews taken together with the journal and the CLES questionnaire, most students responded favorably to the unit. They indicated a preference for learning through active involvement with materials and one another and with content that is relevant to their everyday lives. They also mentioned the involvement and facilitation of the teacher in a way that helped make the classroom atmosphere relaxing and comfortable, allowing them to express their opinions during class exercises. This latter finding is similar to that purposed by Strgar (2007) who contends that teacher involvement and management are major factors in influencing student interest. In addition, several other researchers such as Lawrenz (1976), Fouts & Mayers, 1992, Fraser & Fisher (1986), and Fraser (1994) claimed that positive students' perception of the classroom environment is an important and integral factor in promoting students' learning. Similarly, in this study, most students responded favorably to the constructivist classroom environment. Therefore, it appears that the positive students' perceptions of the constructivist classroom environment also contributed to the success of the FPLU.

5.2.2 Limitations of the Research Study

Based on an interpretative paradigm, this study was therefore conducted without comparing student outcomes with the control group. Even though, the overall findings indicated the successfulness of the learning unit implementation, this present study also has some limitations. Not all of the students were happy with the way the unit was presented. A few students disliked the laboratory exercises which take a few days to complete. Instead, they would prefer an investigation in which they could obtain results immediately or within a class period. It appears that additional readings focusing on the labor intensiveness of most scientific research might help these students see how the FPLU investigation more closely resembles actual scientific studies. In addition, most of students expressed an interest in experimenting with a variety of plant species, rather than only working with hot chili fruits as provided as a

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plant learning model. They felt that using only one plant species did not provide enough information to form a general conclusion about the defense mechanisms of plants. For example, one student said: “I worked with only chilies, how can I know that this kind of chemical defense response will be produced in the same way by others [other plant species]”. To meet this student’s concern, additional tests, like the one done with the chili fruit, could be devised for several species of plants.

By their nature, inquiry-based activities take more time than those that use a more didactic teaching approach. Therefore, it is not surprising that students who are used to merely receiving information tend to reject a more active approach. For example, one student said: “I prefer to learn by being directly told the main idea of the lesson”. Therefore, before using the FPLU, it might be necessary to involve students in some inquiry-based classroom activities and active reading exercises to help them make the transition from passive learning to a more active format. In addition, this current study was carried out with only one group of science students from one school. Therefore, our conclusions would be stronger if the FPLU was used with a different student population and with a larger number of students. In addition, as indicated by several researchers such as Hong, Shim, & Chang. (1998), Prokop, Prokop, & Tunnicliffe (2007), and Trumper (2006) girls have a greater preference of studying about plants than boys. In this study, most of students were girls (71%) reflecting the present gender bias in this study. In the future, perhaps a more equal number of boys and girls should be set up to control this variable.

5.2.3 Educational Implications

This study has raised the issue concerning on the studying about plants in Thai high school students as well as promoting an essential plant science concept of plant defenses for students. Based on the findings in this study, it was found that Thai high school students showed little interest in studying plants than those of animals. Furthermore, they have some alternative conceptions in that plants are just passive and helpless organisms which are unable to defend themselves against invaders. From the findings of plant paucity situation, this study suggests the Thai educators and teachers have to pay more emphasis on the teaching on plants for not only high school students but also all educational levels. Addressing students’ alternative conception of the plant

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science concept is very important and essential to focus on the concept that are potentially confusing and misunderstanding to students. This can help us to develop a proper way to help students develop their understanding correctly. More importantly, we need to provide more coverage of a plant study in biology curriculum and present plants as the viable, interesting and important organisms like animals or even human. In addition, the selected plant content should be relevant to student's everyday experience. Likewise, in this unit, the topic of plant defense was selected to promote a fundamental plant concept that students should learn as well as to alter their alternative understanding of plants. Furthermore, this topic also provided students to learn about a new perspective, and interesting and surprising information of plants. The acquired knowledge of the study can be also applied to use in the eco-friendly agricultural practice.

In the present study, an inquiry-based approach and constructivist perspective were used to frame both the learning unit development and implementation. From the findings, this learning unit has proved to be successful teaching intervention. It can be used as a guideline to develop other guided-inquiry-based instruction on any other topics that focuses on students to acquire the new knowledge by their own construction through the process of questioning, investigating, observing, collecting and interpreting data, discussion and drawing conclusion which is useful for knowledge construction of the students. Students should be more given to learn actively from direct experiences through multi-inquiry-based tasks. Based on the success of designed activities implemented in this unit, it is important to note that not only the laboratory experiment that can be used as the inquiry-based activity, but also other strategies such as data analysis and discussion and gathering information by reading can be designed as the inquiry-based activities which is useful for promoting students' learning. Additionally, for the knowledge construction, students should be encouraged to work collaboratively in doing activities with their peers and in that they can share, compare, critique and discuss ideas to each other. It is also suggested that teachers should have greater involvement in the learning process by playing their role as the facilitators who engage students to learn through the cognitive process, provide learning materials, share ides and discussion, and give suggestions and reflection on students' learning.

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Regarding the classroom learning environment, this particular learning unit was implemented based on the constructivist learning environment (CLE). The results revealed that the students perceived the learning environment in the unit were more constructivist-oriented as evidences from preferences in participating in learning through several characteristics of the CLE. From the findings, it can be noted that the CLE is a factor which contributes to the success of students' learning. This study has led to a suggestion that the teachers should pay attention to create the proper learning environment that fits with student preferences. In addition, teacher should focus on providing students (1) to learn the science content relevant to students' real life, (2) to learn about the provision status of scientific knowledge, (3) to express their critical opinion regarding to the teaching and learning in the classroom, (4) to involve in classroom planning and management, (5) to share, discuss and justify the ideas with their peers, and (6) to participate in the interesting and joyful activities.

After the unit intervention, students' perception of plant study was likely altered to the positive way. They seemed to have more interest in studying with plants. The study suggests that plants can be an interesting topic if the teachers provide appropriate insight of plants, and use appropriate teaching method and environment.

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CHAPTER VI
CONCLUSION

6.1 Scientific Aspect

The research project to investigate the ability of the yeast antagonists isolated from tropical fruits and vegetables to control chili anthracnose has been initialized and reported by Chanchaichaovivat, Panijpan, & Ruenwongsa (2007, 2008). Among several yeast strains, *Pichia guilliermondii* strain R13 isolated from Thai rambutan showed highest efficacy in suppressing anthracnose disease caused by *Colletotrichum capsici* in harvested chili fruit. Its multiple modes of action include nutrient competition, tight attachment to the fungus, and hydrolytic enzyme secretion are involved in the control of anthracnose fungus. However, another possible mechanism of action in which the strain R13 induces host resistance has not yet been elucidated. Thus, this present study aimed to investigate the ability of the *P. guilliermondii* strain R13 to induce resistance against *C. capsici* in chili fruit.

This study have used various scientific evidences to determine the exhibition of induced resistance after yeast treatment including the reduction of disease incidence and rot diameter in chili after subsequent infection by *C. capsici*, and the expression of defensive-related proteins (phenylalanine ammonia-lyase, chitinase, and β-1,3-glucanase) and defensive substance (capsidiol phytoalexin). Based on the findings of this study, the yeast antagonist treatment significantly reduced disease incidence and lesion diameter caused by *C. capsici* in the chili. The yeast treatment also significantly enhanced the activities of phenylalanine ammonia-lyase, chitinase, and β-1,3-glucanase, and the accumulation of capsidiol phytoalexin in chili tissue compared to the controls. The observation under the scanning electron microscope illustrated the abnormality of spores and hyphae of *C. capsici*. It was also found that the fungal pathogen had restricted growth on chili tissue near the yeast-inoculated wounds. In this case, it is possible that the increased production of these defensive compounds resulting in the abnormal *C. capsici* morphology and inhibition

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of *C. capsici* infection and disease. These evidences indicated that the *P. guilliermondii* strain R13 has ability to induce several biochemical defense responses of chili fruit, and the induction of disease resistance may be another possible mechanism of the yeast strain R13 in controlling postharvest chili anthracnose.

6.2 Educational Aspect

As a consequence of the preliminary study, it revealed that Thai high school students expressed less interest in studying of plant. They also possessed an alternative conception in that plants are passive organisms and incapable to defend themselves to the invaders. In addition, the topic of plant defense mechanism seems to overlook in the Thai high school biology curriculum. To promote students' conceptual understanding in the concept of plant defense mechanism as well as their perception on plant study, this study therefore developed a novel inquiry-based learning unit focusing on the topic of plant defense responses for high school students.

The findings from the unit intervention clearly demonstrated that the students could construct their own conceptual understanding through their active participation in the inquiry-based activities as measured by the significantly increase of the post-test score of the conceptual test and concept map. The results from student interview and journals revealed positive students' perception of plant study due to the new perspective and interesting of plant content and activities in the unit. Regarding to the CLES questionnaire, it was shown that students perceived more constructivist-oriented learning in the classroom. When the CLES questionnaire was co-analyzed with the interview and the journals, the preferences toward the learning unit of students were revealed.

The main contribution of this study is the development of an effective learning unit framed with an inquiry approach and constructivist epistemology. The novelty of plant science content and exercises relevant to their everyday experience were incorporated in the learning unit and in that the high school students in grade twelfth were challenged to investigate their new knowledge of plant defense mechanisms through such diverse guided-sequence activities. In addition, this study demonstrates the importance of creating the proper classroom environment implemented with inquiry-based teaching where students are joyful with the activities,

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actively involved in planning classroom activities, expressing critical opinions, communicating with peers and teachers, and where they find personal relevance in what they are studying. Lastly, it is also interesting to note that even though students normally prefer to learn with animals rather than plants, learning activities that are based on appropriate teaching strategies and environment can promote student understanding in plant science concepts as well as enhance their interest and positive perception in topics where they have previously shown little interest like plant science.

6.2.1 Recommendations

6.2.1.1 Recommendations for Further Study

The participants involved in this study were only 31 science students from a high school in the central Thailand. Due to the small sample size used in this study, it may contribute less reliability in the research findings. Therefore, additional group with a larger number of science students in different school areas should be included in a further study to ensure the effectiveness of the learning unit on students' learning achievement.

6.2.1.2 Recommendations for Further Development

Based on the research finding, it indicated that Thai science curriculum for the students in all grade levels should be revised by placing a greater coverage on plant study. Curriculum developer should implement novel and interesting plant contents as well as develop several hands-on activities incorporated in the textbook that present plants as the critical and interesting organism of our world. Concerns on the negative perception of the students on plant study related to students' alternative conception of plants also need to be distributed to the teachers. Teachers should be well trained in teaching about plant regarding the body of plant knowledge and the pedagogy. Additionally, the teachers should use appropriate plant activities as well as teaching method and environment to help students develop their understanding in a unique characteristic of plants and help them realize the importance of plant study.

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APPENDIX

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APPENDIX A

Guided protocol to investigate laboratory experiment

"How can plants fight back?"

Materials:

- red chili fruits
- *S. cerevisiae* suspension ($5x10^8$ cells ml^{-1})
- *R. stolonifer* suspension ($5x10^4$ spores ml^{-1})
- dropper
- cork-borer (6-mm, 10-mm diameter)
- motar and pestle
- separating funnel
- cheesecloth
- TLC solvent system (hexane:acetone, 1:1, v/v)
- glass vial
- microcap (5µl)
- TLC silica plate
- TLC chamber
- hair dryer
- plastic chamber
- tissue paper
- n-hexane
- vanillin reagent

Procedure:

1. Wound the chilies by using a sterile cork-borer (3 wounds per fruit, 2-mm deep and 6-mm wide).
2. Place one drop of yeast suspension (*S. cerevisiae*) on each of chili wound and allow it to air-dry. Then, incubate the chilies at 28°C for 2 days. In the chili control group, place the sterile distilled water on each wound in stead.
3. Remove chili wounds with a sterile cork-borer (10-mm diameter).
4. Homogenize 5g of chili tissue with 15 ml of n-hexane. Then, filter tissue debris through the cheesecloth.
5. Separate the upper layer of chili extract by a separating funnel and transfer to a glass vial. Then, place the extract in a fume hood for two days.
6. Re-suspend the dried extract with 0.5 ml n-hexane and spot the chili extract (5µl) on the TLC silica plate.

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7. Place the TLC plate in the chamber containing a solvent system.
8. When the plate is completely developed, spray vanillin reagent over the plate and then heat it up with a hair dryer. Observe the changes on the plate (A).
9. To perform the fungitoxicity test, after spotting the chili extract on the plate and developing the plate completely, spray the fungal spore over the plate and incubate it the moisten plastic chamber at 28^{o}C for 5 days. Then, observe the changes on the plate (B) compare with the result in step 8 (plate A).

Note: Safety precautions

For safety, students should aware these cautions.

1. Wear laboratory gloves while handling the chilies to avoid skin irritation.
2. Use aseptic techniques while working with the microorganisms.
3. Conduct experiments that work with the organic solvent in fume hood.
4. Wear appropriate protection including laboratory gloves and splash-proof goggles throughout the work with the organic solvent.

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APPENDIX B

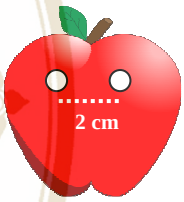
Experimental Data Analysis and Discussion Activity

"Investigation a Scientific Study of Ghaouth, Wilson, & Wisniewski

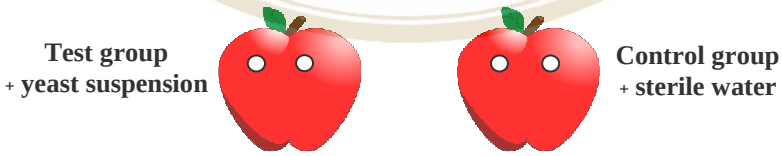
Instruction: The scientific method below is a part of an experiment conducting by a group of plant scientist*. Read the procedure carefully and think critically about the possible result and its explanation.

Procedure

1. Fresh apples were divided into two groups, test group and control group. Each group contained of 10 apples. On each apple, it was made 2 surface wounds by a razor blade (3 mm wide by 5 mm deep) at areas approximately 2 cm apart.



2. In the test group, each wound was inoculated by 35µl of a yeast suspension (*Candida saitoana*), whereas each wound of the controls was placed by the sterile water at the same amount of yeast suspension. Then, the apples were incubated at various time intervals (0, 24, 48 and 72 h) at 20°C.



3. After incubation, a second wound (5 mm wide by 1 mm deep) was made 1 cm apart from the 2 initial wounds and then 10 µl of plant fungal suspension (*Botrytis cinerea*) was placed into the second wound. All apples were kept at 20°C for a week.

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Questions

1. After incubation for one week, do you think what might happen to the second wound of each group of apple? Why do you think like that?

   Prediction:

   ……

   ……

   ……

   Explanation:

   ……

   ……

   ……

Note

Next, you will be provided the experimental result of this research study. Analyze the result and think critically on the possible explanation of the result, and then answer the questions below.

Questions

What did you observe and analyze from the experimental result? Can you explain why the result be like that?

Observation:

……

……

……

Explanation:

……

……

……

* The study of Ghaouth, Wilson, & Wisniewski et al. has published in the journal "Phytopathology" as cited as, Ghaouth, E.I.A., Wilson, L.C., & Wisniewski, M. (2003). Control of postharvest decay of apple fruit with *Candida saitoana* and induction of defense responses. *Phytopathology*, 93, 344-348.

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Experimental Result of Ghaouth, Wilson, & Wisniewski

Procedure After inoculation the apples for a week, the second wound of the apples in each group was measured the lesion diameter. The experimental result is illustrated in Figure 1.

Result

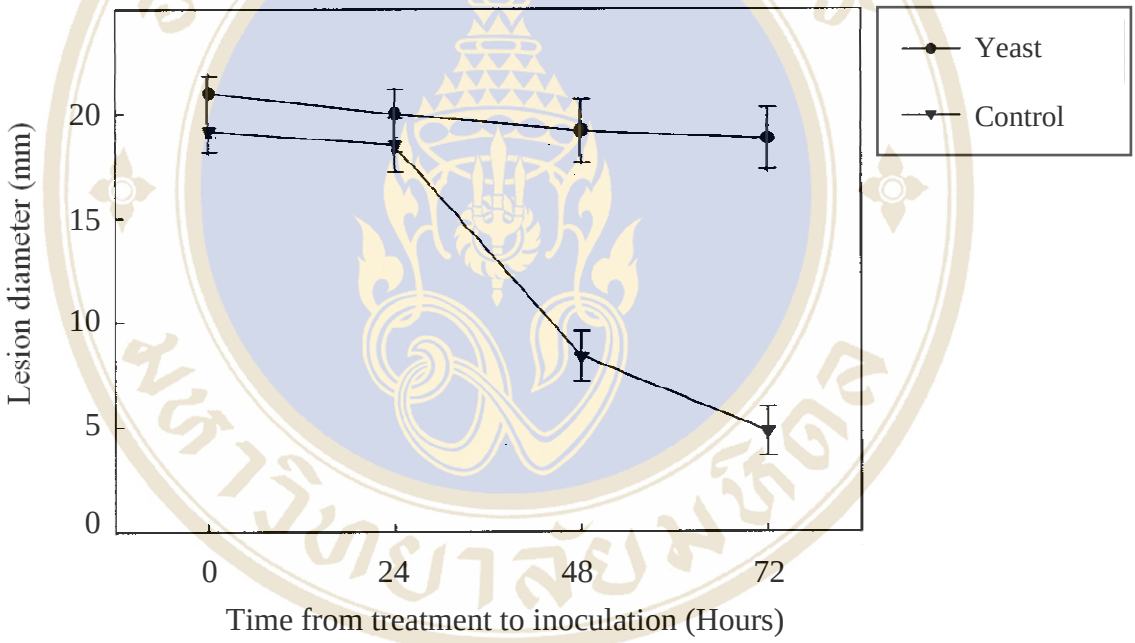

Figure 1 Lesion development of the apples of yeast-treated group compared to the control.

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Experimental Data Analysis and Discussion Activity

"Investigation a Scientific Study of Nantawanit et al."

Instruction: The scientific method below is a part of an experiment conducting by a group of plant scientist*. Read the procedure carefully and think critically about the possible result and its explanation.

Procedure

1. Fresh chilies were divided into two groups, test group and control group. Each group contained of 30 chilies. On each chili, it was made a surface wounds by a cork-borer (6 mm wide by 2 mm deep).

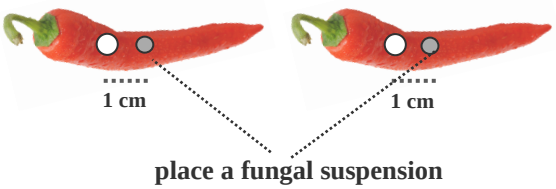
2. In the test group, each wound was inoculated by 30µl of a yeast suspension (*Pichia guilliermondii*), whereas each wound of the controls was placed by the sterile water at the same amount of yeast suspension. Then, the chilies were incubated at various time intervals (0, 24, 48 and 72 h) at 20°C.

Test group
+ yeast suspension

Control group
+ sterile water

3. After incubation, a second wound (5 mm wide by 1 mm deep) was made 1 cm apart from the initial wound. Then, 10 µl of a suspension of plant fungal pathogen (*Colletotrichum capsici*) was placed into the second wound. All chilies were kept at 28°C for 5 days.

Test group



Control group

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Questions

1. After incubation for 5 days, do you think what might happen to the second wound of each group of chili? Why do you think like that?

   Prediction:

   ……

   ……

   ……

   Explanation:

   ……

   ……

   ……

Note

Next, you will be provided the experimental result of this research study. Analyze the result and think critically on the possible explanation of the result, and then answer the questions below.

Questions

1. What did you observe and analyze from the experimental result? Can you explain why the result be like that?

   Observation:

   ……

   ……

   ……

   Explanation:

   ……

   ……

   ……

* The study of Nantawanit et al. will be submitted to publish in the journal "Biological Control" as cited as, Nantawanit, N., Chanchaichaovivat, A., Panijpan, B., & Ruenwongsa, P. (2009). Induction of defense response against *Colletotrichum capsici* in chili fruit by the yeast *Pichia guilliermondii* stratin R13.

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Experimental Result of Nantawanit et al.

Procedure After inoculation the chilies for 5 days, the second wound of the chilies in each group was measured the lesion diameter. The experimental result is illustrated in Figure 1.

Result

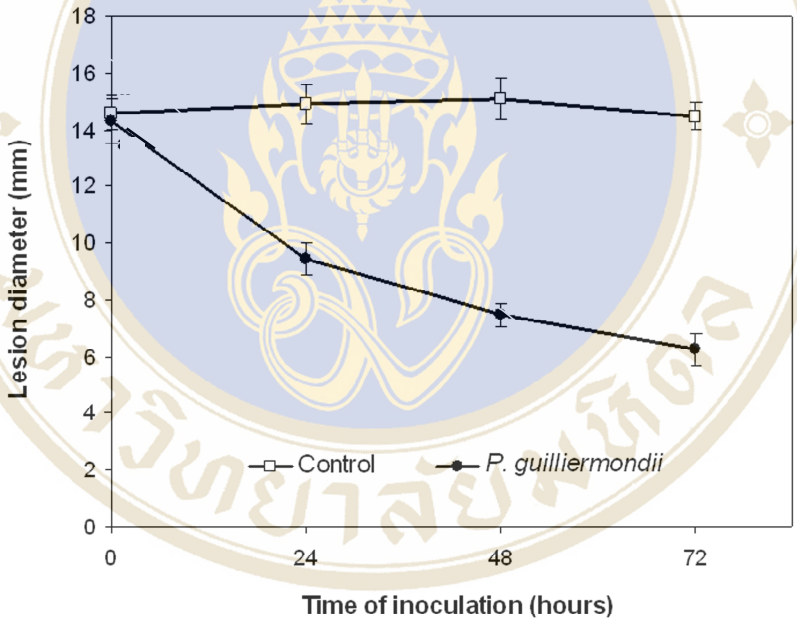

Figure 1 Lesion development of the chilies of yeast-treated group compared to the control.

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APPENDIX C

CONCEPTUAL UNDERSTANDING TEST ON PLANT DEFENSE RESPONSES

Instruction: For each question, please check √ in the box that you think is the best answer and circle the letter as the reason for your choice.

1. Unlike animals, plants are unable to protect themselves against pathogens by producing a defense system.

□ True □ **False**

Reason

a. As organisms, plants are lower than animals, therefore they cannot develop any kind of defense responses.

b. A self-defense response against diseases is a basic response of living organisms which is also found in plants.

c. Plants can protect themselves by producing the defense system as animals with less effectiveness.

d. Plants have no a blood circulation system. Therefore, there are no white blood cells and antibodies against pathogens.

2. Animals are able to warn a signal to their group in many ways such as producing pheromones, making sounds and gestures. Plants are also able to release signal used to warn their friends.

□ **True** □ False

Reason

a. When plants are invaded by insects, they will generate volatile compounds to give a signal to neighboring plants.

b. When plants are invaded by insects, they will generate some chemicals in the pollens. Wind will facilitate these pollens to give a signal to other plants.

c. Although plants are not able to generate and warn a signal by themselves, chemicals generated from the insect pests are able to stimulate other plants for the awareness of this invasion.

d. Plants are not able to make sounds or don't have a sensory system. Therefore, the reception and transmission of signals in plants are impossible.

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3. If pathogens could successfully infect to any area of the plant cells, they will spread out soon and infected other plant cells more easily.

□ True □ **False**

Reason

a. Other cells that have not been intruded will response by generating defensive substances against the pathogens

b. The invasion of pathogens makes the balance of cells lost.

c. If plants are invaded by pathogens, the metabolism of plants will be decreased.

d. The way that plant pathogens used to destroy other areas of plant cells is not changed.

4. The defense system of plants is different from those of animals

□ **True** □ False

Reason

a. Plants and animals are different in terms of physical and biological structures. So, they need to create their own systems that fit for their survival.

b. Like animals, plants produce antibody to resist pathogens.

c. The defense system of plants resists only to the microbial that causes the disease, but the immune system of animals affects all types of invasive microorganisms.

d. Plants do not have the defense system because the composition and structure of plant cells are not developed completely as in animals.

5. Plants cannot protect themselves from the attack of the insects.

□ True □ **False**

Reason

a. Plants protect themselves from the insect attacks by producing substances to stimulate the death of plant cells and in that it makes insects do not continue to eat those plant tissues.

b. Plants can not mobile. Also, they have no organ that is developed as animals. So, they cannot protect themselves from the invasion of insects.

c. Plants can protect themselves from insect attack by producing some defensive chemicals that affect directly to insects.

d. Plant cells are not developed as animal cells. Therefore, plants have no any mechanism of resistance against the insects.

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6. The death of plant tissues around the area invaded by the microbial is an evidence that plants cannot protect themselves from microbial infections.

□ True □ **False**

Reason

a. If plants can protect themselves against the pathogens, plant tissue should continue to look normal or close to normal after pathogen infection.
b. The death of plant tissue makes the tissue around weaker in that the microbial can destroy the plant cells more easily.
c. The death of plant tissue causes the signals to stimulate the production of plant defensive chemicals against microorganisms.
d. The death of plant tissue causes the lack of food sources for the pathogens. It is a strategy that plants use for protecting themselves.

7. When plants are invaded by microorganisms, they will produce some enzymes to digest the microbial cell walls.

□ **True** □ False

Reason

a. It is a kind of plant defense strategy to protect themselves from the pathogens.
b. Plants are unable to produce any kind of enzymes.
c. When plants are invaded by pathogens, plants generate other chemical substances to destroy microbial, not enzymes.
d. In general, plants produce these kinds of enzymes all the times, not only when they are invaded.

8. In general, plants are able to respond to the insect attack.

□ **True** □ False

Reason

a. The response to the invasion of pests is a basic mechanism of plant self-defense.
b. Plants have a defense mechanism which works only against insect larvae.
c. Not all plants can combat pests by producing defense response.
d. Only plants with higher evolutionary level are able to resist pests.

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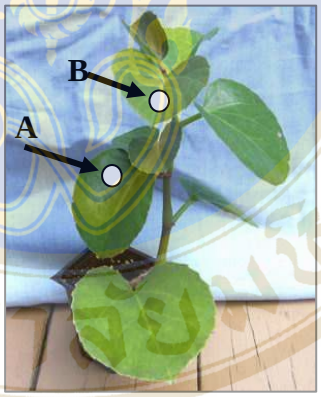
9. The plant defense against pathogens can be elicited by some microbial strains. This strategy is useful for agricultural applications.

□ **True** □ False

Reason

a. This defense mechanism may cause the mutation in plant pathogens and then they are difficult to eliminate the pathogens.
b. This mechanism may cause the adaptation in plants and then plants may develop powerful defense system.
c. This mechanism may cause the mutation of plant genetics, and in long-term, it will be harmful to the consumers.
d. This mechanism may alleviate the plant diseases by inducing plant antimicrobial chemicals.

10. Based on the image below, if a fungus invades a plant leave at the location A. Then three days after, location B is infected by the fungus in the same manner. The size of lesion caused by the fungal infection should be different in that the lesion B should likely to be smaller.



□ **True** □ False

Reason

a. Lesion B is smaller because it is near the plant stem where plant cells are more active and ready to divide.
b. **Lesion B is smaller because there is a signal from the plant area A triggering plant defense system to protect themselves from the subsequent invasion of pathogens.**
c. Lesion A is smaller because it is infected at first time when the plant is still healthy.
d. Lesion A is smaller because the infected leave is at the lower part of the plant. Plant cell walls of the lower leaves are thicker and harder.

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11. The death of plant cells in the area that is invaded by the pathogens is beneficial to plants.

True False

Reason

a. The death of plant cells is a way to restrict a food source for the pathogens.

b. The death of plant cells indicates the severity of the disease.

c. Whenever the plant cell death occurs, it will induce other plant cells die at other areas.

d. Whenever the plant cell death occurs, it will produce the volatile substances that destroy microorganisms.

12. Phytoalexin is a substance that plants generate and adversely affect to the invasive microorganisms.

True False

Reason

a. Phytoalexin is a substance to facilitate the division of pathogens.

b. Phytoalexin could help plant cell walls thicker and protect them from the pathogen invasion.

c. Phytoalexin is a substance that shows defensive activities against pathogens and it is a mechanism for plant protection.

d. Phytoalexin is a substance to facilitate the division of new plant cells after destruction by pathogens.

13. If plants are invaded by insects, plants will produce some volatile organic chemicals (VOCs) releasing to the atmosphere.

True False

Reason

a. The release of VOCs is a defense mechanism of plants in response to insect attack.

b. The release of VOCs is a defense mechanism of plants in response to invasive pathogens.

c. The release of VOCs is a defense mechanism of plants in response to all kinds of plant enemies.

d. The healthy plants generally produce VOCs and release to the atmosphere.

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14. In agriculture, the spray of jasmonic acid to plants should be avoided.

□ True □ **False**

Reason

a. Jasmonic acid is an attractive substance to the insect-herbivores.
b. Jasmonic acid may affect on the genetics of insects.
c. Jasmonic acid could stimulate plants defense system to produce some chemicals against insects
d. Jasmonic acid is an organic compound that insects do not like its smell. So, it helps to repel insects.

15. When a plant is invaded by insects, it is possible that the same plant specie in neighborhood will be less destroyed.

□ **True** □ False

Reason

a. Insects can produce and then release a substance to induce other insects to destroy plants in the neighborhood.
b. Lesions on damaged plants will release some compounds and induce other insects to destroy plants in the neighborhood.
c. Damaged plants can release a substance that helps coating other plants' leaves to prevent the subsequent attack from the insects.
d. Damaged plants will emit a signal to warn neighboring plants to be ready in producing a chemical for protecting themselves.

16. All plant species have defense mechanisms to protect themselves from the enemies such as insects and microbes, but the level of protective mechanism will vary due to the plant species.

□ True □ **False**

Reason

a. The resistance mechanisms of plants can respond to microbial infection but no any effect on insect attacks.
b. The self-protection is the basic response of all plant species.
c. Only plants with higher level of evolution have genes for construction a defense response to pests.
d. The response by producing resistance is a basic mechanism of only plants that were only severely damaged.

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17. Some yeast strains can be used to prevent and control plant diseases as an alternative strategy for plant protection.

□ **True** □ False

Reason

a. Most yeast strains can cause disease in plants.
b. Some yeast strains induce some plants to generate defense mechanisms.
c. Yeasts produce substances that are beneficial to the growth of plant pathogens.
d. Yeasts produce some anti-microbial compounds.

18. Produced oxygen and reactive oxygen species in plant cells are beneficial to microorganisms.

□ True □ **False**

Reason

a. Radical oxygen can cause the death of microbes.
b. Radical oxygen can stimulate plant metabolism.
c. Radical oxygen can enhance the severity of the plant disease.
d. Radical oxygen has no any effect on microbes.

19. After microbial invasion, plant cell membranes work for recognition and sending signal to intracellular plant cell.

□ **True** □ False

Reason

a. Plant cell membranes have receptors that are specific to bind to the microbial cell wall component.
b. The membranes have channels that allow the chemicals produced by pathogens transmit into the plant cells.
c. Specific receptors that bind to the component of microbial cell walls are situated at plant cell walls.
d. Plant cell walls have specific pores that allow the chemicals produced by pathogens transmit into the plant cells.

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20. Synthetic chemicals that have properties similar to volatile organic compounds (VOCs) produced by damaged plants can use to prevent the crop damage caused by insect attack.

□ **True** □ False

Reason

a. These synthetic substances attract the natural enemies of insect pests.

b. These synthetic substances are very toxic to insect pests.

c. These synthetic substances do not affect to the pests because they are non-natural substances.

d. These synthetic substances attract more species of insects to destroy plants.

21. Enzymes that inhibit the protein digestion of the insects (proteinase inhibitor) are produced in plant cells and are useful to plants.

□ **True** □ False

Reason

a. Proteinase inhibitor inhibits the protein digestion and it is toxic to insects.

b. Proteinase inhibitor inhibits the protein digestion in that it causes malnutrition in insects

c. Proteinase inhibitor inhibits the protein digestion and it is toxic to plant cells.

d. Proteinase inhibitor inhibits the protein digestion in that plant cells lack of essential nutrition used for cell growth.

22. The increase of jasmonic acid in plant cells of damaged plants indicates the higher level of plant defense responses against insects.

□ **True** □ False

Reason

a. Jasmonic acid induces more insect pests to damage crops.

b. Jasmonic acid is an indicator of deteriorated cell due to the insect attack.

c. Jasmonic acid induces the defensive chemical production in plant cells that are resistant to insects.

d. Jasmonic acid is not related to any of plant resistant mechanisms to the insects.

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23. Predators of the insect pest receive chemical signals from the damaged plant to attack the insect pests.

□ **True** □ False

Reason

a. Wound lesions in plants caused by insect attack have a specific type of chemicals that attract to the insect-predators.
b. The pheromones released from the insect pests attract the insect-predators to kill the pests.
c. Damaged plants release volatile organic compounds to attract insect-predators.
d. The volatile organic compounds released from the insect pests attract the insect-predators to kill the pests.

24. If antagonistic yeast is sprayed throughout the chilies, the yeast-treated chilies are likely to be low damaged by the pests when compared to the controls.

□ **True** □ False

Reason

a. Yeasts release an enzyme to digest plant cell wall. When the plant cell wall becomes thinner, pathogen can invade through it easily.
b. Yeast induces the production of defensive responses against the pathogens in chili fruit.
c. Yeast releases a specific enzyme to digest pathogen cell wall directly.
d. Yeast help digesting plant cell walls to become a food source of pathogens.

25. Defensive response systems of plants against the plant enemies such as microbial and insects are beneficial to plants.

□ **True** □ False

Reason

a. The defensive responses of plants allow plants to survive in the environment.
b. The defensive responses of plants cause changes of plant genetics.
c. The defensive responses of plants help promoting plant evolution in a better way.
d. The defensive responses of plants cause the loss of plant cell energy.

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APPENDIX D

Constructivist Learning Environment Survey (CLES) Questionnaire

Pre-CLES Questionnaire

Objective:

The objective of this questionnaire is to investigate the learning environment in the biology classroom. Your answers will be kept in secret. It will be used for research study that does not have any effects to individual or school.

Instruction:

Please consider each statement, use ✓ in the space of the table which the best describes your feelings about teaching and learning environment in your previous biology class. The criterion of each level is that:

5 means almost always
4 means often
3 means sometimes
2 means seldom
1 means almost never

Part 1: Personal data

Gender ☐ Male ☐ Female

Student' Number.....

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Part 2: Leaning environment in biology class

In the biology class…	Almost Always (5)	Often (4)	Sometimes (3)	Seldom (2)	Almost Never (1)
1. I learn from other sources outside of school.					
2. I learn that scientific theories are human inventions.					
3. It's OK to ask the teacher "why do we have to learn this?"					
4. I help the teacher to plan what I'm going to learn.					
5. In biology class, I get the chance to talk to other students.					
6. In biology class, I am enthusiastic for joining leaning activities.					
7. New learning starts with problems about the world outside of school.					
8. I learn that science is influenced by people's values and opinions.					
9. I feel free to question the way I'm being taught.					
10. In biology classes, I help the teacher decide how well my learning is going.					
11. In biology classes, I talk with other students about how to solve problems.					
12. The activities in biology classes are among the most interesting at this school.					
13. I learn how science, particularly biology, can be a part of my out-of – school life.					
14. I lean that the views of science have changed over time.					
15. In biology classes, it's OK to complain about activities that are confusing.					
16. In biology classes, I have a say in deciding the rules for classroom discussion.					
17. In biology classes, I try to make sense of other students' ideas related to biology concepts.					
18. The activities in biology class make me interested in science.					
19. I get a better understanding of biology concepts from situations outside of school.					
20. I learn that different sciences are used by people in other cultures.					
21. In biology classes, It's OK to complain about any thing that stops me from learning.					
22. In biology classes, I have a say in deciding how much time I spend on an activity.					
23. In biology classes, I ask other students to explain their ideas.					
24. In biology classes, I enjoy the learning activities.					

(Continued)

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Part 2: Leaning in biology class (Continued)

In the biology class…	Almost Always (5)	Often (4)	Sometimes (3)	Seldom (2)	Almost Never (1)
25. I learn interesting things about the world outside of school.					
26. I learn that biological knowledge can be questioned.					
27. In biology classes, I'm free to express my opinion.					
28. In biology classes, other students ask me to explain my ideas.					
29. In biology classes, I feel confused.					
30. What I learn from biology classes has nothing to do with my out-of-school life.					
31. I learn that science, particularly biology, reveals the secrets of nature.					
32. In biology classes, it's OK to speak up for your rights.					
33. In biology classes, I have a say in deciding what will be on the test.					
34. In biology classes, other students explain their ideas to me.					
35. The learning activities in biology classes are a waste time.					
36. In biology classes, I have a say in deciding what activities I do.					
37. What I learn science particularly biology has nothing to do with the world outside of school.					
38. I learn that scientific knowledge, particularly in biology, is beyond doubt.					
39. In biology classes, I feel unable to complain about anything.					
40. In biology classes, I have a say in deciding how my learning is assessed.					
41. In biology classes, other students pay attention to my ideas.					
42. I feel tense in biology classes.					

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APPENDIX E

Constructivist Learning Environment Survey (CLES) Questionnaire

Post-CLES Questionnaire

Objective:

The objectives of this questionnaire are to investigate the learning environment particularly in the Fighting Plant Learning Unit (FPLU). Your answers will be kept in secret. It will be used for research study that does not have any effects to individual or school.

Instruction:

Please consider each statement, use ✓ in the space of the table which the best describes your feelings about teaching and learning environment in the FPLU. The criterion of each level is that:

5 means almost always
4 means often
3 means sometimes
2 means seldom
1 means almost never

Part 1: Personal data

Gender ☐ Male ☐ Female

Student' Number.....

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Part 2: Leaning in the Fighting Plant Learning Unit (FPLU)

In this class…	Almost Always (5)	Often (4)	Sometimes (3)	Seldom (2)	Almost Never (1)
1. I learn about the world outside of school.					
2. I learn that scientific theories are human inventions.					
3. It's OK to ask the teacher "why do we have to learn this?"					
4. I help the teacher to plan what I'm going to lean.					
5. In the FPLU, I get the chance to talk to other students.					
6. In the FPLU, I look forward to leaning activities.					
7. New learning starts with problems about the world outside of school.					
8. I learn that science is influenced by people's values and opinions.					
9. In the FPLU, I feel free to question the way I'm being taught.					
10. In the FPLU, I help the teacher decide how well my learning is going.					
11. In the FPLU, I talk with other students about how to solve problems.					
12. The activities in the FPLU are among the most interesting at biology class.					
13. In the FPLU, I learn how science can be a part of my out-of –school life.					
14. I learn that the views of science have changed over time.					
15. In the FPLU, it's OK to complain about activities those are confusing.					
16. In the FPLU, I have a say in deciding the rules for classroom discussion.					
17. In the FPLU, I try to make sense of other students' ideas.					
18. In the FPLU, the activities make me interested in science.					
19. In the FPLU, I get a better understanding of biology concepts from situations outside of school.					
20. I learn that different sciences are used by people in other cultures.					
21. In the FPLU, it's OK to complain about any thing that stops me from learning.					
22. In the FPLU, I have a say in deciding how much time I spend on an activity.					
23. In the FPLU, I ask other students to explain their ideas.					
24. In the FPLU, I enjoy the learning activities.					

(Continued)

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Part 2: Leaning in the Fighting Plant Learning Unit (FPLU) (Continued)

In this class…	Almost Always (5)	Often (4)	Sometimes (3)	Seldom (2)	Almost Never (1)
25. I learn interesting things about the world outside of school.					
26. I learn that scientific knowledge can be questioned.					
27. In the FPLU, I'm free to express my opinion.					
28. In the FPLU, other students ask me to explain my ideas.					
29. In the FPLU, I feel confused.					
30. What I learn from the FPLU has nothing to do with my out-of-school life.					
31. In the FPLU, I learn that science reveals the secrets of nature.					
32. In the FPLU, it's OK to speak up for your rights.					
33. In the FPLU, I have a say in deciding what will be on the test.					
34. In the FPLU, other students explain their ideas to me.					
35. In the FPLU, the learning activities are a waste time.					
36. In the FPLU, I have a say in deciding what activities I do.					
37. In the FPLU, what I learn has nothing to do with the world outside of school.					
38. In the FPLU, I learn that scientific knowledge.					
39. In the FPLU, I feel unable to complain about anything.					
40. In the FPLU, I have a say in deciding how my learning is assessed.					
41. In the FPLU, other students pay attention to my ideas.					
42. In the FPLU, I fell tense.					

---- Please answer the questions in the next page ----

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Please answer briefly through the following questions:

1. Were you pleased about the learning environment in the FPLU? Why? Why not?

   ……
   ……
   ……

2. What are the difficulties that you had been faced in the FPLU? Please explain.

   ……
   ……
   ……

3. What thing (s) that you want to change or suggest? Why?

   ……
   ……
   ……

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APPENDIX F

Interview Questions

Questions were used to guide the semi-structured interviews before and after the learning unit implementation. The students were interviewed with a series of open-ended questions around the following questions. Each heading represents a cluster of questions.

Pre-interview:

Students' perception of studying about plants compared to animals

1. What are the differences between plants and animals?
2. What are the similarities between plants and animals?
3. What kinds of animal activities had you been experienced in biology class? Were they interesting for you? Why?
4. What kinds of plant activities had you been experienced in biology class? Were they interesting for you? Why?
5. Between plants and animals, what kinds of organism do you prefer to study in biology class? Why do you prefer to study with that organism?

Students' understanding of plant defense mechanisms

6. What is a plant response?
7. What are plant stimuli?
8. Can plant respond to environmental stimuli?
9. What are the enemies of plants?
10. Can plants protect themselves against the natural enemies such as pathogens and insects? If yes, how can plants defend themselves?
11. In the case of animals, for example, if animals are infected, can they defend themselves against the pathogens? If yes, how?

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Post-interview:

Students' perception of plants and studying about plants

1. From the activities, did you acquire a surprising piece of knowledge that did not match your prior experience? What was it?
2. After completion the FPLU, what did you understand more about plants?
3. What topic do you want to learn more about plants?
4. What are the similarities between plant activities you had been experienced and the activities in this unit?
5. What are the differences between plant activities you had been experienced and the activities in this unit?
6. What are the factors that make plant study more interesting to you?

Students' perception of the learning unit FPLU

7. Does this learning unit differ from other biology class you had been experienced? How?
8. Does this learning unit similar from other biology class you had been experienced? How?
9. How did the unit help you learn?
10. Did your classmate helps you learn? How?
11. Did the teacher help you learn? How?
12. What kinds of teaching and learning strategies did you like and not like in this unit?
13. Did you enjoy participating in the unit? Why?
14. What things should be improved in this unit?

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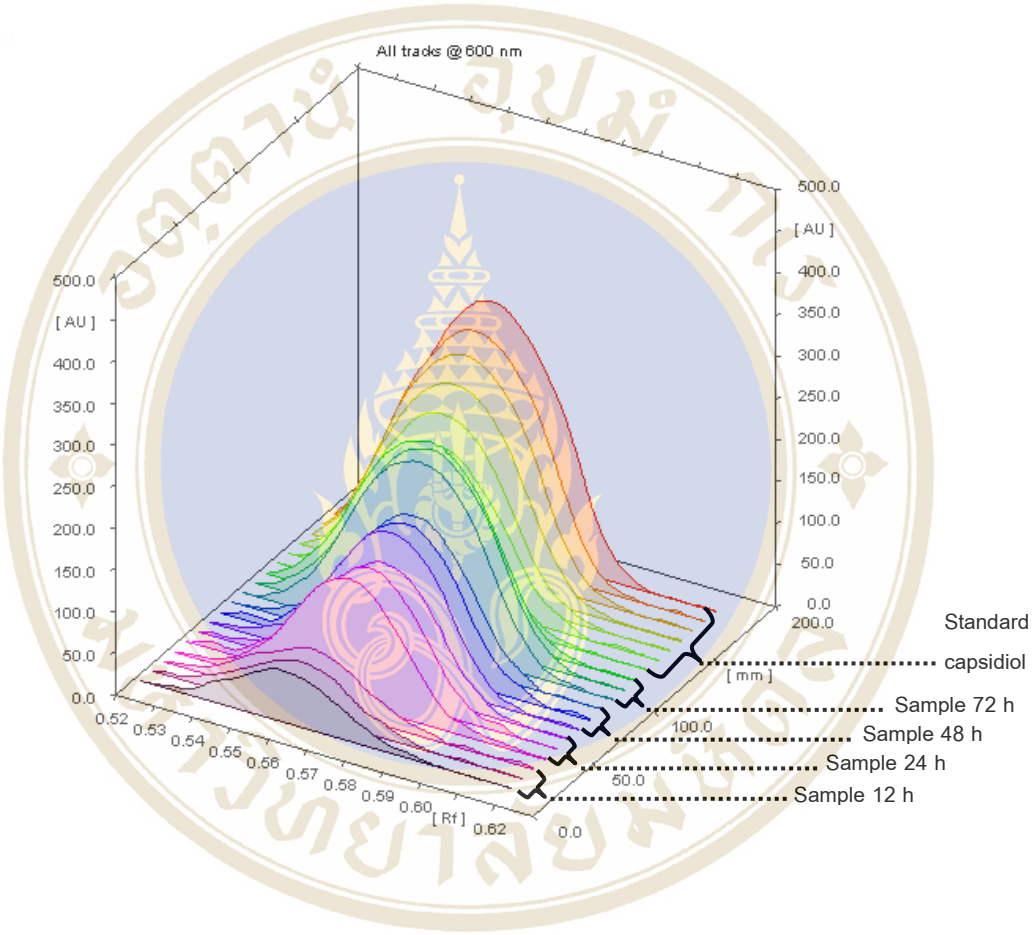
APPENDIX G



Area in absorbance unit (AU) of sample capsidiol extracted from yeast-treated chili (at 12, 24, 48 and 72 h) and AU of authentic capsidiol measured by scanning color band over the plate at 600 nm.

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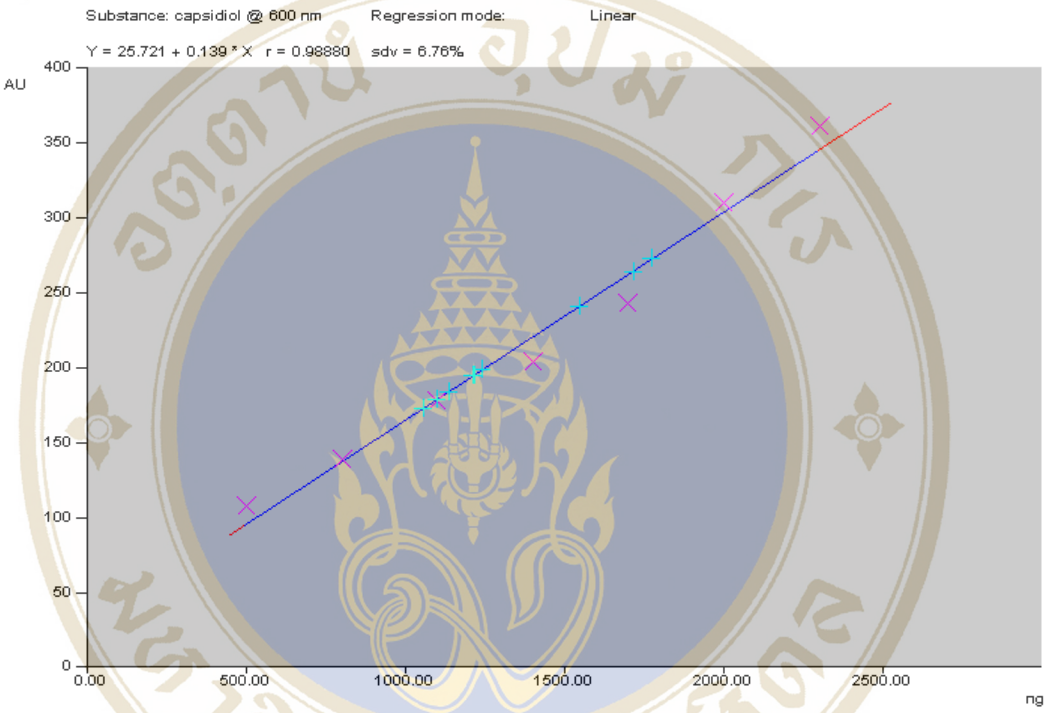
APPENDIX H



Standard curve of authentic capsidiol at maximum absorbance at 600 nm.

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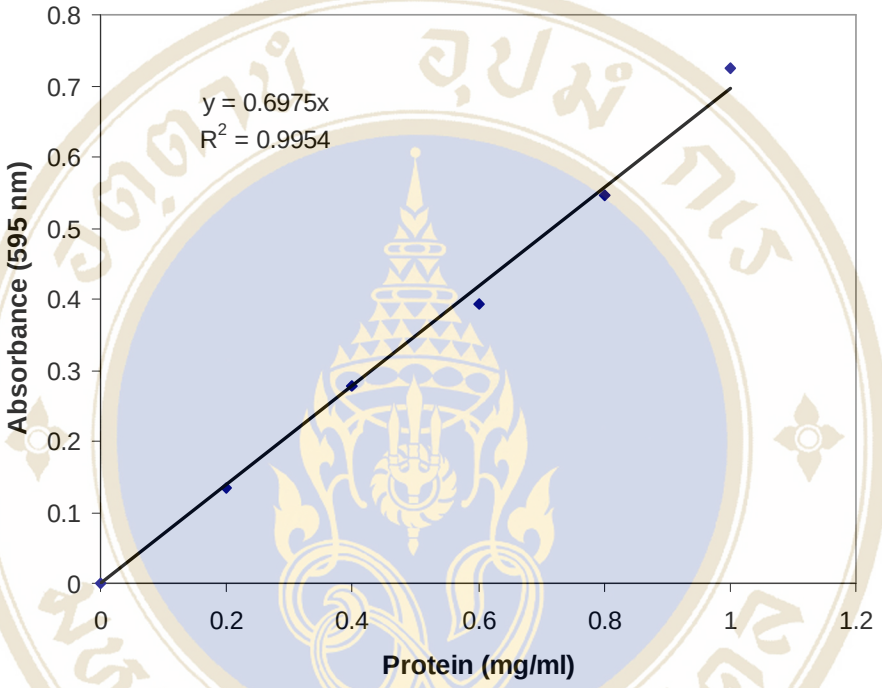
APPENDIX I



Standard curve of protein at maximum absorbance at 595 nm.

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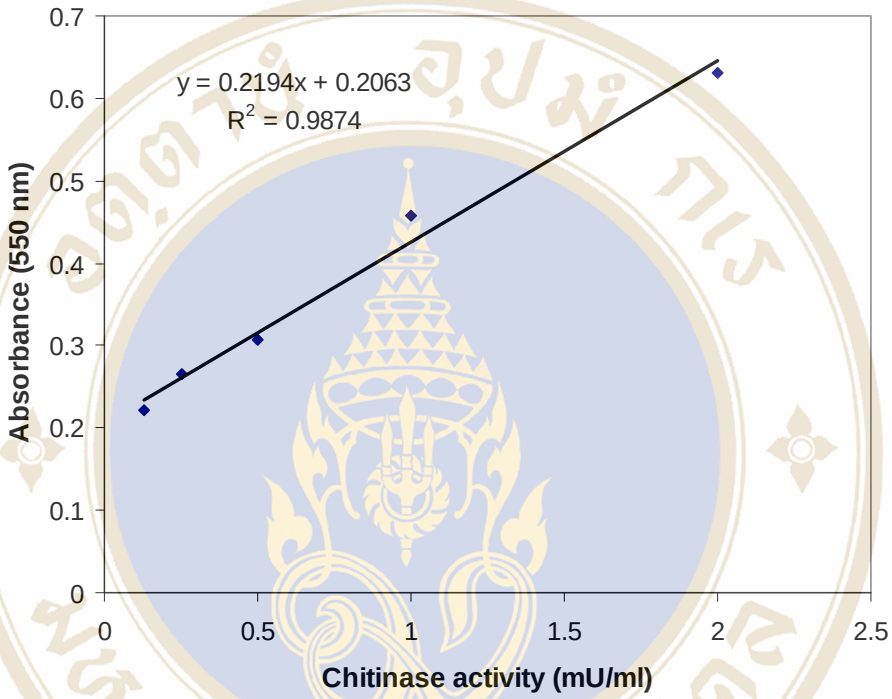
APPENDIX J



Standard curve of chitinase activity at maximum absorbance at 550 nm.

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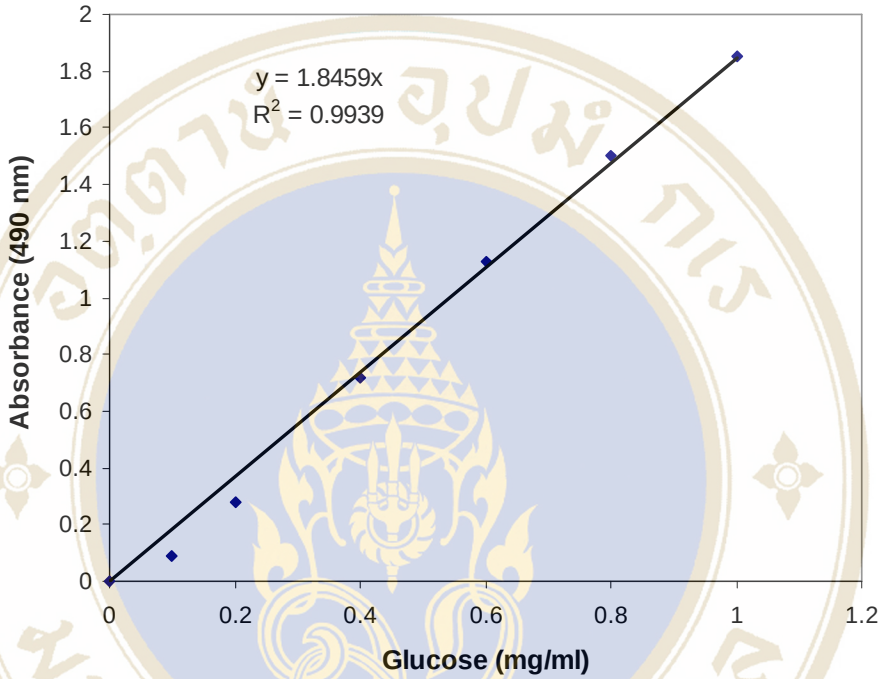
APPENDIX K



Standard curve of glucose at maximum absorbance at 490 nm.

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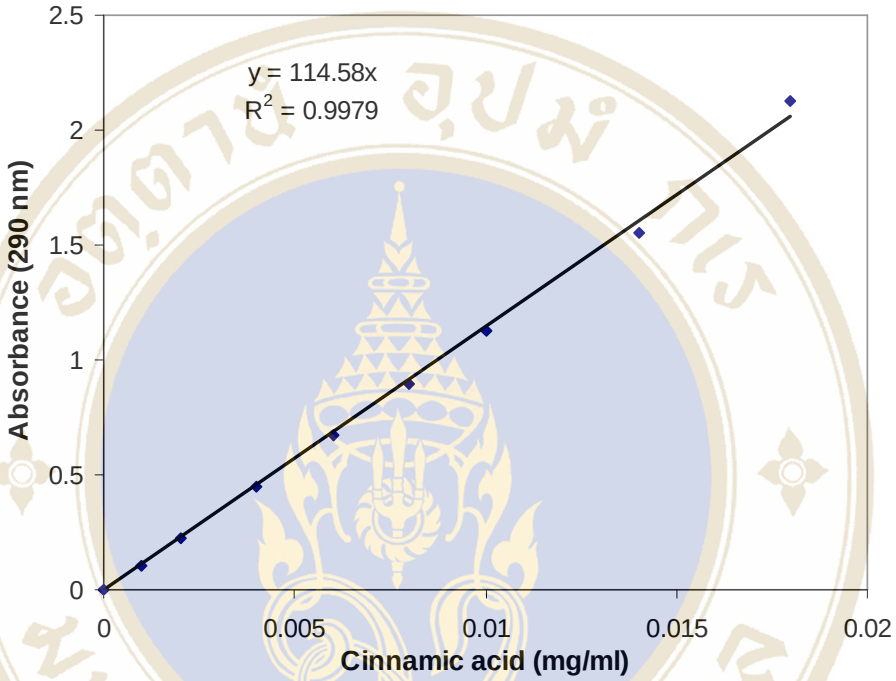
APPENDIX L



Standard curve of cinnamic acid at maximum absorbance at 290 nm.

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BIOGRAPHY

NAME	Miss Nantawan Nantawanit
DATE OF BIRTH	October 15, 1977
PLACE OF BIRTH	Chonburi, Thailand
INSTITUTION ATTENDED	Chulalongkorn University, Bangkok, 1996-2000 Bachelor of Science (Biochemistry) Chulalongkorn University, Bangkok, 2000-2002 Master of Science (Biomedicinal Chemistry) Mahidol University, Bangkok, 2006-2009 Doctor of Philosophy (Science and Technology Education)
SCHOLARSHIP	The Royal Thai Government Scholarship under the Promotion of Science and Mathematics Teacher Project, the Institute for the Promotion of Teaching Science and Technology (IPST) (2006-2009)
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