EVALUATION OF *Bacillus* sp. BASED CHITOSAN FORMULATION TO INDUCE RESISTANCE AGAINST FUSARIUM WILT DISEASE ON TOMATO

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การประเมินประสิทธิภาพสูตรสำเร็จ *Bacillus* sp. ผสมไคโตซานเพื่อชักนำความต้านทานต่อโรคเหี่ยวเหลืองของมะเขือเทศ

นางสาวปริฉัตร นำประดิษฐ์ทรัพย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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คำสำคัญ: ฟูซาเรียม อ๊อกซีสปอรัม/เหี่ยวเหลือง/บาซิลลัส/มะเขือเทศ/ชักนำความต้านทาน

โรคเหี่ยวเหลืองในมะเขือเทศ เกิดจากเชื้อรา *Fusarium oxysporum* f.sp. *lycopersici* (*FOL*) เป็นโรคสำคัญในประเทศไทย ซึ่งก่อให้เกิดความเสียหายกับผลผลิตมะเขือเทศมากกว่า 50-80% เกษตรกรจำเป็นต้องใช้สารเคมีป้องกันกำจัดเชื้อราในการควบคุมโรคในปริมาณมาก การศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษากลไกการชักนำความต้านทานในมะเขือเทศต่อโรคเหี่ยวเหลืองของสูตรสำเร็จ *Bacillus*-based chitosan (BBC) ซึ่งเป็นสูตรสำเร็จของแบคทีเรียบาซิลลัสและไคโตซาน โดยทำการทดสอบประสิทธิภาพของสูตรสำเร็จ BBC จำนวน 3 สูตร ได้แก่ BBC1, BBC2 และ BBC3 ที่ความเข้มข้นของไคโตซานที่แตกต่างกัน ได้แก่ 800, 1000, 1200 และ 2000 ไมโครกรัม/มิลลิลิตร ในการยับยั้งการเจริญของโคโลนีเชื้อรา พบว่า BBC1 ที่ความเข้มข้น 800 ไมโครกรัม/มิลลิลิตร สามารถยับยั้งการเจริญของเส้นใยเชื้อรา *FOL* ได้ดีที่สุดที่ 45.14% ซึ่งสูงกว่ากรรมวิธีการใช้สารเคมี อย่างมีนัยสำคัญ ดังนั้นจึงเลือกสูตรดังกล่าวมาทำการศึกษากลไกด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (environmental scanning electron microscope : ESEM) แสดงให้เห็นโหมดการทำงานของ BBC1 ที่ความเข้มข้น 800 ไมโครกรัม/มิลลิลิตร สามารถยับยั้งการเจริญเติบโตของ *FOL* ได้โดยตรงโดยการหลั่งสารต้านเชื้อรา จากนั้นทดสอบอัตราการรอดชีวิต พบว่าหลังจากเก็บไว้ที่อุณหภูมิห้องเป็นเวลา 2 เดือน ปริมาณเชื้อลดลงเหลือและคงที่ในเดือนที่ 3 มีปริมาณเท่ากับ 10^8–10^6 CFU/g (Log_{10} of 8.51-6.20) หลังจากนั้นมีปริมาณเชื้อมีแนวโน้มลดลง และเมื่อเวลาผ่านไปเป็นเวลา 6 เดือน ปริมาณการอยู่รอดของเชื้อเหลืออยู่เพียง 10^4 CFU/g (Log_{10} of 4.71) เมื่อนำไปใช้ด้วยวิธีการฉีดพ่นทางใบร่วมกับการราดดิน เปรียบเทียบกับสารเคมีเทอร์ราคลอร์ ซุปเปอร์-เอ็กซ์, บาซิลลัสการค้า และไคโตซานการค้า COS® ในสภาพโรงเรือนทดลอง และภายหลังการฉีดพ่นครั้งสุดท้าย 7 วันทำการปลูกเชื้อรา *FOL* สาเหตุโรคเหี่ยวเหลือง พบว่า สูตรสำเร็จ BBC1 สามารถลดการเกิดโรคเหี่ยวเหลืองได้ 78.95% ซึ่งไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติกับสารเคมี ซึ่งลดการเกิดโรคได้ 73.69% นอกจากนี้ BBC1 ที่ความเข้มข้น 800 ไมโครกรัม/มิลลิลิตร สามารถส่งเสริมให้มะเขือเทศมีผลผลิตเพิ่มขึ้น 75.0% และมีน้ำหนักต่อผลเพิ่มขึ้น 32.5% เมื่อเทียบกับกรรมวิธีควบคุม จากนั้นทำการเก็บตัวอย่างใบมะเขือเทศที่ 0, 24 และ 48 ชั่วโมง ภายหลังการปลูกเชื้อ สาเหตุโรค มาทำการตรวจสอบกลไกการปกป้องตนเอง ได้แก่ ปริมาณกรดซาลิไซลิก (SA), ปริมาณกิจกรรมเอนไซม์ chitinase, ปริมาณกิจกรรมเอนไซม์ β-1,3-glucanase และปริมาณ

กิจกรรมเอนไซม์ phenylalanine ammonia lyase (PAL) พบว่า 24 ชั่วโมงหลังการปลูกเชื้อสาเหตุโรคมะเขือเทศมีที่ฉีดพ่นด้วยสารเคมี, สูตรสำเร็จ BBC1, บาซิลลัสการค้า และไคโตซานการค้า COS® มีแนวโน้มที่เพิ่มขึ้นของปริมาณ SA สูงสุดที่ 9.38, 9.23, 9.15 และ 9.09 ไมโครกรัมต่อกรัมน้ำหนักสด เช่นเดียวกับมะเขือเทศที่ฉีดพ่นด้วยสูตรสำเร็จ BBC1, สารเคมี, ไคโตซานการค้า COS® และ บาซิลลัสการค้า มีปริมาณกิจกรรมของเอนไซม์ chitinase เพิ่มขึ้นสูงสุดที่ 24 ชั่วโมงหลังการปลูกเชื้อสาเหตุโรค 1.00, 0.98, 0.98 และ 0.96 ไมโครโมลต่อมิลลิกรัมโปรตีน ขณะเดียวกันปริมาณกิจกรรมของเอนไซม์ β-1,3-glucanase มีแนวโน้มเพิ่มขึ้นสูงสุดที่ 30.34, 28.42, 27.07 และ 24.63 ไมโครโมลต่อมิลลิกรัมโปรตีน เมื่อฉีดพ่นด้วยสารเคมี, สูตรสำเร็จ BBC1, ไคโตซานการค้า COS® และ บาซิลลัสการค้า เช่นเดียวกับปริมาณกิจกรรมของเอนไซม์ PAL มีแนวโน้มเพิ่มขึ้นสูงสุดที่ 92.65, 87.18, 84.83 และ 79.97 ไมโครโมลต่อมิลลิกรัมโปรตีน เมื่อฉีดพ่นด้วยสารเคมี, ไคโตซานการค้า COS®, สูตรสำเร็จ BBC1 และ บาซิลลัสการค้า นอกจากนี้ เมื่อนำใบมะเขือเทศที่ฉีดพ่นด้วยสิ่งกระตุ้นสูตรสำเร็จ BBC1 และปลูกเชื้อสาเหตุโรคที่เวลา 24 ชั่วโมงภายหลังการปลูกเชื้อมาศึกษาการเปลี่ยนแปลงสารชีวเคมีด้วยเทคนิค synchrotron FT-IR microspectroscopy พบว่า สูตรสำเร็จ BBC1 ส่งเสริมให้มะเขือเทศมีการสังเคราะห์สารในกลุ่มไขมัน C=O ester, โปรตีน (Amide I, II) และ C-H bending ในเนื้อเยื่อสูงกว่ากรรมวิธีควบคุม อย่างมีนัยสำคัญทางสถิติ จากการทดลองนี้แสดงให้เห็นว่าสูตรสำเร็จ BBC1 มีประสิทธิภาพในการควบคุมโรคเหี่ยวเหลืองของมะเขือเทศทั้งทางตรงและทางอ้อม โดยมีประสิทธิภาพในการยับยั้งการเจริญของเชื้อราสาเหตุโรคโดยตรงและสามารถชักนำให้มะเขือเทศเกิดกระบวนการปกป้องตนเองให้มีความต้านทานต่อการเข้าทำลายของเชื้อก่อโรค ดังนั้น การชักนำความต้านทานพืชโดยใช้สูตรสำเร็จ BBC1 จึงเป็นแนวทางที่ดีในการนำไปใช้ในระบบการผลิตมะเขือเทศปลอดภัยและลดการใช้สารเคมีในการควบคุมโรคซึ่งเป็นอันตรายต่อมนุษย์และสิ่งแวดล้อม

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา .....

ปีการศึกษา 2564 ลายมือชื่ออาจารย์ที่ปรึกษา .....

PARICHAT NUMPARDITSUB : EVALUATION OF *Bacillus* sp. BASED CHITOSAN FORMULATION TO INDUCE RESISTANCE AGAINST FUSARIUM WILT DISEASE ON TOMATO. THESIS ADVISOR: ASST. PROF. NATTHIYA BUENSANTEAI, 104 PP.

Keyword: *Fusarium oxysporum*/Fusarium wilt/Bacillus/Tomato/Induced resistance

Fusarium wilt disease on tomatoes is caused by *Fusarium oxysporum* f.sp. *lycopersici* (*FOL*). It is a serious disease of tomatoes in Thailand, which can cause tomato yield loss up to 50-80%. Farmers need to use a lot of chemical fungicides to control the disease. The objective of this study was to investigate the resistance induction mechanisms against Fusarium wilt disease in tomato plants after being treated with *Bacillus*-based chitosan (BBC) formulation, which is a formulation of *Bacillus* sp. and chitosan. The efficacy of 3 BBC formulations including BBC1, BBC2, and BBC3 at different concentrations of 800, 1000, 1200, and 2000 µg/ml was tested for inhibiting the growth of Fusarium colonies. The result showed that BBC1 at a concentration of 800 µg/ml was the most effective for inhibiting *FOL* mycelial growth by 45.14%, which was not significantly different from the positive control (terraclor super-x®). Therefore, this formulation was selected for a further study of the mode of action by environmental scanning electron microscope (ESEM). The result pointed out that the BBC1 at a concentration of 800 µg/ml demonstrated the direct mode of action that inhibited *FOL* growth by secretion of antifungal agents. Then, the *Bacillus* viability was evaluated after storage at room temperature until 6 months. The *Bacillus* sp. strain CaSUT008 in the encapsulated form was decreased by 10^8–10^6 CFU/g (Log_{10} of 8.51-6.20) at 1-3 months after storage. After that, the bacteria's survival was decreased to 10^4 CFU/g (Log_{10} of 4.71) at the 6th month. Moreover, the BBC1 at a concentration of 800 µg/ml was applied by foliar spraying and soil drenching to control Fusarium wilt disease and compared with terraclor super-x®, *B. subtillis* commercial, and COS® under greenhouse conditions. The tomato plants were inoculated with *FOL* at 7 days after the last application. It was found that the encapsulated BBC1 was able to reduce the Fusarium wilt disease severity by 78.95%, which was not significantly different from the terraclor super-x® at 73.69%. In addition, the tomato fruit weight and yield of the BBC1 application was significantly increased by 34.5 and 75.0%, respectively when compared with the control treatment. Moreover, the defense mechanisms were investigated in

tomato leaves, including salicylic acid (SA) content, chitinase, β-1,3-glucanase, and phenylalanine ammonia-lyase (PAL) activity at 0, 24, and 48 hours after fungal challenge inoculation (HAI). In addition, the accumulation of SA was significantly increased at 24 HAI in the terraclor super-x®, encapsulated BBC1, *B. subtillis* commercial and COS® application at 9.38, 9.23, 9.15, and 9.09 µg g^{-1} fresh weight, respectively. Besides, the tomatoes treated with the encapsulated BBC1, the terraclor super-x®, COS®, and *B. subtillis* commercial showed the highest increase of chitinase activity at 24 HAI as 1.00, 0.98, 0.98, and 0.96 µmol mg^{-1} protein, respectively. The amount of β-1,3-glucanase increased to the highest at 30.34, 28.42, 27.07, and 24.63 µmol mg^{-1} protein treated with terraclor super-x®, encapsulated BBC1, COS®, and *B. subtillis* commercial, respectively. Similarly, PAL activity significantly increased at 24 HAI to levels of 92.65, 87.18, 84.83, and 79.97 µmol mg^{-1} protein when treated with terraclor super-x®, COS®, encapsulated BBC1, and *B. subtillis* commercial, respectively. In addition, the leaf samples treated with BBC1 at 24 HAI were investigated for biochemical changes by Synchrotron FT-IR microspectroscopy. It was found that the encapsulated BBC1 promoted the synthesis of C=O ester lipids, proteins (Amide I, II), and C-H bending in the epidermis, which were significantly higher than those of the control treatment. These results suggested that the encapsulated BBC1 had the efficiency in direct and indirect control of Fusarium wilt on tomatoes by inhibiting fungal pathogen and inducing the plant defense mechanism during host-pathogen interactions. Therefore, the encapsulated BBC1 is a good approach for tomato production systems to reduce the chemical fungicides used for disease control and also their harmful effects on humans and the environment.

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Academic Year 2021 Advisor's Signature

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LIST OF ABBREVIATIONS

°C	=	Degree Celsius
µL	=	Microliter (s)
µm	=	Micrometer (s)
min	=	Minute (s)
ml	=	Milliliter (s)
mM	=	Millimolar (s)
kV	=	Kilovolt
IPM	=	Integrated pest management
IR	=	Induced resistance
ISR	=	Induced systemic resistance
SAR	=	Systemic acquired resistance
PR	=	Pathogenesis related
DMRT	=	Duncan's multiple range test
FAO	=	Food and Agriculture Organization
FTIR	=	Fourier-transform infrared spectroscopy
PCA	=	Principal component analysis
ROS	=	Reactive oxygen species
AOS	=	Active oxygen species
PGPR	=	Plant growth promoting rhizobacteria
BCAs	=	Biological control agent
HAI	=	Hours after inoculation
JA	=	Jasmonic acid
BABA	=	*β*-aminobutyric acid
ASM	=	Acibenzolar-S-methyl
BTH	=	Benzothiadiazole
SA	=	Salicylic acid
ET	=	ethylene
PAL	=	Phenylalanine ammonia-lyase

LIST OF ABBREVIATIONS (Continued)

PPO	=	Polyphenol oxidase
POD	=	Peroxidase
GA	=	Gibberellin
ESEM	=	Environmental scanning electron microscope
IAA	=	Auxin
SR	=	Synchrotron
NGA	=	Nutrient glucose agar

CHAPTER I
INTRODUCTION

1.1 Background of the selected topic

The tomato (*Lycopersicon esculentum* Mill.) is a member of the Solanaceae family that is endemic to South America, Ecuador, and the Galapagos Islands (Soithong, 1999). It is another widely consumed vegetable. Because tomato are nutrient-dense vegetables that are abundant in vitamins and minerals, particularly vitamin C. It also contains 80–90% lycopene in all carotenoid components (Shi and Maguer, 2000). In Thailand, there are numerous types of tomato, including Plum Tomato and Seeda Tomato, which are extensively used in processing such as tomato extracts, ketchup, and tomato juice. The cherry tomato are popular for eating raw because of their high antioxidants lycopene content. As a result, customers are increasingly requesting tomato (Towanna *et al.*, 2009). In 2020, the harvest area of tomato plants was approximately 5,703.68 hectares, and tomato yield was approximately 125,368 tons valued at 2,015 million baht in Thailand (Agriculture Statistics of Thailand, 2020). Currently, Thailand imports fresh and processed tomato more than 20 million tons external with total value more than 600 million baht. Tomato can be cultivated in all regions of Thailand, especially in the Northeast region such as Nakhon Ratchasima, Nakhon Phanom, Nong Khai, Sakon Nakhon, Mukdahan, Ubon Ratchathani, and Sisaket provinces. The tomato diseases are easy to outbreak in tropical environments and climates including Thailand. The most frequent tomato diseases are Fusarium wilt disease, tomato yellow leaf curl disease, bacterial wilt disease, and late blight disease (Wang and Lin, 2005). Moreover, the insect has also infected tomato. As a result, tomato quality and yield have declined Fusarium wilt disease is caused by *Fusarium oxysporum* f.sp. *lycopersici* (*FOL*) plays an important role in the growth and reduces the yield of tomato by 50-80% because the pathogen can infect all stages of plant growth (Fazio *et al*., 1999; Larkin and Fravel, 1998). Farmers prefer to use chemicals to prevent plant disease because the chemicals give quick and effective results (Singh *et*

al., 1989; Rakphol, 2012). Fungicides had copper as an important component, resulting in residues in the soil and plants that are dangerous to farmers and consumers (Cambrolle, 2014). In Thailand or ASEAN countries, fungicide residue contamination of agricultural goods has become a severe concern (Kongtragoul and Nalumpang, 2010; Jaipin and Nalumpang, 2014; Phukang and Chaisuk, 2016; Suwannarat *et al.*, 2017). At present, there are several ways for controlling Fusarium wilt disease on tomato, including cultural practices, biological management, and Integrated Pest Management (IPM) (Department of Agricultural Extension, 2012). Furthermore, the induced resistance (IR) method is a technique that allows plants to immunize themselves against pathogenic invasions. This will be a crucial method for effectively reducing the usage of chemical pesticides (Prathuangwong *et al*., 2011; Buensanteai *et al*., 2009).

The plant was induced for resistance pathogen invasion by pre-treated biotic elicitors including *Trichoderma* spp. (Hoitink et al., 2006; Palmieri et al., 2012), plant growth-promoting rhizobacteria (PGPR) bacteria such as *Pseudomonas fluorescens* (Vleesschauwer *et al.*, 2008; Verhagen *et al.*, 2011), *Bacillus subtilis* and *B. amyloliquefaciens* (Buensanteai *et al*., 2009; Rudrappa *et al*., 2010; Prathuangwong *et al*., 2011). In addition, abiotic elicitors including salicylic acid (SA), jasmonic acid (JA), and *β*-aminobutyric acid (BABA), thiamine (vitamin B1), acibenzolar-S-methyl (ASM), benzothiadiazole (BTH) and chitosan can also be used to induce disease resistance (Holmes *et al*., 2001; Compant and Mathieu, 2016). These elicitors stimulate the production and accumulation of phytoalexin, which is toxic to pathogens at the site of its infestation. In addition, other antimicrobial compounds are generated to prevent phytopathogens spread throughout the plant stem (Wongkaew *et al*., 1989). The using biotic elicitor on tomato could reduce disease incidence via induced systemic resistance of bacterial wilt disease caused *R. solanacearum* by 41.1 to 80.0% (Boonsuebsakul *et al.*, 2005). The usage of antagonistic fungus *Trichoderma* has potential to protect crops against disease (Weindling *et al.*, 1934; Weindling and Fawcett, 1936). The antagonistic fungus has mechanisms similar to antagonistic bacteria, such as competition, colonization, antibiotic, enzyme synthesis, enhanced growth promotion, and induced systemic resistance.

At present, induced resistance studies have been conducted in Thailand, which there are not many studies on tomato mechanisms of resistance, both structural and biochemical resistance mechanism. The study was performed to evaluate the effect and mechanism of *Bacillus*-based chitosan formulations (BBC) on induced resistance against Fusarium wilt disease on tomato.

1.2 Research objectives of this study

1.2.1 To evaluate *Bacillus*-based chitosan formulations (BBC) on induced resistance against Fusarium wilt disease on tomato

1.2.2 To study the resistance induction mechanisms against *Fusarium oxysporum* f. sp. *lycopersici* (*FOL*) after treated with BBC elicitor in tomato plants

CHAPTER II
LITERATURE REVIEWS

2.1 Background of the problem

Economic crop losses due to plant diseases cause a critical threat to food security. In Thailand, the majority of producers of fresh market vegetables including tomato, cucumber, and pepper are highly dependent on soil fumigation with chemical fungicide as methyl bromide and foliar spraying with chemical fungicide as carbendazim and terraclor super-x® (Kokalis *et al.*, 2002; Nonthapot, 2017). Tomato production in Thailand for the 2008–2018 growing season had an estimated value of more than 1,583 million Thai baht (FAO, 2009). Tomato cultivation in Thailand is often infected with several diseases and insect infestations, resulting in reduced planting area and productivity (Sribut, 2015). Tomato wilt caused by *Fusarium oxysporum* f. sp. *lycopercisi* (*FOL*), a diverse soil-borne pathogen with several economic host ranges, is a plant vascular disease (Fazio *et al*., 1999; Nonthapot, 2017). At present, no effective fungicides are available for Fusarium wilt. Then, alternative methods such as biological control agents (BCAs) have performed effectiveness and have been increasingly applied in the tomato field to control the pathogens (Singburaudom, 2005; Buensanteai *et al.*, 2009). These BCAs such as *Bacillus* sp., *Paenibacillus* sp., and *Pseudomonas* sp. have been used successfully in many pathogens to control diseases, enhance plant growth, and induced systemic resistance and improve crop yield (Cook and Baker, 1993; Buensanteai *et al.*, 2009). By the way, *B. subtilis* and B. *amyloliquefaciens* have been demonstrated to boost plant development and protect plant roots against fungal diseases through several mechanisms, including antibiotic synthesis, siderophores, and induced systemic resistance (Kloepper *et al*., 1980; Weller, 1988; Zehnder *et al.*, 2001; Buensanteai *et al.*, 2009). The formulation of biocontrol agents based on organic amendments (biopolymer, agricultural and industrial by-products) can contribute to longer shelf life and high efficacy to control soil-borne diseases in many crops by acting as inducers of plant innate immune system and plant

defense responses (Buensanteai *et al*., 2009; Fawzy and Monaim, 2013). This study was performed to evaluate the effect and mechanism of *Bacillus*- based chitosan formulations (BBC) on induced resistance against Fusarium wilt disease on tomato.

2.2 Importance of tomatoes

Tomato (*Solanum lycopersicum*) originated from Western South America and is an important vegetable for human life. Tomato is high nutrients as a source of vitamins and minerals necessary for humans. The fruit of tomatoes could be used for several consumption ways such as fresh fruit, and processed tomatoes were used to make soup, juice, and other products (Tsedeke, 1986). More interestingly, the important nutrients including vitamins (A, C, E, B, and K) and minerals content (*ß*-carotene, calcium, magnesium) were contained in tomato fruits (Cohen and Antignu, 1994). In addition, tomatoes are also an important economic crop in Thailand because approximately 70 seed companies are operating in the country, which is expected to be worth more than 20,000 million baht/year with export value reaching 5,452.44 million baht in 2017. Especially, the Northeastern region has a very suitable climate for tomato seed production, which contributes significantly to the export of tomato seeds to Thailand (FAO, 2019). The tomato-growing areas are Nong Khai, Sakon Nakhon, Nakhon Phanom, Kalasin, and Khon Kaen provinces (Nonthapot, 2017). It is the largest source of fresh tomato seed production in Thailand. The seed production process for export must have a phytosanitary certificate that specifies certain disease- free conditions in the field and diseases attached to seeds. Fusarium wilt disease of tomatoes is one of the most important diseases in the cultivation and production of tomato seeds for export, caused by *FOL*. It can severely damage tomatoes and may be transmitted through seeds. This made the country import a lot of tomato seeds. Moreover, the quality and quantity of tomato production around the world were reduced by this disease (Agrios, 1997).

2.3 Tomatoes important diseases

2.3.1 Bacterial wilt disease

Bacterial wilt is one of the major diseases of tomatoes caused by *Ralstonia solanacearum*. The early stages of the disease begin leaves wilt still green, and the

perennials die. A cross-section reveals that the vascular tissue is brown. When the cut stalks of the affected plants are soaked in the water, a milky stream of bacteria oozes from the stem (**Figure 2. 1**) (Allen *et al.*, 2005; Adeltruda *et al.*, 2012; Suresh *et al.*, 2017).

Figure 2.1 Bacterial wilt symptoms of tomatoes. (A) early stages of the disease, (B) the stem is brown, and (C) bacterial ooze. Source: Allen *et al*. (2005) and Adeltruda *et al*. (2012).

2.3.2 Early blight disease

Early blight (*Alternaria solani*) can affect the stems, leaves, and fruits of tomato plants**.** The symptom of this disease firstly appeared on older leaves. *A. solani* lived in soil and can be attached to seeds**.** The initial symptoms when the fungus infestation was characterized by small black spots on the mature leaves, round, brown, and the tissues around the spot often turn yellow (**Figure 2.2**) (Karthika *et al*., 2020). Severely infected leaves and stalks turned brown, dry, and dead**.** The disease developed at optimum temperature (27-30 °C), with 90% humidity greater (Suresh *et al*., 2017).

Figure 2.2 Early blight symptoms of tomatoes. (A) symptoms of the disease on leaves and (B) stem turn brown. Source: Karthika *et al*. (2020).

2.3.3 Fusarium wilt disease

Fusarium wilt of tomato caused by *FOL*. This fungus lived in soil and can destroy the tomato tree from the seedling stage to the reproductive stage. *FOL* can produce asexual spores of 3 types: microconidia, macroconidia, and chlamydospores. Therefore, it could be epidemic quickly and is difficult to prevent (Mohammadi *et al.*, 2004; Pasquali *et al.*, 2004). The *FOL* spores were spread out at optimum soil temperature (20-34 °C). Particularly, the tropical area including Thailand is the suitable condition for *FOL* outbreaks (Sibounnavong *et al.*, 2012). The infected seedlings were stunted, and the oldest leaves were turned yellow and wilt on one side of the plant or one side of a leaf (**Figure 2.3**) (Lim *et al*., 2006). Usually, diurnal wilting was displayed during sunny days and recovered at night when the temperature was cold, but as it progresses, the plants will wilt and not recover (Suresh *et al*., 2017).

Figure 2.3 Fusarium wilt symptoms of tomatoes. (A) leaf yellow and wilt on one side of a leaf, (B) browning of tomato leaf, (C) symptoms wilting of tomatoes. Source: Lim *et al*. (2006).

2.4 The pathogen *Fusarium oxysporum* on tomato

Fusarium oxysporum is a phytopathogen caused the loss of tomato yields, and farmers can't control the disease sufficiently. Wilt diseases could be caused by many phytopathogens including fungi, bacteria, viruses, and nematode. Therefore, the nature of the symptoms must be considered and caused by any factors to determine the appropriate management method. Fusarium wilt at an early stage could be observed in seedlings. The plants stunting, the lower leaves yellow, the leaves and stems wither, the leaf margins were dry, and the necrotic patches were observed throughout the tomato Fusarium wilt plant. *F. oxysporum* has more than 100 forms, each with a specific host to which *F. oxysporum* can cause disease (Nika *et al*., 2005).

Fusarium is common in warm climates. It is contained in soil and old plant debris. The best way to prevent infection in crops or plants is rotation, sterilization, and chemical fungicide. Nowadays, chemical control affects humans and the environment. So, biological control is the best way to control Fusarium wilt disease.

2.5 Cultural practices

There are several methods of prevention of tomato diseases such as cleaning the garden, climbing plants (tying branches up), and leaf pruning to allow the air inside

the canopy to circulate easily. Able to reduce humidity accumulation during epidemic periods (Kaosiri, 1986). Crop rotation alternately with non-Solanaceae plants, destroying weeds and removing diseased plant debris. In addition, when harvesting from the field, all tomatoes including diseased tomato plants should be removed so that they are not a breeding ground for disease. Tomatoes pre-harvesting and in transit tomatoes should be stored in a cold room under constant temperature (Wisetsang, 2006). In 1984, Saranak *et al*. found that leaf pruning and tying branches up in tomatoes can effectively reduce the infestation of pathogens in tomatoes. Likewise, Tumwine *et al*. (2002) found that pruning diseased tomato leaves can reduce pathogen infection in tomatoes plant.

2.6 Chemical control

The chemicals prochloraz and captan were able to inhibit the growth of *FOL* (Khamsap, 2003; Sompong, 2010). However, Sozzi and Gressler (1980) reported that the fungicide captan to control *FOL* at an ever-increasing rate led to the fungi being resistant to capstan by 2.5 times and must be used at concentrations greater than 100 ppm. Moreover, Bunkoed *et al*. (2017) found that the use of 6% of prochloraz and etridiazole together with 24% quintozene can inhibit the mycelium growth of *FOL*, the cause of Fusarium wilt disease up to 95%. In greenhouse conditions, prochloraz was highly reducing disease damage by 81.71%. Lebsing *et al*. (2021) found that the chemicals mancozeb, carbendazim, and benomyl were able to inhibit the mycelium growth of pathogen fungi by 100%. At present, a large number of fungicides are used to kill tomato wilt each year in Thailand. Fungicides were popularly used by farmers such as terraclor super-x (24% quintozene and etridiazole EC) with recommended rate of 30-60 cc/ 20 L, and carbendazim (50% WP) with a recommended rate of 20-30 g/ 20 L, which was recommended for spraying every seven days (Wisetsang *et al*., 2004; Plodpai, 2008). Terraclor super-x is a fungicide that has the best efficiency inhibit the pathogen hyphae but does not inhibit yeast-like colonies. The mechanism of action of terracol super-x was able to inhibit *FOL* hyphae but not inhibit sporulation.

2.7 Biological control

Biological control is using BCAs to destroy or control pathogens including fungi, bacteria, and nematodes (Pal and Gardener, 2006; Dicklow, 2013). The mode of action of biological agents is to compete with pathogens for nutrients, antibiosis by the production of toxins or antibiotics, parasitism, and induced resistance. Oftentimes, different mechanisms were worked together to make an organism effective (Dicklow, 2013).

2.8 Disease resistance mechanisms in plants

2.8.1 Pre-formed resistance

Plants have developed several defense mechanisms to protect themselves from pathogens. The plant's structure, and chemistry were improved before pathogens infection to avoid or prevent diseases. Some of them are reactions to the presence of pathogens through the categories that overlap substantially: including plant structures such as surface wax, cuticle, cell walls (thickness), and casparian strip (endodermis) (Moore *et al*., 2018). Some plants synthesize phytoanticipin has the effect of inhibiting the growth of pathogens. These structures are like the first layer of plant protection (Buchanan *et al.*, 2000). However, pre-formed resistance is controlled by many genes. In addition, plants also need time and energy before the infection is destroyed. Plants could be protected themself from phytopathogens attacking.

2.8.2 Induced resistance

Induction of plant resistance against pathogen infestation can be accomplished by stimulating elicitors. The biotic elicitors including less- or non-virulent pathogens, antagonistic microorganisms (*Bacillus*, *Pseudomonas*, and *Trichoderma*), and abiotic elicitors including organic, inorganic extracts were used to stimulate plant defense to increase resistance to disease (Jimkuntod, 2018). At present, elicitors inducing plant defense have been widely evaluated for the control of plant diseases (Choudhary *et al.*, 2007; Buensanteai *et al.*, 2009; Buensanteai and Athinuwat, 2012). Induced resistance is a pre-treated elicitor before phytopathogen infection, which is divided into 2 types induced systemic resistance (ISR) and systemic acquired resistance (SAR). SAR is also dependent on salicylic acid (SA) signaling and the systemic expression of

pathogenesis-related (PR) proteins genes, acquired disease resistance is a process for existence in the form of plant memory (Sticher *et al.*, 1997; Hammerschmidt, 1999). Another feature of SAR is its broad-spectrum and unique resistance to pathogens which include viruses, bacteria, and fungi (Vallad and Goodman, 2004; Conrath, 2006). ISR is induced by biotic elicitors, including plant growth-promoting rhizobacteria (PGPR), which is characterized by species belonging to the genus *Pseudomonas* that do not cause visible damage to the root system of plants. ISR is dependent on the jasmonic acid (JA) and ethylene (ET) pathway, not involved with pathogenesis-related proteins (PR Protein) (**Figure 2.4**) (Heil and Bostock, 2002; Van and Bakker, 2005; Choudhary *et al*., 2007; Walters *et al*., 2008; Levy *et al*., 2015).

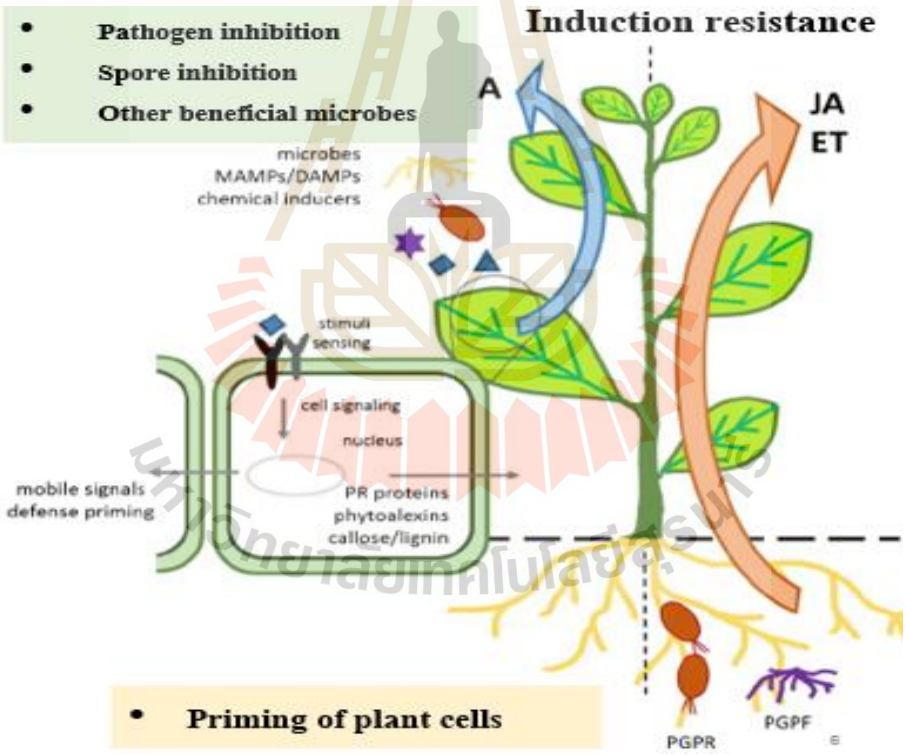


Figure 2.4 Induction of resistance for plant protection against pathogens (Burketova *et al*., 2015).

2.8.2.1 Mechanism of defense resistance

Plants are continuously revealed with pathogen attacks, but the plant showed no symptoms of infection. This is because many plants and microorganisms have interactions that can be described by the gene-for-gene concept, through resistance (R) genes, which can recognize pathogens with the corresponding avirulence (Avr) gene. This interaction causes a hypersensitive response (HR), together with localized cell death around the infection site and resulting in a large number of cloning of the R gene from plants (Hammond-Kosack and Jones, 1996; Dangl and Jones, 2001; Meyers *et al*., 2005; González-Lamothe *et al.*, 2006; Ponce de León and Montesano, 2013). Induced resistance is defined in two forms 1) ISR relies on JA and ET as signaling factors and 2) SAR relies on SA signaling and the accumulation of defense enzymes and PR proteins (**Figure 2.5**) (Vallad and Goodman, 2004; Buensanteai *et al*., 2009, Buensanteai and Athinuwat, 2012). SAR can be occurred by exposing plants to virulent pathogens and avirulent pathogens and nonpathogenic microbes, or by chemical stimulation, such as SA, 2,6-dichloro-isonicotinic acid (INA), or benzo (1,2,3) thiadiazol-7- Carbotioic acid S-Methyl ester (BTH).

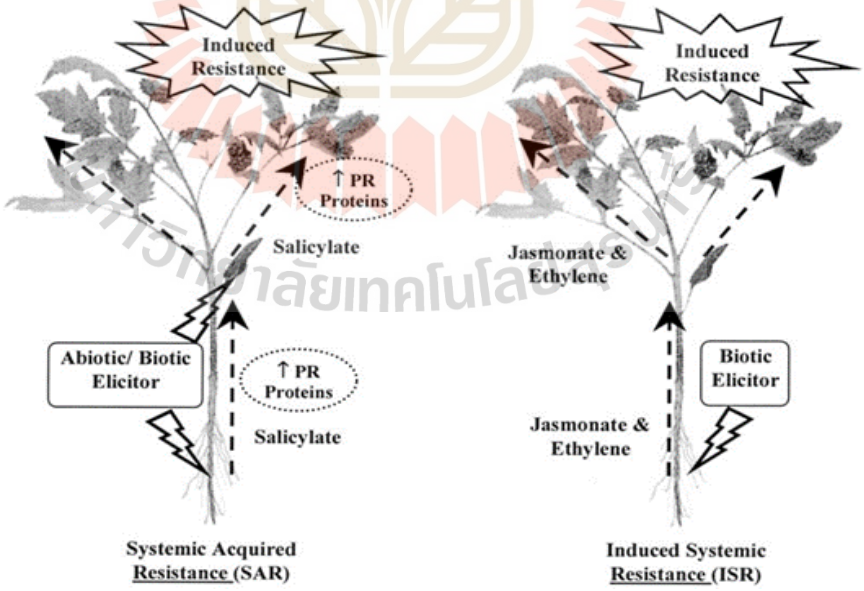


Figure 2.5 The comparison of two types of induced resistance in plants including systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Vallad and Goodman, 2004).

2.8.2.2 Characteristics of elicitors

The term elicitor is often used for compounds that can stimulate the production of phytoalexins against disease (Thakur and Sohal, 2013). Each elicitor is unique, including carbohydrate polymers, lipids, glycopeptides, and glycoproteins. Usually, the plant defense response occurs after the plant detected the microorganism through recognizing elicitor molecules. Elicitor molecules are released between plants and pathogens according to the perception of the elicitor. Typically, activation signaling leads to active oxygen species (AOS), phytoalexin, biosynthesis, strengthening of plant cell walls associated with phenylpropanoid compounds, calloused, defense enzymes synthesis, and PR protein deposition, some of which have antimicrobial properties (Thakur and Sohal, 2013). The mechanism caused plants' resistance against phytopathogens (Prakongkha, 2011; Phiwthong, 2014). The examples of the elicitors are showed in **Table 2.1**.

Table 2.1 Example of the elicitor for the biotic elicitor and abiotic elicitor.

Type of elicitor	Elicitor	Plants	Effects of elicitor	References
Biotic	*Bacillus subtilis*	Grape	Induced resistance *Botrytis cinere*	Rodgers, 1989
			Induced resistance *Eutypa lata*	Ferreira *et al*., 1991
			Induced resistance *Fusarium oxysporum* and *Botryodiplodia theobrome*	Swain *et al*., 2008
	Trichoderma harzianum T39	Grape	Resistance downy mildew	Palmieri *et al*., 2012
	Trichoderma xylanases	*Arabidopsis thaliana*	Induced resistance *Pseudomonas syringae*	Ron and Avni, 2004
	Trichoderma peptaibol	Cotton	Induced resistance *Botrytis cinerea*	Li *et al*., 2009
Abiotic	Salicylic acid (SA)	Tomato	Promotes strength for susceptible species to pathogens and abiotic stress.	Shirasu *et al*., 1997
		Banana	Delays banana fruit ripening.	Srivastava and Dwivedi, 2000

Type of elicitor	Elicitor	Plants	Effects of elicitor	References
Abiotic	Salicylic acid (SA)	Tomato	Synthesis PR-Protein which increases chilling tolerance and resistance to pathogens.	Magallon *et al*., 2002
		Tomato	Increased transcription of PR1 and BLG2 genes and increasing H_2O_2 related to the resistance of *Helicoverpa armigera.*	Peng *et al*., 2004
		Okra	Synthesis of phenolic compound and increasing PAL activity.	Vimala and Suriachandraselvan., 2009
	SA and 4-aminobutaryc acid	Peas	Increased phenol metabolizing POD, PPO, and PAL activity. That helps in the process of resistance to *Erysiphe polygony* in beans	Katoch *et al*., 2005
	SA, Chitosan, methylsalicylate, methyljasmonate	Eggplant	Increased PAL, POD, and polyphenol oxidase to help resist infection of *Ralstonia solanacearum.*	Mandal, 2010

Type of elicitor	Elicitor	Plants	Effects of elicitor	References
Abiotic	Benzothiadiazole	Rice	Resistance powdery mildew	Gorlach *et al*., 1996
		Sunflower	Synthesis of phytoalexin, PR-protein chitinase, H_2O_2	Sauerborn *et al*., 2002
		Cauliflower	Induced resistance to downy mildew.	Godard *et al*., 1999
	Benzothiadiazole and humic acid	Soybean	Reduced disease incidence of root rot and wilt diseases.	Abdel-Monaim *et al*., 2011
	Chitosan	Tomato	Induced resistance Fusarium crown and Root rot in the seedling.	Benhamou *et al.,* 1994
		Tomato	Induced resistance *Fusarium oxysporum* and *Phytophthora capsica*	Ortega-Ortiz *et al*., 2003
	Chitosan	Tomato	Induced resistance to Fusarium crown and Root rot in the seedling.	Benhamou *et al*., 1994
		Tomato	Induced resistance to *Fusarium oxysporum* and *Phytophthora capsica*	Ortega-Ortiz *et al*., 2003

Type of elicitor	Elicitor	Plants	Effects of elicitor	References
Abiotic	Chitosan	Potato	Induced resistance to *Fusarium sulphureum*	Sun *et al*., 2008
		Sorghum	Induced resistance to downy mildew and increase the synthesis of NO (seed priming)	Manjuntha *et al*., 2009
		Rice	increase the synthesis of H_2O_2	Pongorayoon *et al*., 2013
	β-amino butaric acid	Orange	Inhibit germination and growth of *Penicillium italycum* spores.	Tavallali *et al*., 2008

2.8.3 Abiotic elicitors

Induced resistance of plants against pathogen infection can be activated by elicitors. Several chemical compounds have been discovered that can induce plant defense systems. The activator-acibenzolar-S-methyl (BION) has been reported against many fungi, bacteria, and viruses (Oostendorp *et al*., 2001). Moreover, *ß*-Aminobutyric acid (BABA) or 3-(S)- enantiomer could be induced crops against downy mildew disease (Cohen and Tosi, 1995). Previous, probenazole is registered as a seedling or granular treatment in rice against rice blast and bacterial blight disease (Sticher *et al*., 1977). Le Thanh (2015) reported that SA applied as seed soaking and foliar spraying can be reduced rice bacterial blight disease by 55.35%. Thakur and Sohal (2013) reported that benzoic acid, benzothiadiazole, chitosan, methyl salicylate, and SA affected the production of phenolic compounds and defense-related enzymes in plants.

2.8.3.1 Salicylic acid (SA)

SA is a phytohormone and plays an important role in the resistance signaling pathway, primarily in defense to induce local and systemic resistance in plants against pathogens (Hammerschmidt and Smith., 2000; Hussain *et al.*, 2015; Le Thanh *et al.*, 2017). It can also be synthesized from cinnamate produced by phenylalanine ammonia-lyase (PAL) (Chen *et al*., 2008).

There is a study of resistance stimulation by SA in tomatoes. Shirasu *et al*. (1997) found that SA can reduce disease in weak tomato varieties. Garcia-Magallon *et al*. (2002) found that SA stimulated the formation of PR-Protein that helps in disease resistance. Peng *et al*. (2004) found that SA has an effect on increasing transcription of pr1 and bgl2 genes, which are marker genes in the SA pathway, and also increases the amount of hydrogen peroxide (H_2O_2) that affects the resistance to *Helicoverpa armigera*. Katoch (2005) reported that SA helps to increase the activity of polyphenol oxidase (PPO), peroxidase (POD), and Phenylalanine ammonia-lyase (PAL) enzymes related to resistance to *Erysiphe polygony* in beans. Likewise, Vimala and Surichandraselvan (2009) reported that SA increases the synthesis of phenolic compounds and increases the PAL enzyme activity in the okra to resist *Erysiphe*

cichoracearum. Mandal (2010) found that SA promotes the bacterial wilt disease resistance caused by *Ralstonia solanacearum* in eggplant. In addition, Le Thanh *et al*. (2017) reported that rice seed treatment and foliar sprays with exogenous SA reduced bacterial leaf blight severity caused by *Xanthomonas oryzae* pv. *oryzae* by more than 38%.

2.8.3.2 Chitosan

Chitosan is a carbohydrate substance that is used to control plant diseases. It is a derivative of chitin which was cut in the acetylene group of sugar N-acetyl-D-glucosamine that can be degraded naturally, non-toxic to plants, safe to humans and the environment, and can promote plant growth and beneficial microorganisms (Lemma, 2002). Chitin is a component of hard shells that cover the cells of mold, yeast, and many types of microorganisms. Chitin contains nitrogen as an element, nitrogen is released slowly from the molecules, stimulating the immune system of the plant and stimulating the use of minerals (Surawattanawan, 2011; Phiwthong, 2014). Chitosan can inhibit pathogens and act as an elicitor in the SAR simulation process to establish a disease resistance (Prakhong, 2012).

There are reports that chitosan can stimulate the activity of PPO, POD, PAL, chitinase, and PR protein 1 (Pr1), *β*-1,3-glucanase, and chalcone synthase activities. Fajardo *et al*. (1994) found that peanuts seed dressing with chitosan can increase the synthesis of phenolic acids and ferulic acids which can induce resistance to peanuts against *Aspergillus flavus*. Sathiyabama *et al*. (1998) found that spraying chitosan at a concentration of 1,000 ppm can stimulate SA formation and increase *β*-1,3-glucanase and chitinase activity that can reduce the occurrence of rust disease in peanuts. In 2000, Dalila Paz-Lago *et al*. found that foliar spraying chitosan hydrolysates and MSB showed the best results in reducing Fusarium wilt disease. It suggested that the induction of systemic resistance plays a major role as a defense mechanism of tomato against *FOL* attack. Iriti *et al*. (2010) reported that the stomatal uptake chitosan compound is determinant for the induction of plant defense mechanisms against accumulations of callose causing the plant disease resistance. Chakraborty *et al*. (2015)

found that foliar applications chitosan in chili increased *ß*-1,3 glucanase, POD, PPO, PAL, catalase, and phenolics. In 2016, Jahan *et al*. found that all tested concentrations have significantly reduced the mycelia growth of the phytopathogen. And the highest disease reduction of 78.61% was obtained with chitosan application at 800 ppm.

2.8.4 Biotic elicitors

Biotic elicitor is an alternative tool fungicide for protecting the crops against disease with high safety and sustainability. The beneficial microorganisms including *Agrobacterium radiobacter*, *Coniothyrium minitans*, *Trichoderma harzianum*, *Gliocladium catenulatum*, and *Pseudomonas* sp. have a high ability to control plant disease (Feng Wei *et al*., 2016). Laurent *et al*. (2016) reported that disease severity caused by *Fusarium* sp. could be reduced by treating *B. subtilis* strain RC218 associated with mycotoxin accumulation under field conditions. Moreover, *Bacillus* sp. strain HN09 isolated from rhizosphere soil of neem could inhibit mycelium growth, and spore germination, and reduce Fusarium crown and root rot disease (*F. oxysporum* f. sp. *radicis-lycopersici*) on tomatoes under greenhouse conditions (Lin *et al.*, 2017). And the *Bacillus* sp. strain B44 isolated from rhizosphere tomato could reduce Fusarium wilt (*FOL*) by 36% under greenhouse conditions (Jangir *et al*., 2018). On the other hand, *Trichoderma harzianum* inhibited fungal pathogen *Phythium ultimum* and *Phytophthora capsici* under *in-vitro* conditions. In addition, no effect on the pith, vascular bundle, and cortex of the host plant inoculates with *T. harzianum* and pathogens (Uddin *et al*., 2018).

2.8.4.1 *Bacillus* sp.

Bacillus sp. can synthesize various kinds of antibiotics and enzymes. These compounds can inhibit the growth of plant pathogens which is especially important in the agriculture industry (Chen *et al*., 2008). The mechanisms of microorganisms against fungi include obstruction, disturbance, and the formation of holes in the fungal cell wall. Moreover, some peptides are involved in the disintegration of fungal intracellular such as nucleic acid and mitochondria. In addition, *Bacillus* synthesizes many potential

amphiphilic and biological surfactants lipopeptides comprising bacillomycin, mycosubtilin (iturin family), and plipastatin (fengycin family) (Gong *et al.*, 2015). The existence of characterization L amino acids and D amino acids and bacterial hydrophobicity were revealed. These are divided into 2 categories including iturin and macrolactones. The iturin has a destructive effect on the fungal wall by causing the cell to leak by inserting the hydrophobic tail into the cytoplasm and undergoing auto-aggregation to form pores in the cell membrane (Maget-Dana *et al*., 1985).

B. subtilis strain CaSUT008 isolated from the soil can inhibit the pathogen, plant growth promotion and acts as an elicitor in the induced resistance mechanism. These mechanisms can reduce disease incidence and disease severity in plants (Saengchan, 2014). Buensanteai *et al*. (2012) found that the use of *B. subtilis* strain CaSUT008 was able to promote cassava and cucumber via biosynthesis of indole-3-acetic acid (IAA) a plant hormone. The hormone IAA promotes the cassava growth by more than 30%, and can stimulate cassava and cucumber to express biochemical composition and various proteins in the defense mechanism to resistance the invasion of pathogens. Similarly, *Bacillus* sp. strain NB111 was able to enhance cucumber seedling growth including root length and plant height compared to the control (Buensanteai *et al*., 2014).

2.8.4.1.1 Indirect plant growth promotion by *Bacillus* sp.

The *Bacillus* sp. has a role to plant growth promotion by synthesis of certain plant hormones such as gibberellin (GA) and auxin (IAA), which are hormones that are important for plant growth (Cavaglieri *et al*., 2005). The results of Nemutanzhela *et al*. (2014) study showed that the phytohormone (GA, IAA) and defense enzyme (PO, PPO, superoxide dismutase) was induced by treating tomato seeds with *B. subtilis* OTPB1. In addition, *B. subtilis* colonize on plant rhizosphere has the ability-enhancing nutrient uptake by increasing plant hormones and decreasing ethylene (Baysal *et al*., 2008).

2.8.4.1.2 Systemically induced disease resistance by *Bacillus* sp.

When plants are attacked by pathogens, the plant tissues can resist the attack pathogens. This type is known as SAR which has a broad spectrum and long-term capabilities of plants (Qi *et al.*, 2010). Moreover, nonpathogenic microorganisms colonization on roots could also induce plants against phytopathogen that is ISR (Maget-Dana *et al*., 1985). Like SAR, the ISR has also been shown in many crops and is effective against a broad range of diseases (Cao *et al*., 2012). In the study of Cao *et al*. (2011), *B. subtilis* SQR 9 was applied as a bio-organic fertilizer, it could reduce Fusarium wilt incidence disease by 49–61% when compared with the control.

2.8.4.1.3 Activation of plant-resistance activators by *Bacillus* sp.

Induced resistance by rhizobacteria results in a phenotypic response similar to the pathogenically induced systemic resistance. PGPR elicited plant resistance against fungi, bacteria, and viruses under *in-vivo* conditions in which the rhizobacteria and pathogen were kept separately. The degree of inducing resistance was different depending on PRPG and plant species (Vadas *et al*., 1989). The determinants of systemic induced resistance by rhizobacterial were lipopolysaccharide, JA, ethylene, and SA perception (Gong *et al.*, 2014). The degree of resistance was highly effective under challenging conditions thus induced systemic resistance (ISR) is recommended for the biological control of plant disease. In 2004, Ryu *et al*. studied the efficacy of bacterial volatile for ISR activation on Arabidopsis seedlings. The result found that the volatility of *Bacillus* sp. at 4 days after culture could decrease the soft rot disease significantly.

2.8.4.1.4 Enzymes produced by *Bacillus* sp.

In addition to the production of antibiotics, *Bacillus* sp. could also produce enzymes such as chitinase and *β*-1,3-glucanase with a very strong lytic activity (Xu *et al.*, 2013). Lytic enzymes synthesized by *Bacillus* sp. play a significant role in the decomposing of the fungal cell walls (Volpon *et al*., 2000). *Bacillus* sp. could produce many defense enzymes including PPO, PO, and PAL that were reported in previous

research (Thasana *et al.*, 2010). In addition, the researcher also found that the PAL activity plays an important role in plant protection via the phenylpropanoid pathway. At the same time, PPO, *ß*-1,3-glucanases, and chitinases activity were important to induce plant defense against many plant pathogens while PO and PAL were involved in phenylpropanoid breakdown in plant tissues (Ohno *et al.*, 1995). In 2012, Ye *et al*. investigated the *Bacillus subtilis* strain EPCO16 for inducing resistance against *FOL* in tomatoes. The liquid and telic formulation of *B. subtilis* strain EPCO16 were able to induce defense-related enzymes and proteins in diseased tomato plants including PAL, catalase, and phenolics lyase.

Some types of *Bacillus* sp. could directly antagonistic fungal pathogens by producing fungal toxic compounds, or by competing for niches and essential nutrients (Dessalegne *et al.*, 1992). Chitinases and chitinolytic microorganisms have been used for controlling fungal pathogens (Balem, 2008). In 2012, Gomaa *et al*. showed the efficacy of chitinase purified from *B. thuringiensis* strain NM101-19 in soybean seed treatment against *Fusarium* sp. The result found that the chitinase purified from *B. thuringiensis* strain NM101-19 has the potential to break down the cell walls of the fungal pathogen (Horticulture research and development in Ethiopia, 1992).

2.9 Application of biotic and abiotic elicitors in plants

Plants treated with elicitors increase the resistance of plants due to activating multiple signaling pathways of intracellular defense. Biotic and abiotic elicitors induced immune defense responses in plants, which depend on elicitor, concentrations, and timings of application. The use of elicitors to control plant disease can be applied as a seed soaking, drenching, or foliar spray (**Figure 2.6**). In addition, the application of biotic and abiotic elicitors could contribute for managing and enhancing plant physiology (Berninger *et al*., 2018).

Mishra *et al*. (2014) reported that the tomato leaves were sprayed with the bacterial *Pseudomonas* sp. cell pellet mixed chitosan solution (5%) which enhanced the biocontrol efficacy against *Tomato leaf curl virus* (ToLCV) and could increase the

plant height by 45.33%, biomass by 41.21%, chlorophyll content by over 39.48%, and yield by 74.57%.

Ashley *et al*. (2007) reported the efficiency of chitin and chitosan to induce Fusarium wilt disease in celery. The result showed reducing disease incidence and disease severity when compared with the control. Chitosan used for root dipping did not reduce Fusarium wilt disease incidence but significantly reduced disease severity when used with resistant varieties of celery.

Benhamou *et al*. (1997) reported the efficiency of *B. pumilus* strains SE 34 combined with chitosan, for inducing defense in tomatoes. The results found that the tomato roots could be stimulated by plant physiology changes due to the effect of chitosan. In addition, the combinations between PGPR strain SE 34 with chitosan could be implemented as biocontrol strategies.

Bakeer *et al*. (2016) studied the combination applying of bio-agents and chitosan against fusarium soil- borne diseases. The result showed that the most effective treatment was a combination of *T. harzianum*, and *B. subtilis* with chitosan at a concentration of 500 ppm as foliar spraying which reduced the incidence and severity of the disease of Fusarium crown and root rot, and Fusarium wilt on tomatoes. These combination treatments could provide sustainable management of tomato diseases under field conditions.

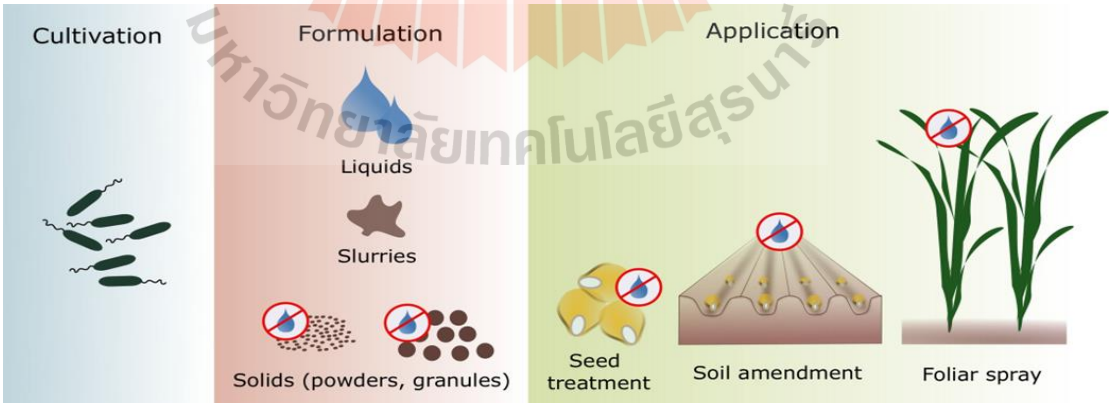


Figure 2.6 Formulation possibilities and inherent application techniques (Berninger *et al*., 2018).

2.10 Fourier Transform Infrared (FTIR) microspectroscopy

Fourier transform infrared (FTIR) microspectroscopy is a technique commonly used in molecular analysis which consists of atoms adhering to chemical bonds. Usually, these atoms are in constant motion or vibration. There are 2 types of vibration in chemical bonds: stretching and bending, using medium infrared light in the range of 2.5-25 nm or 4000-670 cm^{-1} (Saarakkala *et al*., 2010; Le Thanh *et al*., 2017). Synchrotron (SR) based FTIR microspectroscopy has been developed as a rapid, direct, non-destructive, and bioanalytical technique (Yu, 2004; Thumanu *et al*., 2015). It could analyze samples at the micron level or micro-sample areas that cause the molecules to absorb light and then measure the light transmitted to show the relationship of frequency or wavenumber with the transmittance of light, called IR Spectrum (Yu, 2004; Kastyak *et al*., 2012; Wang *et al*., 2015). SR-FTIR microspectroscopy is a new way to look at plant cell walls (Thumanu *et al*., 2015). The absorbance spectrum characteristics of each substance will have specific properties which enable the molecular fingerprint of biochemical components in cell samples or tissue (Kenneth and Lawrence, 2005; Yu, 2008; Thumanu *et al*., 2015; Thumanu *et al*., 2017). The spectral characteristics obtained from biological samples will indicate the characteristics of biochemical compounds within cells or tissues such as carbohydrates, proteins, lipids, and phospholipids can be divided into 6 groups including:

1. The range of 4000-3100 cm^{-1} is mainly due to the absorbance of OH (3400 cm^{-1}) and NH Stretching mode (Amide A ~ 3300 cm^{-1} and Amide B ~ 3030 cm^{-1}).

2. The range of 3100-2800 cm^{-1} the absorption of light C-H Stretching vibration of CH_3, CH_2, which is caused by light absorption of lipids.

3. The range of 1800-1500 cm^{-1} is the absorbance of Amide I and Amide II of protein groups. In particular, the secondary structures of proteins such as alpha-helix, beta-sheet, beta-turn as well as C=O groups from the lipid ester group.

4. The range of 1300-1500 cm^{-1}, the absorbance of C-H bending vibration of CH_3, CH_2 caused by lipid absorption, as well as stretching vibration COO^- from Amino acid side chains.

5. The range of 1230 cm^{-1} is due to the absorption of P=O asymmetric stretching vibrations of phosphodiester, free phosphate, and monoester phosphate, mainly due to the absorption of DNA/RNA Polysaccharide backbone structures.

6. The range of 1200-900 cm^{-1} is due to PO_2- from nucleic acid and C-OC, C-O-P Stretching vibrations of oligo-polysaccharide.

This technique could be used to study the accumulation of substances in plant biochemical processes involved in plant growth- promoting mechanisms, plant immunity, and the promotion of plant growth. At present, this technology has begun to be used to detect the differences between each type of microorganism and is also able to identify plant pathogens because it is accurate, hassle-free, and inexpensive (Buensanteai *et al*., 2012). Few reports have been published on the application of FTIR in plants. According to McCann *et al*. (1992), the FTIR spectroscopy technique could detect large structural changes in pectic polymers on deportation and drying from the cell wall of both single onions (*Allium cepa*). FTIR spectroscopy provides an accurate and fast assay for wall components and cross-links by identifying polymers and functional groups. Yu (2008) used SR light sourced FTIR microspectroscopy to characterize the protein molecular structure of plant tissue. The cluster analysis and principal component analysis of yellow and brown *Brassica canola* seeds within the cell did not show differences were shown obviously in terms of protein amide I structure demonstration that they were related. However, the study showed that both yellow and brown canola seeds contain the same protein but with different ratios. Thumanu *et al*., (2017) have been investigating the effect of *B. subtilis* strain D604 on inducing resistance mechanisms of chili using SR-FTIR. The result found that the changing cell chili was observed in higher integral areas for the C=O ester from lipids, lignin, or pectin (1770–1700 cm^{-1}) as well as polysaccharides (1200–900 cm^{-1}).

CHAPTER III
RESEARCH METHODOLOGY

3.1 Preparation of *Bacillus*-based chitosan formulations (BBC)

The *Bacillus* sp. (acid-base tolerance) strain CaSUT008 was obtained from the stock culture of Plant Pathology and Biopesticide Laboratory, Suranaree University of Technology. The experiment was carried out in completely randomized design (CRD) with 3 supplement mixtures including CHIZA4 (chitosan formulation 1; BBC1), CHIZA7 (chitosan formulation 2; BBC2) and 2% chitosan (chitosan formulation 3; BBC3) at 800, 1,000, 1,200, 2,000 µg/ml. The *Bacillus* sp. strain CaSUT008 was cultured in 100 ml of nutrient broth (NB) in conical flasks on a rotary shaker at 200 rpm and 37°C. Then, the BBC was prepared by mixing each supplement mixture with 2% sodium alginate in miscible liquids (Ke Tong, 2017). Next, the encapsulated BBC were packed in plastic bags and keep at room temperature for the next experiments (Wongchalee, 2015).

3.2 Evaluation of the antifungal activity of encapsulated BBC with *F. oxysporum* f.sp. *lycopersici* (*FOL*) in *in-vitro* conditions

The experiment was carried out in CRD with 12 encapsulated BBC treatments, 2 positive control, 1 negative control, and 3 replications (**Table 3.1**). The cork borer with a diameter of 0.5 cm was used to pierce the agar containing *FOL* mycelium, which was placed at 2 cm from the edge of the petri dish. On the opposite side, a disc of BBC encapsulates treatments, terraclor super-x® (positive control), *Bacillus* sp. strain CaSUT008 (positive control), and distilled water (negative control) was placed similarly with keep a distance of 5 cm between the two dishes. The plates were incubated for 72 h at 28 °C and measured the diameter of the mycelium pathogen. The percentage inhibition of radial growth (PIRG) was calculated by the formula of Tronsmo (1992):

$$\text{PIRG} = \frac{R1-R2}{R1} \times 100$$

R1 = The radial fungal mycelium of *FOL* in the negative control petri dish.

R2 = The radial fungal mycelium of *FOL* in encapsulated BBC treatment and positive control.

Table 3.1 The describe treatments in this study.

Treatment	Concentration	Note
BBC1 (*Bacillus*-based CHIZA4)	800, 1,000, 1,200, and	12 encapsulated BBC
BBC2 (*Bacillus*-based CHIZA7)	2,000 µg/ml	treatment
BBC3 (*Bacillus*-based 2% chitosan)		
Bacillus sp. strain CaSUT008	10^8 cfu.ml^{-1}	Positive control
Terraclor super-x®	20 ml / 20 L	Positive control
Distilled water		Negative control

3.3 Environmental scanning electron microscope (ESEM) analysis

The *FOL* sample from the highest inhibition encapsulated BBC treatment, positive and negative control at 24 hours after incubation (HAI) was prepared similarly to the previous experiment. The 2.5% glutaraldehyde was added to a phosphate buffer solution at 4 °C for 6 h. Then, the samples were washed with sodium phosphate buffer for 2 h at 4 °C and repeated 3 times (Mihali *et al*., 2012; Fernando *et al*., 2017). The *FOL* samples were analyzed at 10 kV. by Environmental scanning electron microscope (ESEM) at Synchrotron Light Research Institute (Public Organization).

3.4 Evaluation of survival *Bacillus* sp. on encapsulate

The experiment was carried out in CRD with 3 replications. The highest effective encapsulated BBC from experiment 3.2, 1 formulation was kept at room temperature (26-30 °C) in an opaque bottle. The 0.05 g of encapsulated BBC was dispersed in 10 ml of distilled water. The dilute with 1.64 M of potassium dihydrogen phosphate (KH_2PO_4) was conducted to obtain 8 concentrations (10^{-1}-10^{-8}) (Wiwattanapatapee, **2011**). Then, 100 µl of each concentration was spread plated over

the entire nutrient glucose agar (NGA) surface and calculated survival bacterial colonies after incubation for 24 h. The samples were collected every month at 0, 1, 2, 3, 4, 5, and 6 months after storage. The bacterial concentration was performed to observe trends in bacterial survival rates in the encapsulate formulations, using the formula of Pupakdeepan (2009), as follows:

$$\text{Survival rate of bacterial (CFU/g)} = \frac{\mathbf{N \times DF \times V_0 \times 1000}}{\mathbf{V_a \times W_0}}$$

N = number of colonies counted

DF = dilution factor

V_0 = amount of initial stock suspension

V_a = amount of suspension used in the spread plate

W_0 = weight of the BBC formulations assessed

Then, the survival rate of bacterial (CFU/g) was transferred and showed as log (survival rate of bacterial (CFU/g)).

3.5 Evaluation of encapsulated BBC for induced resistance in tomato against Fusarium wilt disease under greenhouse conditions

The experiment was carried out on 3x5 factorial in CRD, and 4 replications with 10 plants per replication (**Table 3.2**). The tomato seeds surface was sterilized for 1 min by washing with 95% ethanol. Then, the seeds were rinsed with distilled water for 5 min (2 times) before planting into the mixture of soil and organic fertilizer. The tomato seedlings were transplanted into the plastic bag containing 5 kg of SUT soil. The highest effective encapsulated BBC treatment was used to evaluate the induced resistance of tomatoes against Fusarium wilt disease. The treatments were divided into 3 methods: soil drenching 150 ml/pot at 7, 28, and 49 days after transplanting; spraying at 7, 14, 21, 28, 35, 42, and 49 days after transplanting; and a combination of both methods (Lahlali *et al.*, 2013). At 63 days after transplanting, the plants were inoculated with spores suspension of *FOL* at 1 x 10^6 conidia/ml. The disease severity

and plant growth parameters including plant height and fresh weight were recorded 7 days after inoculation *FOL* (Herman and Perl, 2007; Karimi *et al.*, 2010).

Table 3.2 List of treatments on evaluation of encapsulated BBC for induced resistance against tomato Fusarium wilt disease under greenhouse conditions.

Treatment	Application (Factor A)	Elicitor (Factor B)
1	Soil drenching[1/]	The highest effective encapsulated BBC
2		COS®
3		*B. subtillis* commercial
4		Terraclor super-x® *(*positive control*)*
5		Water (negative control)
6	Foliar spray[2/]	The highest effective encapsulated BBC
7		COS®
8		*B. subtillis* commercial
9		Terraclor super-x® *(*positive control*)*
10		Water (negative control)
11	Combination[1,2/]	The highest effective encapsulated BBC
12		COS®
13		*B. subtillis* commercial
14		Terraclor super-x® *(*positive control*)*
15		Water (negative control)

[1/] Soil drenching at 7, 28, and 49 days after transplanting.

[2/] Foliar spraying at 7, 14, 21, 28, 35, 42, and 49 days after transplanting.

3.6 Study of tomato defense mechanisms against Fusarium wilt disease by treated encapsulated BBC

3.6.1 Salicylic acid content

Tomato leaves were collected from the experiment 3.5. The 0.5 g of tomato leaves was pulverized in a cold mortar with liquid nitrogen and added 500 µl of 90% (v/v) methanol. The mixture was centrifuged for 15 min at 12,000 rpm, 4 °C in a 1.5 ml micro centrifuges tube. Then, the 100 µl of supernatant and 0.02 M Ferric ammonium sulfate were mixed into a 96 well plate, and incubated for 5 min at 30 °C. After that, the mixture was read by Spectrophotometry with an absorbance of 530 nm. The salicylic acid content (µg g^{-1} fresh weight) was analyzed and compared with the standard curve (Raskin *et al.*, 1989; Phiwthong *et al.*, 2014).

3.6.2 Assay of defense enzymes

3.6.2.1 Protein extraction

The total protein extracted from tomato leaf samples was collected from experiments 3.5 at 0, 24, and 48 HAI. The 0.5 g of tomato leaves were pulverized in a cold mortar with liquid nitrogen, which was put into micro centrifuges tube and added 1 ml of homogenization buffer (1mM phenylmethylsulfonyl fluoride, 0.1 M Tris-HCl at pH 7.0, triton X-100, 0.1 M KCl, 3% (w/v) polyvinylpyrrolidone). Then, the mixture was centrifuged at 12,000 rpm, 4 °C for 30 min. The supernatant was transferred to a new tube to detect total protein concentration according to the description by Bradford (1976) and Prakongkha *et al.* (2013). The total protein was used for assay chitinase, *β*-1,3-glucanase, and phenylalanine ammonia-lyase (PAL) activity.

3.6.2.2 Assay of chitinase

The 0.4 ml of the total protein was mixed with 0.1% colloidal chitin and 0.05 M sodium acetate buffer (pH 5.0) with a ratio of 1:1, which was incubated at 37 °C for 2 h. After that, the 200 µl of the mixture was dripped into 96 well plates to measure chitinase activity at the absorbance of 585 nm. The N-acetyl glucosamine (GlcNAc) at 0, 0.5, 1, 2, 5, 10, 20, 50, 100, 150, and 200 µg/ml was used as standard. One unit of chitinase activity was calculated as the product of 1 µmol GlcNAc formed

min^{-1} mg^{-1} protein (Reissig *et al.*, 1955; Dumas *et al.*, 2009; Prakongkha *et al.*, 2013; Carmen and Roncero, 2016).

3.6.2.3 Assay of *β*-1,3-glucanase activity

The 62.5 µl of the total protein was mixed with 62.5 ul of 4 mg/ml laminarin in sodium acetate buffer (pH 5.0), which was boiled in hot water and gently shaken at 40 °C for 10 min. After that, the reaction was stopped by adding 375 µl of 1% 3,5-Dinitrosalicylic acid and boiled in hot water for 5 min. The mixture was measured at an absorbance of 500 nm. The *β*-1,3-glucanase activity was calculated as µmol glucose released min^{-1} mg^{-1} protein (Pan *et al.*, 1991; Buensanteai *et al.*, 2009; Prakongkha *et al.*, 2013).

3.6.2.4 Assay of phenylalanine ammonia-lyase (PAL)

The 50 µL of homogenate was used to determine the L-phenylalanine ammonia-lyase activity. The reaction consisted of 0.5 ml of borate buffer, 20 mM L-phenylalanine, 0.25 ml, and 0.25 ml of distilled water was incubated at 30 °C for 4 h. The absorbance was measured at a wavelength of 290 nm. The PAL activity was calculated based on standard curves using cinnamic acid at 0, 0.5, 1, 2, 5, 10, 20, 50, 100, 150, and 200 µg/ml. One unit of PAL activity was defined as 1 µmol trans-cinnamic acid min^{-1} mg^{-1} protein (Giorgi *et al.* 2009; Kim and Hwang, 2014; Riaz *et al.*, 2014).

3.7 Analysis of biochemical changes of tomato against Fusarium wilt disease by Synchrotron Fourier-transform infrared (SR-FTIR) microspectroscopy

3.7.1 Samples preparation

Biochemical changes in tomato leaves were studied by Synchrotron Fourier-transform infrared (SR-FTIR) microspectroscopy. The tomato leaf samples were selected from the highest effective encapsulated BBC treatment and control (water) of the experiment 3.5. The tomato leaf samples were frozen and fixed in optimal cutting temperature compound (OCT, Tissue-Tek®, Electron Microscopy Science) by liquid nitrogen and stored at -80°C for 24 hours. The frozen sample was cut with a cryostat to a size of 20 microns. The cutting sample was placed on infrared transparent

BaF_2 windows with a size of 13x2 mm. The moisture was removed by vacuum desiccator for approximately 30 min to 1 h (Thumanu *et al*., 2017).

3.7.2 Analysis by SR-FTIR microspectroscopy

The samples were analyzed by SR- FTIR microspectroscopy at BL4. 1 IR spectroscopy and imaging at Synchrotron Light Research Institute (Public Organization). The samples were measured with the range of 4000-800 cm^{-1}, aperture size 10x10 μm, and 64 scans. The spectral data in epidermis tissues were collected and separated by using the OPUS 7.2 program (Bruker Optics Ltd, Ettlingen, Germany), and the Cytospec program (version 1.3.4) (Cytospec Inc., USA). The principal component analysis (PCA) was analyzed by using Unscrambler 9.7 (CAMO, Norway). The integral area of the biochemical functions group was detected by OPUS 7.2 program (Bruker, Germany) (Lasch *et al*., 2003; Thumanu *et al*., 2017).

3.8 Data analysis

The results of the experiments were analyzed by using Duncan's Multiple Range Test (DMRT) and/or factorial 2 factor in SPSS program version 16.0.

CHAPTER IV

RESULTS

4.1 Preparation of BBC formulations

The three BBC formulations including BBC1, BBC2, and BBC3 have been prepared from three supplement mixtures at 800, 1000, 1200, and 2000 µg/ ml encapsulated with the coating materials of 2% sodium alginate (w/v) and *Bacillus* sp. strain CaSUT008 cultured. All BBC formulations have a form of dry and round shape clear granules, which were similar. The size of the BBC formulations was consistent at approximately 2.2 mm (**Figure 4.1**).

Figure 4.1 The morphology of BBC formulations encapsulated with 2% sodium alginate (SA) and Bacillus sp. strain CaSUT008 cultured on the solution of A) BBC1 800 µg/ml B) BBC1 1,000 µg/ml C) BBC1 1,200 µg/ml D) BBC1 2,000 µg/ml E) BBC2 800 µg/ml F) BBC2 1,000 µg/ml G) BBC2 1,200 µg/ml H) BBC2 2,000 µg/ml I) BBC3 800 µg/ml J) BBC3 1,000 µg/ml K) BBC3 1,200 µg/ml and L) BBC3 2,000 µg/ml.

4.2 Antifungal effect of encapsulated BBC against *FOL* under *in-vitro* conditions

The 12 BBC treatments were used to assess the antifungal efficacy against *FOL* mycelia growth. The BBC1 (800 µg/ml) was shown the highest effective for inhibiting *FOL* mycelial growth by 45.14%. In contrast, the BBC2 (2000 µg/ml) showed the lowest efficacy with 32.37%. The other treatments showed similar antifungal efficacy with percent inhibition of radial growth 36.97-41.74% compared with negative control (**Table 4.1; Figure 4.2**).

Table 4.1 The inhibition percentage of mycelia growth of *FOL* by BBC treatments at 7 days after incubation.

Treatments[1/]	Mycelia radial [2/] (cm)	Percent inhibition of radial growth[2/] (%)
BBC1 800 µg/ml	2.14±0.10c	45.14a
BBC1 1000 µg/ml	2.43±0.14bc	37.82ab
BBC1 1200 µg/ml	2.37±0.13bc	39.35ab
BBC1 2000 µg/ml	2.32±0.17bc	40.72ab
BBC2 800 µg/ml	2.31±0.12bc	40.89ab
BBC2 1000 µg/ml	2.43±0.20bc	37.73ab
BBC2 1200 µg/ml	2.36±0.22bc	39.52ab
BBC2 2000 µg/ml	2.65±0.13b	32.37b
BBC3 800 µg/ml	2.44±0.10bc	37.73ab
BBC3 1000 µg/ml	2.28±0.24bc	41.74ab
BBC3 1200 µg/ml	2.47±0.16bc	36.97ab
BBC3 2000 µg/ml	2.31±0.13bc	40.89ab
Bacillus strain CaSUT008	2.43±0.14bc	37.90ab
Terraclor Super-X®	2.34±0.03bc	40.29ab
Distilled water	3.91±0.09a	0.00c
F-test	**	**
CV (%)	7.54	12.72

[1/] Tomatoes plants were challenged with *FOL* for 7 days.

[2/] Means in the column followed by the same letter are not significantly different according to the DUNCAN test (α = 0.05)

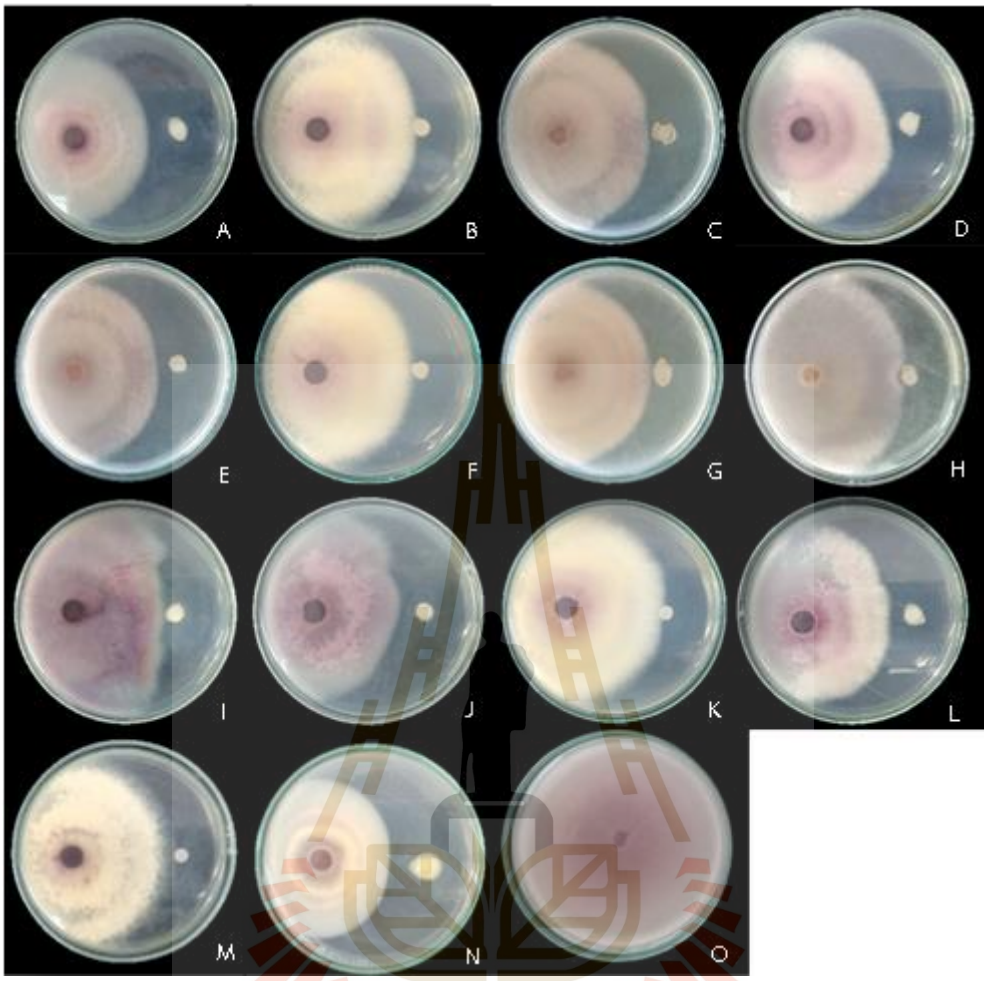


Figure 4.2 The antifungal efficacy of BBC treatment for inhibiting *FOL* under *in-vitro* conditions. A) BBC1 800 µg/ml B) BBC1 1,000 µg/ml C) BBC1 1,200 µg/ml D) BBC1 2,000 µg/ml E) BBC2 800 µg/ml F) BBC2 1,000 µg/ml G) BBC2 1,200 µg/ml H) BBC2 2,000 µg/ml I) BBC3 800 µg/ml J) BBC3 1,000 µg/ml K) BBC3 1,200 µg/ml L) BBC3 2,000 µg/ml M) *Bacillus* strain CaSUT008 N) Terraclor Super-X® and O) Control.

4.3 ESEM observation

ESEM observation revealed that BBC1 has a stronger antagonistic effect on the morphology of the *FOL* structures when compared with terraclor super-x® (positive control) and untreated control. The pathogen hyphae were wrinkled, twisted, and shrunken as a result of antifungal chemicals by direct contact (**Figure 4.3 B**). Bacterial cells had adhered to hyphae. In the untreated control, pathogen hyphae appeared complete with a smooth surface, occasionally displaying a contour of the septum, but no bacterial cells were visible (**Figure 4.3 A**).

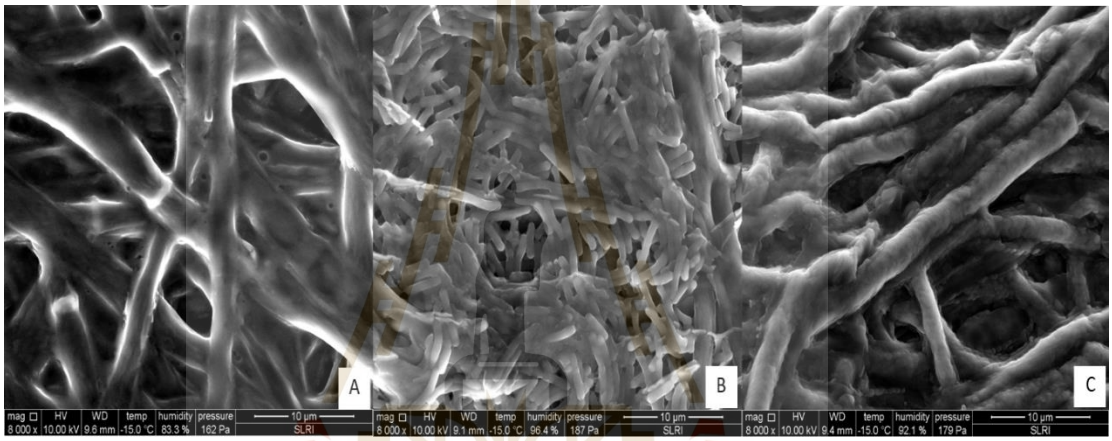


Figure 4.3 Environmental scanning electron micrographs of *FOL* with no bacterial treatment (A), treated with BBC1 800 µg/ml (B), and treated with chemical fungicide (terraclor super-x®) (C).

4.4 Survival of *Bacillus* sp. on encapsulated BBC

The survivability of *Bacillus* sp. strain CaSUT008 on BBC1 formulation was examined every month until 6 months after storage. The *Bacillus* sp. strain CaSUT008 was shown stable in the encapsulate formulation with 10^8 CFU/g (Log_{10} of 8.51) at one month after storage. At 2 and 3 months, the survival *Bacillus* sp. strain CaSUT008 was approximately 10^7 CFU/g (Log_{10} of 7.22), and 10^6 CFU/g (Log_{10} of 6.20), respectively. There was a decline that remained steady, and it tended to diminish at 4, 5, and 6 months after storage with the survival bacteria was approximately 10^5, 10^5, and 10^4 CFU/g (Log_{10} of 5.70-4.71), respectively (**Figure 4.4**). This suggests that the encapsulated BBC1 can be kept at room temperature (26-30 °C) in an opaque bottle for 3 months,

containing a sufficient amount of *Bacillus* sp. active to control Fusarium wilt disease in tomatoes.

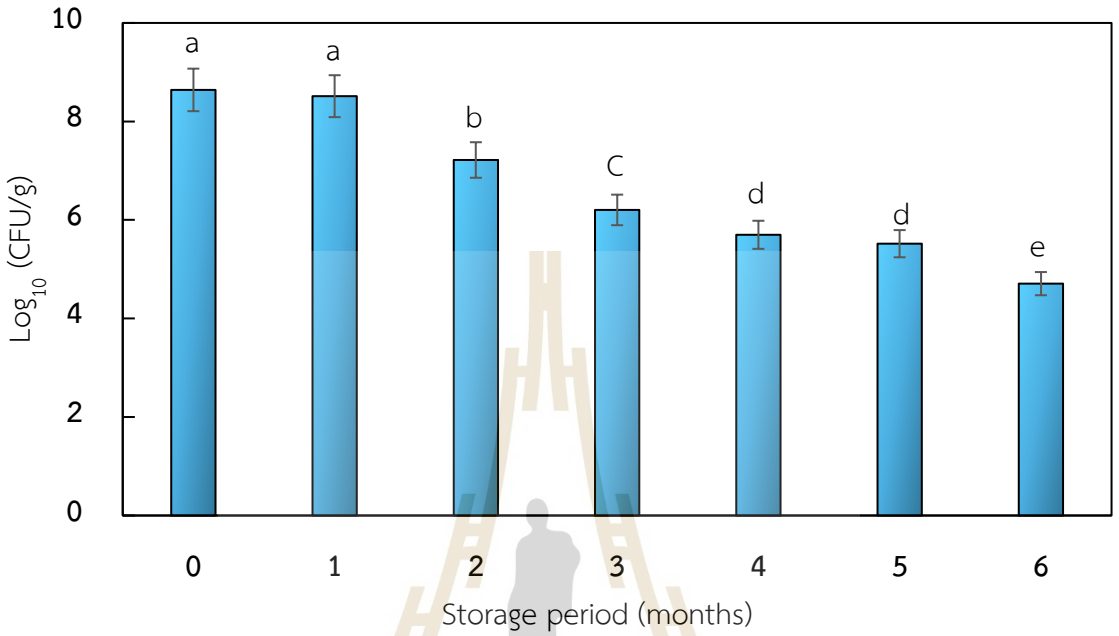


Figure 4.4 Microbial survival rates of *Bacillus* sp. strain CaSUT008 in the BBC1 encapsulate.

4.5 Effect of encapsulated BBC1 to control Fusarium wilt disease and plant growth promotion under greenhouse conditions

The tomato plants were pre-treated with encapsulated BBC1 by soil drenching and foliar spray until 49 days after transplanted. Then, the tomato plants were inoculated with *FOL* for 7 days after the last treatment time. The combination of soil drenching and foliar spraying BBC1 800 µg/ml could reduce Fusarium wilt disease by 78.95% at 7 days after inoculation similar to fungicide treatment (terraclor super-x®) at 73.69%. The efficacy of the combination of both methods BBC1 was significantly higher than only soil drenching or foliar spraying (**Table 4.3**).

The combination of both methods was evaluated for tomato growth promotion including plant height, fresh weight, and yield. The plant height of the BBC1 treatment was significant increased by 38.8% compared with the control treatment. Meanwhile,

B. subtillis commercial treatment was nonsignificant compared to control treatment. The fruit weight and yield of the BBC1 treatment significantly increased by 34.5 and 75.0% compared with the control treatment. The yield of BBC1 treatment was 6,707 Kg per rai, which is also higher than positive control as COS® at 4,885 Kg per rai and *B. subtillis* commercial at 4,320 Kg per rai (**Table 4.4**). The results showed that the BBC1 encapsulate was able to promote tomato growth and yield under greenhouse conditions.

Table 4.2 Effect of application and elicitor on the reduction of Fusarium wilt disease under greenhouse conditions.

Treatment[1/]	Disease reduction (%)
Application (A)	
Soil drenching	43.29
Foliar spray	43.16
Soil drenching + Foliar spray	49.48
F-test	ns
Elicitor (B)	
BBC1	49.12a[2/]
COS®	50.87a
B. subtillis commercial	57.89a
Terraclor super-x®	64.91a
Water	0.00b
F-test	**
A*B	**
CV (%)	35.92

[1/] Tomato plants were challenged with *FOL* at 56 days after transferring.

[2/] Means in the column followed by the same letter are not significantly different according to the DUNCAN test (α = 0.05).

Table 4.3 Efficacy of encapsulated BBC1 on the reduction of Fusarium wilt disease under greenhouse conditions.

Treatments[1/]	Disease reduction (%)[2/]
Soil drenching BBC1 800 µg/ml	26.32c
Soil drenching COS®	58.55abc
Soil drenching *B. subtillis* commercial	63.16abc
Soil drenching terraclor super-x® (positive control)	68.42abc
Soil drenching water (negative control)	0.00d
Foliar spray BBC1 800 µg/ml	42.11bc
Foliar spray COS®	63.16abc
Foliar spray *B. subtillis* commercial	57.90abc
Foliar spray terraclor super-x® (positive control)	52.63bc
Foliar spray water (negative control)	0.00d
Soil drenching + Foliar spray BBC1 800 µg/ml	78.95a
Soil drenching + Foliar spray COS®	26.32cc
Soil drenching + Foliar spray *B. subtillis* commercial	68.42abc
Soil drenching + Foliar spray terraclor super-x® (positive control)	73.69a
Soil drenching + Foliar spray water (negative control)	0.00d
F-test	**
CV (%)	32.95

[1/] Tomato plants were challenged with *FOL* at 56 days after transferring.

[2/] Means in the column followed by the same letter are not significantly different according to the DUNCAN test (α = 0.05).

Table 4.4 Efficacy of encapsulated BBC1 on tomato growth under greenhouse conditions.

Treatments[1/]	Plant growth parameters[2/]		
	Plant height (cm)	Fresh weight (g/fruit)	Yield (kg/rai)
Soil drenching + Foliar spray BBC1 800 µg/ml	96.6a	29.76a	6,707a
Soil drenching + Foliar spray COS®	85.8ab	23.64ab	4,885bc
Soil drenching + Foliar spray *B. subtillis* commercial	77.2b	23.07ab	4,320c
Soil drenching + Foliar spray terraclor super-x® (positive control)	85.2ab	24.61ab	6,320ab
Soil drenching + Foliar spray water (negative control)	69.6b	22.13b	3,833c
F-test	*	*	*
CV (%)	14.84	15.03	17.50

[1/] Tomato plants were challenged with *FOL* at 56 days after transferring.

[2/] Means in the column followed by the same letter are not significantly different according to the DUNCAN test (α = 0.05)

4.6 Effect of encapsulated BBC1 on tomato defense mechanisms against Fusarium wilt disease

4.6.1 Salicylic acid content

The combination of both methods was evaluated for enhancing the defense mechanism against tomato Fusarium wilt disease. The salicylic acid content was investigated at 0, 24, and 48 HAI *FOL*. At 0 HAI, the result showed that terraclor super-x® treatment was the highest SA content at 8.93 µg g^{-1} fresh weight, following by chitosan commercial COS® and *B. subtillis* commercial at 8.85 and 8.81 µg g^{-1} fresh weight, respectively. The control treatment was the lowest significant SA content at 8.56 µg g^{-1} fresh weight. At 24 HAI, the terraclor super-x®, encapsulated BBC1, *B. subtillis* commercial, and chitosan commercial COS® increased SA content with 9.38, 9.23, 9.15, and 9.09 µg g^{-1} fresh weight, respectively higher than the control treatment at 8.17 µg g^{-1} fresh weight. The SA content of treatments decreased at 48 HAI while control treatment was increased. However, this SA content was also significantly higher than

the control treatment (**Table 4.5**). That means the tomato plants pretreated with BBC1 encapsulated and *B. subtillis* commercial could response to SA faster and stronger than the negative control treatment.

Table 4.5 Effect of encapsulated BBC1 on SA content of tomato leaves.

Treatment	Salicylic acid[1/] µg g^{-1} fresh weight		
	0 HAI[1/]	24 HAI	48 HAI
Soil drenching + Foliar spray BBC1 800 µg/ml	8.38±0.01b[2/]	9.23±0.01ab	9.09±0.00a
Soil drenching + Foliar spray COS®	8.85±0.02a	9.09±0.00b	8.43±0.02d
Soil drenching + Foliar spray *B. subtillis* commercial	8.81±0.01a	9.15±0.01b	9.05±0.01a
Soil drenching + Foliar spray terraclor super-x® (positive control)	8.93±0.02a	9.38±0.05a	8.70±0.00b
Soil drenching + Foliar spray water (negative control)	8.56±0.03b	8.17±0.00c	8.59±0.01c
F-test	**	**	**
CV (%)	12.47	9.50	11.63

[1/] HAI: hours after inoculation.

[2/] Each value represents a mean of three replicate plants with two leaves per plant. The mean in the column followed by the same letter is not significantly different according to the DUNCAN test (α= 0.05).

4.6.2 Chitinase activity

The combination of both methods was evaluated for enhanced defense mechanism against tomato Fusarium wilt disease. The Chitinase activity was investigated at 0, 24, and 48 HAI *FOL*. At 0 HAI, chitosan commercial COS® treatment had the highest chitinase activity at 0.98 µmol GlcNAc formed min^{-1} mg^{-1} protein, following by *B. subtillis* commercial at 0.95 µmol GlcNAc formed min^{-1} mg^{-1} protein. The BBC1 treatment was the lowest significant chitinase activity at 0.91 µmol GlcNAc formed min^{-1} mg^{-1} protein. The terraclor super-x® and water control treatments were similar, which was significantly higher than the BBC1 treatment. The BBC1 treatment strong increased chitinase activity at 24 HAI with 1.00 µmol GlcNAc formed min^{-1} mg^{-1} protein, which was significantly higher than the rest of treatments. At 48 HAI, all of

treatments were similar chitinase activity but significantly higher than control treatment (**Table 4.6**).

Table 4.6 Effect of encapsulated BBC1 on chitinase activity of tomato leaves.

Treatments	Chitinase activity (μmol GlcNAc formed min^{-1} mg^{-1} protein)		
	0 HAI[1/]	24 HAI	48 HAI
Soil drenching + Foliar spray BBC1 800 μg/ml	0.91d[2/]	1.00a	0.98a
Soil drenching + Foliar spray COS®	0.98a	0.98b	0.98a
Soil drenching + Foliar spray *B.subtillis* commercial	0.96b	0.96c	0.99a
Soil drenching + Foliar spray terraclor super-x® (positive control)	0.95c	0.98b	0.98a
Soil drenching + Foliar spray water (negative control)	0.94c	0.95c	0.96b
F-test	**	**	**
CV (%)	3.02	1.91	2.97

[1/] HAI: hours after inoculated;

[2/] Each value represents a mean of three replicate plants with two leaves per plant. Mean in the column followed by the same letter are not significantly different according to the DUNCAN test (α= 0.05)

4.6.3 *β*-1,3-glucanase activity

The combination of both methods was evaluated for enhancing defense mechanism against tomato Fusarium wilt disease. The *β*-1,3-glucanase activity was investigated at 0, 24, and 48 HAI *FOL*. At 0 HAI, the results showed that terraclor super-x®, chitosan commercial COS®, and control treatment was highest *β*-1,3-glucanase activity at 27.64, 27.15, and 27.10 μmol glucose released min^{-1} mg^{-1} protein, following by BBC1 treatment at 24.63 μmol glucose released min^{-1} mg^{-1} protein, respectively. The *B. subtillis* commercial treatment was the lowest significant *β*-1,3-glucanase activity at 23.17 μmol glucose released min^{-1} mg^{-1} protein. At 24 HAI, the BBC1 treatment increased *β*-1,3-glucanase activity to 28.42 μmol glucose released min^{-1} mg^{-1} protein, which was significantly lower than terraclor super-x® at 30.34 μmol glucose released min^{-1} mg^{-1}protein which higher than *B. subtillis* commercial, chitosan commercial COS®, and a control treatment with 24.63, 27.07, and 27.53 μmol glucose

released min^{-1} mg^{-1} protein, respectively. The *β*- 1,3- glucanase activity of BBC1 treatment was similar with chitosan commercial COS®, and control, which was significantly lower than terraclor super-x® at 31.18 µmol glucose released min^{-1} mg^{-1} protein which higher than *B. subtillis* commercial at 25.64 µmol glucose released min^{-1} mg^{-1} protein (**Table 4.7**).

Table 4.7 Effect of encapsulated BBC1 on *β*-1,3-glucanase activity of tomato leaves.

Treatments	*β*-1,3-glucanase activity (µmol glucose released min^{-1} mg^{-1} protein)		
	0 HAI[1/]	24 HAI	48 HAI
Soil drenching + Foliar spray BBC1 800 µg/ml	24.63b[2/]	28.42b	28.31b
Soil drenching + Foliar spray COS®	27.15a	27.07c	27.61b
Soil drenching + Foliar spray *B. subtillis* commercial	23.17c	24.63d	25.64c
Soil drenching + Foliar spray terraclor super-x® (positive control)	27.64a	30.34a	31.18a
Soil drenching + Foliar spray water (negative control)	27.10a	27.53c	27.83b
F-test	**	**	**
CV (%)	2.95	3.44	1.51

[1/] HAI: hours after inoculated.

[2/] Each value represents a mean of three replicate plants with two leaves per plant. Means in the column followed by the same letter are not significantly different according to the DUNCAN test (α= 0.05)

4.6.4 Phenylalanine ammonia-lyase (PAL) activity

The combination of both methods was evaluated for enhanced defense mechanism against tomato Fusarium wilt disease. The PAL activity was investigated at 0, 24, and 48 HAI *FOL*. The result showed that chitosan commercial COS® treatment had the highest PAL activity at 88.82 µmol trans-cinnamic acid min^{-1} mg^{-1} protein at 0 HAI, following by terraclor super-x® treatment at 80.88 µmol trans-cinnamic acid min^{-1} mg^{-1}protein. The PAL activity of BBC1 treatment was 74.41 µmol trans-cinnamic acid min^{-1} mg^{-1} protein but significantly higher than *B. subtillis* commercial at 71.07 µmol trans-cinnamic acid min^{-1} mg^{-1} protein and control treatment at 70.07 µmol

trans-cinnamic acid min^{-1} mg^{-1} protein. At 24 HAI, the BBC1 treatment increased PAL activity to 84.83 µmol trans-cinnamic acid min^{-1} mg^{-1}protein, which was significantly lower than terraclor super-x® at 92.65 µmol trans-cinnamic acid min^{-1} mg^{-1}protein which higher than *B. subtillis* commercial, and control treatment with 79.97 and 72.59 µmol trans-cinnamic acid min^{-1} mg^{-1} protein, respectively. The PAL activity of BBC1 and terraclor super-x® treatment decreased at 48 HAI with 80.07 and 77.66 µmol trans-cinnamic acid min^{-1} mg^{-1} protein, respectively, which was significantly lower than that of chitosan commercial COS® at 100.4 µmol trans-cinnamic acid min^{-1} mg^{-1}protein and *B. subtillis* commercial at 87.18 µmol trans-cinnamic acid min^{-1} mg^{-1}protein which higher than control at 72.71 µmol trans-cinnamic acid min^{-1} mg^{-1}protein (**Table 4.8**).

Table 4.8 Effect of encapsulated BBC1 on PAL activity of tomato leaves.

Treatments	Phenylalanine ammonia-lyase (PAL) (µmol trans-cinnamic acid min^{-1} mg^{-1} protein)		
	0 HAI[1/]	24 HAI	48 HAI
Soil drenching + Foliar spray BBC1 800 µg/ml	74.41c[2/]	84.83b	80.07c
Soil drenching + Foliar spray COS®	88.82a	87.18b	100.4a
Soil drenching + Foliar spray *B.subtillis* commercial	71.07d	79.97c	87.18b
Soil drenching + Foliar spray terraclor super-x® (positive control)	80.88b	92.65a	77.66c
Soil drenching + Foliar spray water (negative control)	70.07d	72.59d	72.71d
F-test	**	**	**
CV (%)	1.49	1.60	1.75

[1/] HAI: hours after inoculation.

[2/] Each value represents a mean of three replicate plants with two leaves per plant. The mean in the column followed by the same letter is not significantly different according to the DUNCAN test (α= 0.05).

4.7 Effect of the encapsulated BBC1 on biochemical change of tomato against Fusarium wilt disease

The combination of soil drenching and foliar spraying of BBC1 and control treatment was compared to detect biochemical change at 24 HAI by using SR-FTIR.

The aperture size 10x10 μm of cryosection tomato leaf samples were measured with a range 4000-800 cm-1, and 64 scans. The spectra of epidermis tissues were collected and selected by hierarchical clustering analysis (**Figure 4. 5**). These spectra were performed by PCA. The results found that the PC1 axis was able to separate spectral groups in the epidermis tissues of tomato leaf between the BBC1 (horizontal axis, negative) and control (horizontal axis, positive) with different of 82% (**Figures 4.6**). The 2nd derivative analysis showed the different at peaks 1736 cm^{-1} (lipid), 1653 and 1542 cm^{-1} (protein Amide I, II), 1451 and 1375 cm^{-1} (CH bending), 1244 cm^{-1} (hemicelluloses), 1143 and 1099 cm^{-1} (polysaccharides) between BBC1 and control treatments (**Figure 4. 7**). These spectra were calculated for the integral areas of biochemical function group. The results showed that the integral areas of the C=O ester from lipids, protein (Amide I, II), and C-H bending of BBC1 treatment were significantly higher than control treatment by 1. 6- 27. 0 folds. While the integral areas of hemicelluloses and polysaccharides were no significance between two treatments (**Table 4.9, Figure 4.8**).

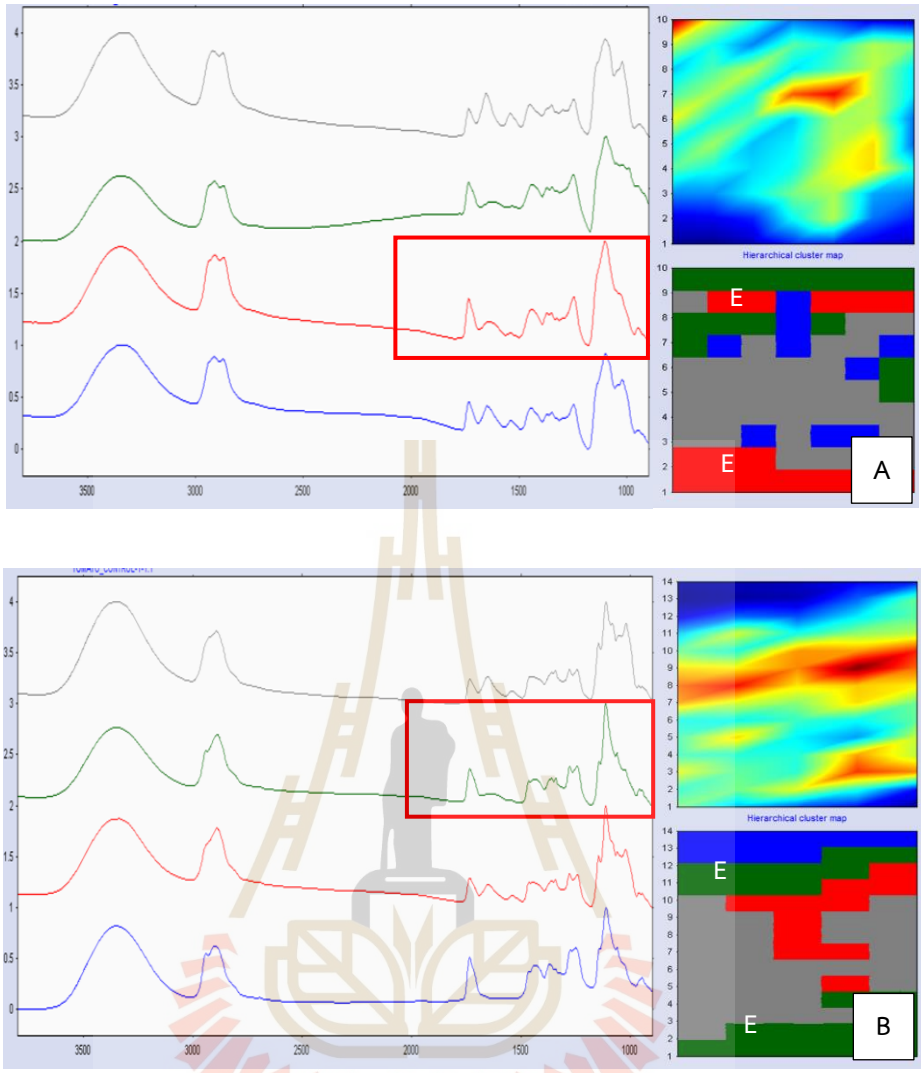


Figure 4.5 The hierarchical clustering analysis of tomato leaves. A) BBC1 treatment, and B) Control. Note: E: epidermis tissues.

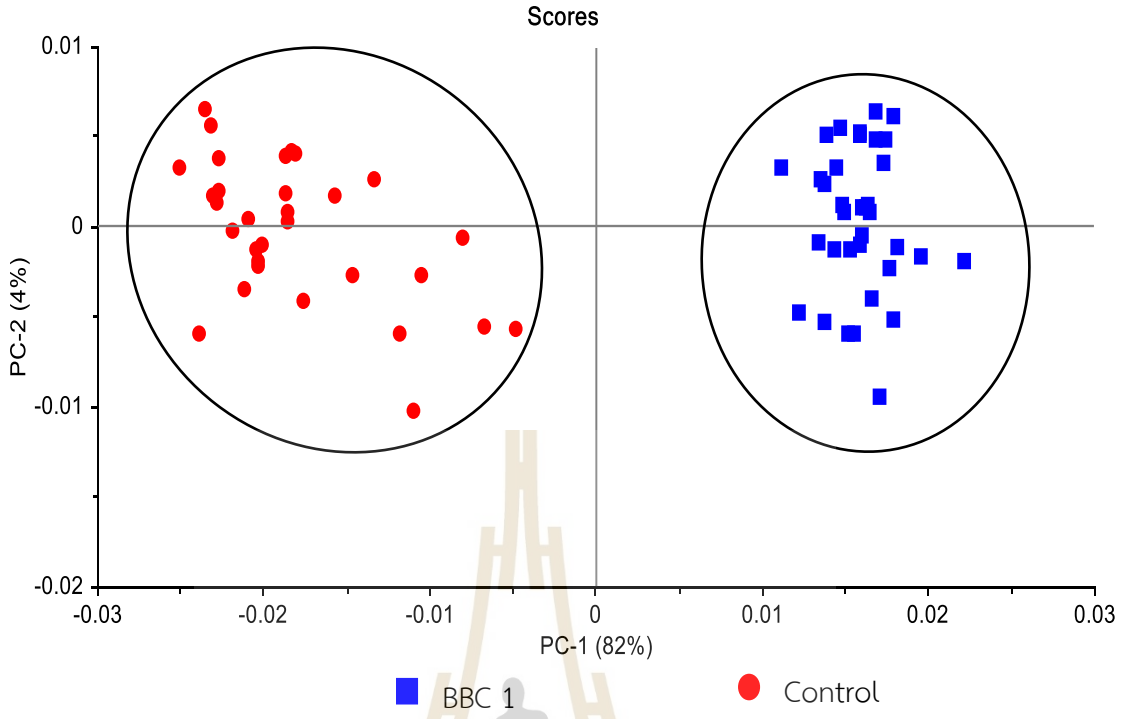


Figure 4.6 The principle component analysis (PCA) of epidermis tissues of BBC1 and control treatment.

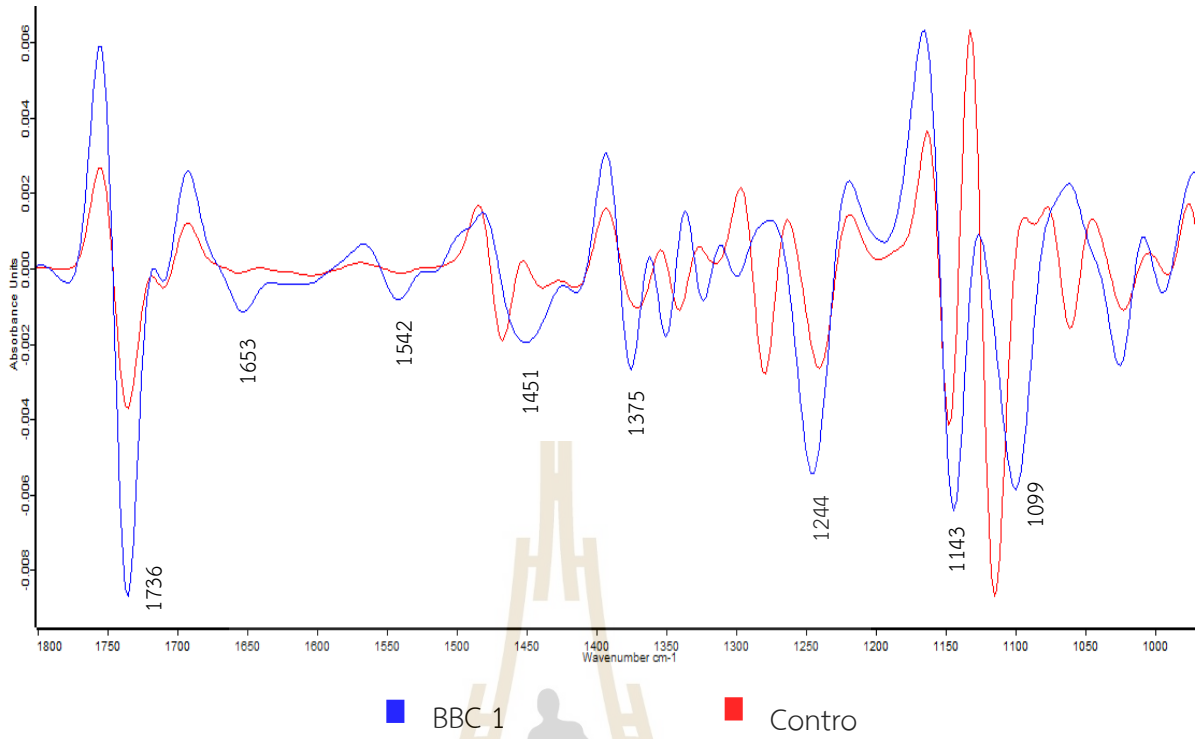


Figure 4.7 The 2nd derivative analysis of epidermis tissues of BBC1 and control treatment. The lipid group C=O ester (~1740-1700 cm^{-1}); the protein group Amide I (~1700- 1600 cm^{-1}), and Amide II (~1600- 1500 cm^{-1}); the carbohydrate group CH bending (~1470-1350 cm^{-1}), hemicellulose (~1300-1200 cm^{-1}), and polysaccharide, (~1200- 1000 cm^{-1}) of BBC1 (blue) and control treatment (red).

Table 4.9 The integral areas of biochemical function groups in epidermis tissues of tomato leaves.

Treatments	Functional groups[1/]					
	1	2	3	4	5	6
	C=O ester	Amide I	Amide II	CH bending	Hemicelluloses	Polysaccharides
	1740-1700	1700-1600	1600-1500	1470-1350	1300-1200	1200-1000
BBC1	0.101±0.003a	0.063±0.004a	0.027±0.003a	0.061±0.006a	0.073±0.003	0.178±0.01
Control	0.065±0.004b	0.003±0.003b	0.001±0.002b	0.020±0.009b	0.067±0.01	0.168±0.02
F-test	*	**	*	**	ns	ns
%CV	0.00	3.25	5.48	6.21	4.00	0.00

[1/] Means in the column followed by the same letter are not significantly different according to the DUNCAN test (α = 0.05).

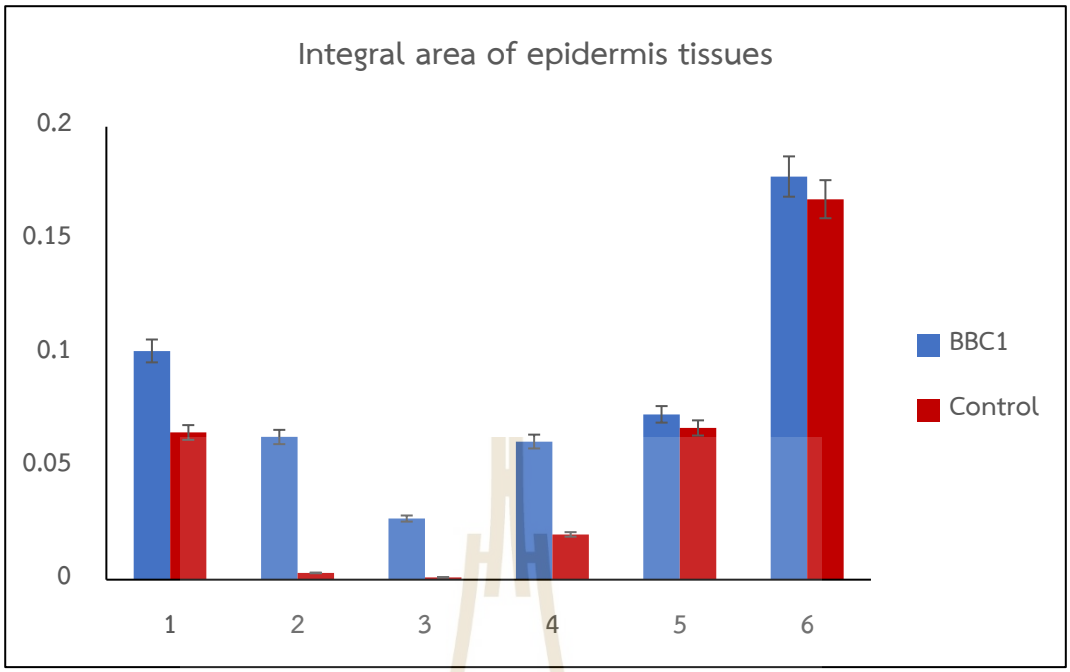


Figure 4.8 The integral areas of biochemical function groups in epidermis tissues of tomato leaves. 1.lipid group C=O ester (~1740-1700 cm^{-1}); 2. protein group Amide I (~1700-1600 cm^{-1}); 3. protein group Amide II (~1600-1500 cm^{-1}); 4. CH bending (~1470-1350 cm^{-1}); 5. hemicellulose (~1300-1200 cm^{-1}); 6. polysaccharide (~1200-1000 cm^{-1}) of BBC1 (blue) and control treatment (red).

CHAPTER V
DISCUSSION AND CONCLUSIONS

The efficacy and their mechanisms of BBC formulations on controlling tomato Fusarium wilt disease were evaluated in this research. The step of preparing BBC formulations, antifungal test, induced resistance test, and mechanism characterization were summarized as follows:

5.1 The encapsulate production of BBC formulations

The encapsulated BBC formulations with 2% sodium alginate showed a consistent grain size. Due to using high concentrations of sodium alginate, the rigid gel-bead structure or the tight connection of the polymer chains was obtained. When the mixture was dropped through the syringe into the calcium chloride solution leading to form the clear pellet with homogenous grain size. Similarly, Wiwattanapatapee (2011) reported that the optimum formula and conditions for preparing alginate gel beads were 1-3 % (w/v) alginate, 0.03 M calcium chloride, and syringe tip size 2 mm. This gel beads form has good physical characteristics and could release *B. megaterium*. However, during encapsulate biologics, there are several limitations including the large diameter of the gel beads and low productivity, making it difficult to scale up the production of microorganisms to the industrial level (Krasaekoopt *et al.*, 2003). The bacteria are slow-released from the gel bead, necessitating the use of a solvent or a substance capable of dissolving sodium alginate. Then, the releasing bacteria could reproduce to increase their density in the environment (Wongchalee, 2015). Pau *et al*. (1988) reported that the production of dry formulations was often used by microorganisms capable of producing spores and would require the addition of a cell protection agent to protect cells from heat during the drying process such as gelatin, etc. Some compounds improve the ability of cells and aid in the stability of the product during storage in opaque bottle. It's also crucial to use the correct packaging. The packaging serves to control airflow and humidity, which can cause mold contamination and bacteria non-stability (Wongchalee, 2015). According to Mung *et al*.

(2008), the probiotic bacterial pellets *L. plantarum* strain LP64 coated with calcium alginate were dried in a fluidized bed dryer for 15-45 minutes at 30 °C with an air velocity of 1 m/min. After 60 days of storage, it was found that the dry bacteria stored at 4 °C showed a slight decrease in the number of viable cells. The samples were kept at room temperature lead to decrease in the cell viability by 2-3 log cycles. Likewise, Thammasat *et al*. (2012) discovered that *B. amyloliquefaciens* isolate DGg13 had bacterial viability in two powdered formulations including soil powder and talcum powder. The number of bacteria was 4×10^{11}-6.3×10^{12} cfu.g^{-1} in the first month after storage. The quantity of *B. amyloliquefaciens* isolate DGg13 was decreased to 10^{8} - 10^{11} cfu.g^{-1} at 6 months after storage at 4-10 °C. The antagonistic bacteria in the soil powder formulation had the highest concentration of 2.5×10^{11} cfu.g^{-1}. In 2007, Pengnoo *et al*. developed granules and effervescent tablet forms of antagonistic bacteria *B. megaterium* to use for sowing or spraying. Bacteria could persist at least a year in each type of biological product. The product's packaging makes it easy to transport. It's possible to keep at room temperature. Farmers could easily and effectively control rice sheath diseases of rice.

5.2 Antifungal effect of encapsulated BBC against *FOL* under *in-vitro* conditions

The efficacy of BBC formulations in fungal growth inhibition was different depend on mixture composition. The BBC1 a combination of *Bacillus* sp. strain CaSUT008 and CHIZA4 at 800 ppm showed the most effective in inhibiting *FOL* under *in-vitro* conditions. Similarly, Luo *et al*. (2019) reported that the microcapsules of *Bacillus* sp., alginate, and chitosan reduced Fusarium growth by 100% compared with the control. The *Bacillus* sp. could act as inhibition agents to inhibit phytopathogen activity. Each antibiotic is unique, based on the bacteria and media that produce chemical properties (Berg and Ballin, 1994). *Bacillus* sp. has been reported their aability to generate antifungal metabolites including lipopeptides (bacillomycin, fengycin, surfactin), volatile chemicals, hydrolytic enzymes, and siderophores (Yuan *et al.*, 2012; Li *et al.*, 2016; Lee *et al.*, 2017; Hanif *et al.*, 2019). Ongena *et al*. (2005) reported that fengycin produced from *B. subtilis* strains S499 and M4 inhibited *F. oxysporum*, *R. solani*, and *B. cinerea*. Similarly, Chung *et al*. (2008) reported that the

B. subtilis strain ME488 efficiently inhibited Fusarium wilt disease of cucumbers and Phytophthora blight of peppers by producing bacilysin, iturin, and mersacidin. According to Younes *et al*. (2020), *B. amyloliquefaciens* fungitoxic activity results in wrinkled with depressions in their surfaces, ruptured rinds, deformed, shrunken, twisted, coiled, and collapsed *S. cepivorum* mycelium. In addition, Manjula *et al*. (2004) reported that fresh cells, cell-free culture filtrate of *B. subtilis* strain AF1 and their chitinase was able to inhibit uredospore germination of *Puccinia arachidis* caused peanut rust by 90.2, 86.4%, and 96.0%, respectively. The length of the germ tube was reduced by causing granular cytoplasm, degrading germ tube, and breaking down chitin in the cell walls. Moreover, *B. subtilis* strain AF1 cells from log phase, alginate formulation, cell-free culture filtrate, and their chitinase has able to control peanut rust disease. The using fresh cells of *B. subtilis* strain AF1 showed the most effective control, followed by alginate formulation, NAGase, and CCF, respectively. Moreover, Ciampi *et al*. (2007) reported that the *B. subtilis* bio encapsulated alginate beads and their free liquid cell suspension could reduce potato black scurf caused by *Rhizoctonia solani* under field conditions by 58% and 12%, respectively.

5.3 Survival of *Bacillus* sp. on encapsulated BBC

After one month of storage, the *Bacillus* sp. strain CaSUT008 was shown stable in the encapsulate formulation. At 2 and 3 months, there was a decline that remained steady, and at 4, 5, and 6 months, it tended to diminish. Consistent with Wongchalee (2015), the survival of *Bacillus* sp. strain D604 was stable in the first period after 2 months of storage. Then, the number of bacteria decreased in the 3 and 4 months. In addition, Musiksang (2008) studied the number of microencapsulating starter cells in bacterial gel tablets incubated for 3 hours at 37 °C under acidic conditions. The number of cells reduced to 10^6 cfu.ml^{-1} from the initial bacterial cell count of 10^9 cfu.ml^{-1} was an acceptable quantity of cells, while the final cell count of 10^5 cfu.ml^{-1} was too low. The number of bacterial start cells is another important factor in their implementation of microencapsulation or the production of various forms of bacterial formulations. Moreover, Bacillus cells were used instead of endospores in this research. As a result, the preserved bacteria have a low survival rate. In the future, bacterial endospores should be used instead of vegetative cells (Suarez *et al*., 2011; Chen *et*

al., 2012). *Bacillus* was capable to produce one endospore per cell, which was antagonistic microorganism, more resistant to chemicals, radiation, and heat than normal cells. That lead to enhance the survival rate in formulations (Kloepper *et al.*, 2004).

5.4 Effect of encapsulated BBC1 on controlling Fusarium wilt disease and plant growth-promoting under greenhouse conditions

Three methods including soil drenching, foliar spraying, and their combination were evaluated for reducing tomato Fusarium wilt disease under greenhouse conditions. The soil-drenching combined with foliar spraying BBC1 800 µg/ml showed the highest effective reducing disease by 78.95%, which was similar to fungicide (terraclor super-x®) treatment (73.69%). Similarly, Luo *et al.* (2019) reported that the *Bacillus* sp. alginate-chitosan microcapsules were applied as soil drenching combined with soil microcapsules embedding has able to reduce Fusarium wilt disease by 14.04%. According to Vinodkumar *et al.* (2017), the carnation was treated by root dipping combined with soil drenching *B. amyloliquefaciens* could reduce stem rot by 87.9% compared with the control. Carnation cuttings treated with *B. amyloliquefaciens* also increased the number of shoots, length of flower stalks, and flower yield compared to the untreated control. The beneficial antagonist bacterium *Bacillus* spp. is effective with a multifaceted mode of action including competition, colonization of the rhizosphere, and secretion of volatile, non- volatile antifungal agents or antimicrobial peptides (**Figure 5.1**). In addition, *Bacillus* could transfer phosphorus to soluble form, more usable by plants. This bacteria also enhanced nitrogen uptake, produced phytohormones (auxin, cytokinin, gibberellin), siderophores to promote plant growth (Trivedi *et al.*, 2007; Teaumroong *et al.*, 2012). In present research, the plant height, fruit weight, and yield of this treatment were also increased by 38.8, 34.5, and 75.0%, respectively. In previous research, Domenech *et al.* (2006) also reported that *B. subtilis* and *B. licheniformis* could enhance the plant growth of peppers and tomatoes. In addition, the combination of soaking and soil drenching *B. subtilis* E7-17 with sugarcane stalks before transplanting could increase the average weight up to 3.12 kg/plant, which was significantly higher than the control (1.75 kg/plant). The *B. amyloliquefaciens* strain Ba13 could enhance tomato fresh weight on days 14, 21, and

28 by 19.52%, 33.30%, and 20.15%, respectively (Chenglong *et al.*, 2021). Moreover, the shoot and root length of tomatoes treated by *Bacillus* sp. strains B1, B2, and B3 were increased by 57-85 and 68-71.2%, respectively (Mengistie *et al.*, 2022). In a research of Suárez *et al.* (2011), microencapsulates of *B. subtilis* strains B1, J1, and B1J1M2 were able to suppress damage of *R. solani* and *FOL* disease in tomatoes and also enhance tomato yield compared with the control or fungicide treatment.

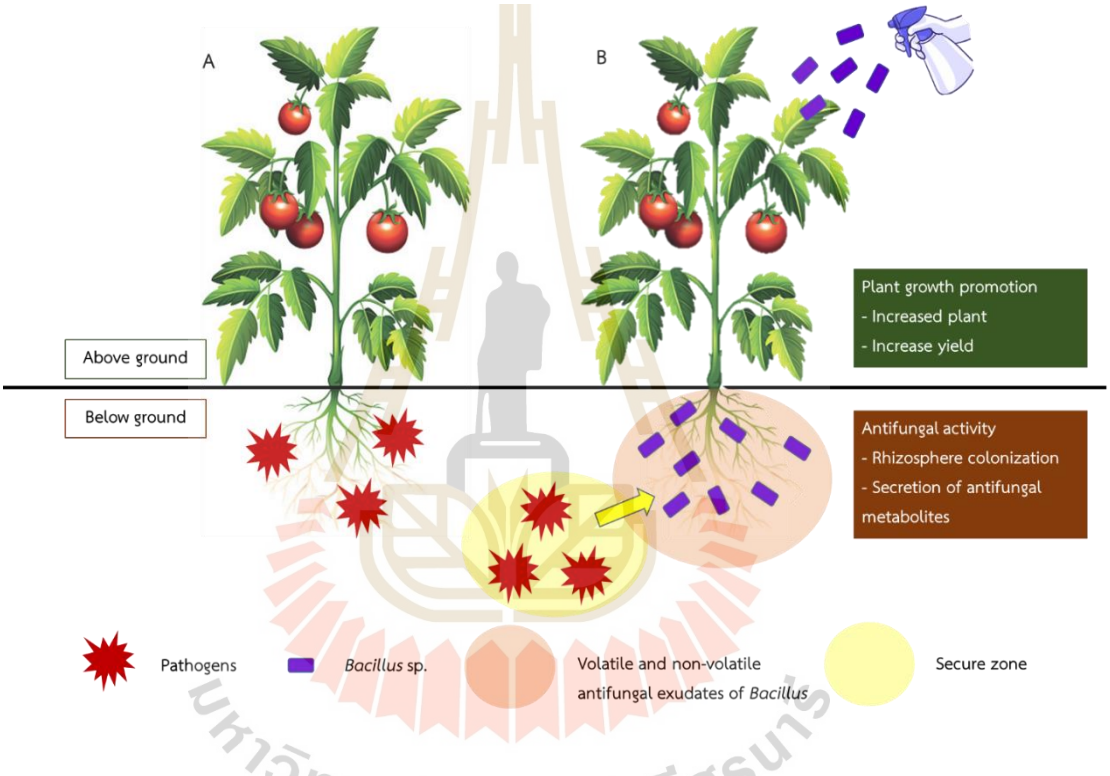


Figure 5.1 Hypothetical model on the mode of action of *Bacillus* sp. in the management of tomato diseases. (A) untreated plant, (B) plant treated with *Bacillus* sp. (Vinodkumar *et al.*, 2017).

5.5 Mechanisms of encapsulated BBC1 induced resistance against tomato Fusarium wilt disease

The tomato plants were treated with BBC1 800 µg/ml by soil-drench combined foliar spraying until 49 days after transplanting. Then, the plants were inoculated at 56 days after transplanting by spraying *FOL* spore suspension. The accumulation of salicylic acid and defense enzyme activity including chitinase, *β* -1,3-glucanase, and PAL were investigated at 0, 24, and 48 HAI. The salicylic acid content increased at 24 HAI and decreased at 48 HAI. Similarly, Khan *et al*. (2020) reported that spraying *B. cereus* on tomato plants could enhance biochemical change at 24 HAI, which is related to inducing resistance signaling. In a research of Huihui *et al*. (2022), tomatoes treated with *B. amyloliquefaciens* strain SN16-1 were enhanced defense system against *R. solani* though SA signaling. Dongdong *et al*. (2015) also reported SA content was increased in Arabidopsis resistance disease by treating *B. cereus* strain AR156. The SA is an important signaling molecule that mediates plant defense responses against many biotrophic/hemibiotrophic pathogens. In the other hand, the defense enzyme PAL was increased at 24 HAI *FOL*. The PAL activity induced by the *CaPAL1* gene in pepper, which impacts to free salicylic producing, a signaling molecule involved in plant defense mechanisms. Meanwhile, the PAL enzyme activity was low when the *CaPAL1* gene was non-expressed, which was related to reduce salicylic acid production. Moreover, the PAL was related to the phenylpropanoid pathway, which lead to induce phenolic compounds and affect plant defense (Kim and Hwang, 2014). The PAL activity was associated with polyphenol oxidase (PPO) activity. The PPO enzyme was defense one, which catalyzes phenol compounds to quinones (Thipyapong *et al*., 1995; Ruiz *et al*., 1999). Similarly with PAL activity, chitinase and *β* -1,3-glucanase were increased at 24 HAI and decreased at 48 HAI. In a research of Poonam *et al*. (2012), these two enzymes were also increased at 24 HAI in susceptible lettuce varieties against *Alternaria brassicicola*. In a previous report of Ward *et al*. (1991), the *β* -1-3 glucanase was expressed higher than in melon resistance Fusarium compared with susceptible variety. The precursors of the enzyme are chitin and *β* -1-

3 glucans, which are the main components of the cell walls of many fungi (Wessels *et al*., 1981). *β* -1-3 glucanase and chitinase release oligosaccharides from the fungal cell walls and act as a signal to induce host defense response (Ham *et al*. 1991).

The tomato defense could be enhanced and rapid responded by pretreating BBC1 at 800 µg/ml. Tomatoes undergo the process of recognizing microbial-associated molecular patterns (MAMP). The chitin as a component of the fungal cell walls could be detected by extracellular receptor-like kinases (RLKs) on the plasma membrane. Then, the signal was transmitted through the MAP kinase cascades, and transcription takes place rapidly through WRKY transcription factors to synthesize PR-NP24, a type of protein thaumatin-like protein associated with tomato resistance (Dry *et al.*, 2010; Mengnan *et al.*, 2019). The early response is open and interupts the ion channels between the plasma membrane, leading to generate active oxygen species (AOS) including oxidative burst, phosphorylation, or dephosphorylation of protein kinases that are important signaling network transmission to stimulate other defense mechanisms (Thakur and Sohal, 2013). At the same time, the SA is also induced by the isochorismate pathway with *PR1* and *NPR1* expressed in chloroplasts, where ICS1 plays an important role in plant defense (Wildermuth *et al.*, 2001; Seyfferth *et al.*, 2014). The proteins and enzymes are synthesized to enhance defenses system including glycoprotein, fatty acid, carbohydrates, peptides, and phytohormones. It is associated with inducing structural and biochemical defenses including celluloses, hemicelluloses, lignins, chitinases, *β*-1,3-glucanases, chitinases, PPO, PAL, and POD (Vickers *et al*., 2009; Loreto *et al*., 2001; Belinky *et al*., 2003; Miedes *et al*., 2014; Huihui *et al.*, 2022). In present study, the tomato treated by BBC1 800 µg/ml were early induced for generating SA compound and PAL enzyme at 24 HAI, which was able to form lignins, lead to strengthen cell walls. The activity of chitinase and *β* -1,3-glucanase was also enhanced, which directly degraded chitin and *β* -1-3 glucan in fungal cell walls led to suppress pathogen infection. The role of BBC1 800 µg/ml in tomato defense was summarized and described in **Figure 5. 2** (Trouvelot *et al.*, 2014; Jimkuntod, 2018).

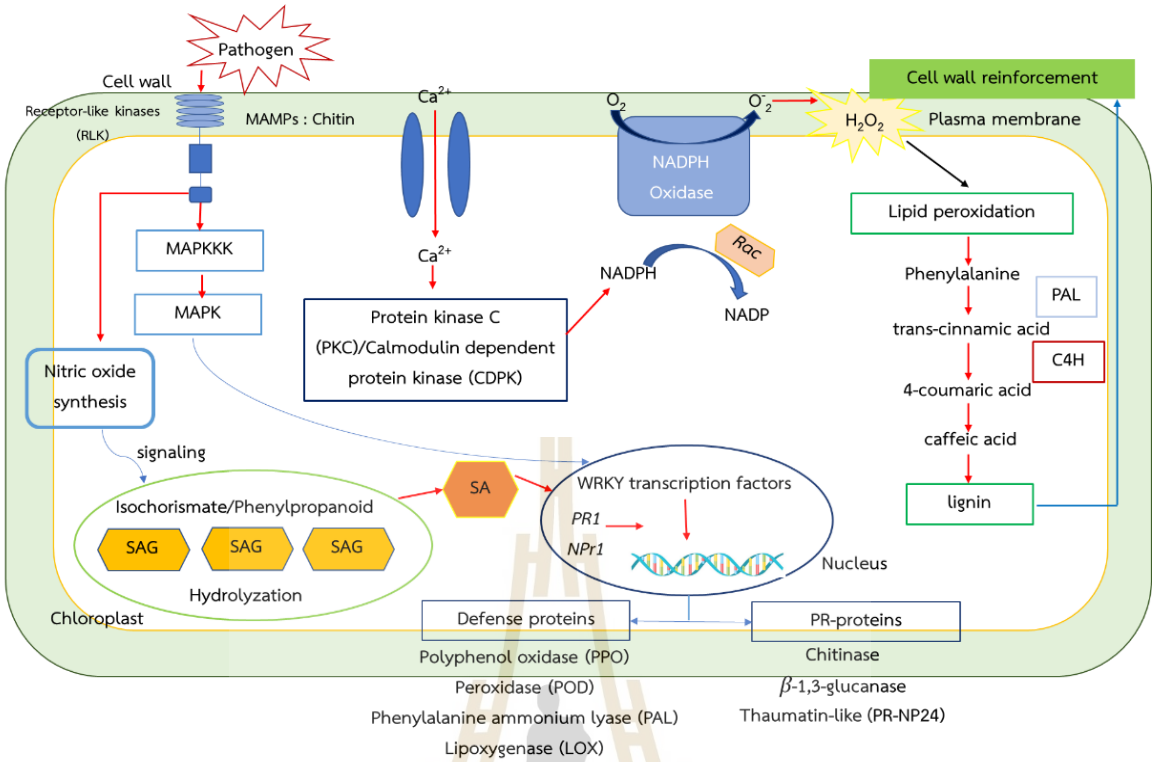


Figure 5.2 The self-defense mechanisms process of tomato after treated with BBC1 800 µg/ml formulation and inoculated with *FOL* (Trouvelot *et al.*, 2014; Jimkuntod, 2018).

5.6 Biochemical changes by SR-FTIR microspectroscopy technique

The biochemical change was studied by SR-FTIR microspectroscopy. The tomato leaves treated with BBC1 800 µg/ml showed a significant higher content of lipid groups C=O ester, Amide I, Amide II, CH-bending, and similar of hemicelluloses and polysaccharides. The plant cells contain lipid compounds including phospholipid, glycolipid, and lipoprotein, which are important components of plant cell membranes. Likewise, the protein is responsible for regulating metabolism, and intracellular activities and acting as enzymes or subunits of enzymes in plant cells. Carbohydrates have a significant role in the function and structure of plants. The celluloses, hemicelluloses, chitin, lignin, and pectin are frequently present in cell walls and cell membranes that are formed from polysaccharides or carbohydrates (Theerakulpisut, 2002; Wongchalee, 2015). These biomolecules were enhanced in epidermis tissues by

pretreating BBC1 800 µg/ml to stimulate flavonoids – a antifungal compound and accumulate carbohydrates to strengthen plant cell walls. As a result, the pathogen infection was defensed leading to reduce symptoms (Kim and Hwang, 2014). Saengchan (2014) studied the accumulation of biochemical related to the plant growth-promoting after stimulation with *Bacillus* sp. strain CaSUT007 and SA by FTIR technique. The results showed that the lipid group including C-H stretching, and C=O ester was increased in *Bacillus* sp. CaSUT007 and SA treatments. However, the carbohydrate group including C-H bonding, C-O stretching, and polysaccharide was decreased in *Bacillus* sp. CaSUT007 and SA treatments. These biochemical changes affected growth-promoting and resistance against soft rot disease on Chinese mustard. Similarly, Phiwthong (2014) studied the defense mechanism of grapes after *S. ampelinum* inoculation by the FTIR technique. The results found that the changing substances of the lipid group, C=O ester, amide protein, CH bending, celluloses, hemicelluloses, and carbohydrates in the SecCaSUT007 and chitosan treatments helped plant resistance against diseases. In addition, Wongchalee (2015) examined the biochemical changes of chili leaves sprayed with *Bacillus* sp. strain D604 by SR FTIR microspectroscopy technique. The amount of C=O ester, CH-bending, hemicelluloses, and polysaccharides were also higher than in *Bacillus* sp. strain D604 treatment, which enhanced plant growth, increased yield, and induced resistance against anthracnose disease. In present research, the hemicellulose and polysaccharide groups in tomato leaves treated with BBC1 800 µg/ml were not significantly different compared to the control treatment. This might be due to the fact that carbohydrates are a key source of energy in the mechanism of SAR. Furthermore, carbohydrates is used as an energy source for the production of ROS and PR proteins, which leads to generate HR (Saengchan, 2014; Wongchalee, 2015). In addition, this might decrease hemicelluloses and increase celluloses, and lignin synthesizing, which are packed together into lignocellulose after plants self-defense processes. Then, the hemicellulose was hydrolyzed to form and accumulate phenolic compounds, causing pathogen toxic (Vogt, 2010; Jönsson and Martín, 2016). As a result, the BBC1 800 µg/ml treatment did not cause different polysaccharide and hemicellulose groups compared with the control treatment.

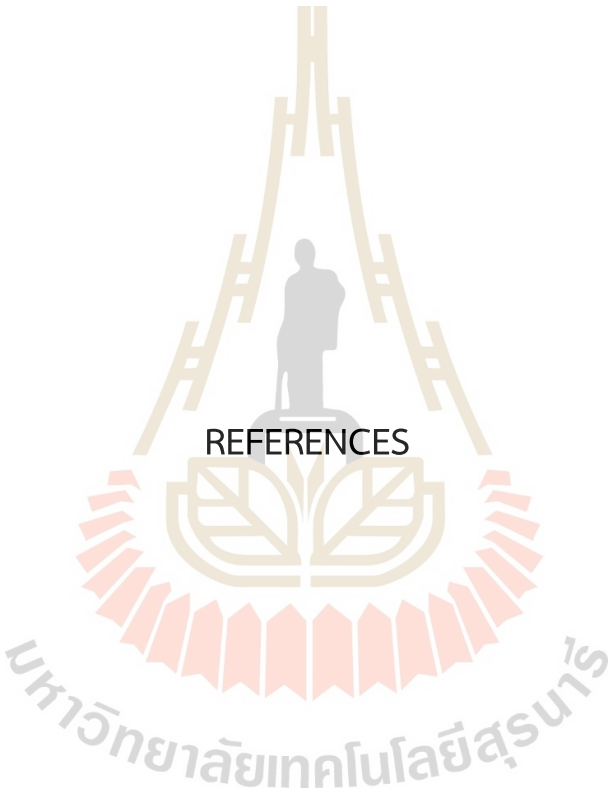
5.7 Conclusion

The encapsulated BBC1 is an effective solution for the management of Fusarium wilt disease by pre or post treatment when *FOL* is infected. Moreover, BBC1 encapsulate can induce systemic resistance by producing endogens SA, enzymes defense activity including chitinase, *β* -1-3 glucanase, and PAL to prevent *FOL* infection. In addition, encapsulated BBC1 can improve tomato yield and enhance tomato growth parameters.

5.8 Guidelines for the application of encapsulated BBC1

The BBC1 encapsulate showed the efficiency on suppressing the Fusarium wilt disease on tomatoes in two ways, including direct antifungal action and induced resistance mechanisms against phytopathogen. The application of the encapsulated BBC1 by soil drenching every 2 weeks combined with foliar spraying every 7 days showed similar efficacy in controlling Fusarium wilt disease compared with chemicals under greenhouse conditions. This encapsulated BBC1 has able to alternative pesticides in crop production. The encapsulation of BBC1 is low productivity. Therefore, scaling up manufacturing to an industrial level is challenging. The bacteria are released from the gel bead slowly in practice, which required the use of a solvent or a substance capable of dissolving sodium alginate for releasing bacteria. The development of other stable forms, high survival rates, and easy industrial production is the guideline for alternating chemicals in the control of tomato Fusarium wilt disease in the future.

REFERENCES



REFERENCES

Abdel, M. M. F., Ismail, M. E., & Morsy, K. M. (2011). Induction of systemic resistance of benzothiadiazole and humic acid in soybean plants against Fusarium wilt disease. *Mycrobiol.*, 39, 290-298.

Adeltruda, M., Mansuet, T., Martin, K., Richard, M., & Elisiana K. (2012). Damping off disease management in tomatoes - fact sheets for farmers, Plantwise, Horti Tengeru, Tanzania.

Agrios, G. N., (1997). 4th Academic Press New York. *Plant Pathol*. 635 pp.

Agriculture Statistics of Thailand. (2020). Office of agricultural economics ministry of agriculture and cooperatives, Bangkok, Thailand.

Ashley, A., Hubbard, J. C., & Liu, L. (2007). Effects of Chitin and Chitosan on the incidence and severity of Fusarium yellows of celery. *Plant. Dis*. 82(3), 322-328.

Balem, T., (2008). Response of tomato cultivars differing in growth habit to nitrogen and phosphorus fertilizers and spacing on vertisol in Ethiopia. *Acta Agri*., 2, 1.

Baysal, E.O., Calıskan, M.Y., & Silova, E.O. (2008) An inhibitory effect of a new *Bacillus subtilis* strain (EU07) against *Fusarium oxysporum* f. sp. *radicis- lycopersici*. *Physiol. Mol. Plant Pathol*., 73(1), 25–32.

Bakeer, A., Riad, S.R., Mohamedy, E.L., Saied, M. & Kareem, F, (2016). Field suppression of Fusarium soil borne diseases of tomato plants by the combined application of bio agents and chitosan. *Br. Biotechnol J*., 3(3), 1-10.

Belinky, P. A., Flikshtein, N., Lechenko, S., Gepstein, S., & Dosoretz, C. G. (2003). Reactive oxygen species and induction of lignin peroxidase in *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol*., 69(11), 6500-6506.

Benhamou, N., Lafontaine, P.J., & Nicole, M. (1994). Seed treatment with chitosan induces systemic resistance to Fusarium crown and root rot in tomato plants. *Phyto*., 84, 1432-1444.

Benhamou, N., Rey, P., Cherif, M., Hockenhull, J., & Tirilly, Y. (1997). Treatment with the mycoparasite, Pythium oligandrum, triggers the induction of defense-related reactions in tomato roots upon challenge with *Fusarium oxysporum* f. sp.

radicis-lycopersici. *Phytopathology.*, 87, 108–122.

Berg, G., & Ballin, G. (1994). Bacterial Antagonists to *Verticillium dahliae* Kleb. *J. Phytopathol*., 141(1), 99-110.

Berninger, T., López, O. G., Bejarano, A., Preininger, C., & Sessitsch, A. (2018). Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. *Microb. Biotechnol.*, 11(2), 277–301.

Boonsuebsakul, W., Thammakitchawat, P., Puawongphaet, B., Buaart, S., Kraikruan, & Nimkingrat, T. (2005). Biological control of tomato bacterial wilt. *DAO*., 1012-1022 p.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., 72(1-2), 248-254.

Buchanan, B. B., Gruissem, W., & Jones, R. L. (2000). Biochemistry and molecular biology of plants. Rockville, MD: American Society of Plant physiologists.

Buensanteai, N., Yuen, G. Y., & Prathuangwong, S. (2009). Priming, signaling, and protein production associated with induced resistance by *Bacillus amyloliquefaciens* KPS46. *J. Microbiol. Biotechnol*., 25, 1275–1286.

Buensanteai, N., & Athinuwat, D. (2012). The antagonistic activity of *Trichoderma virens* strain TvSUT10 against cassava stem rot in Thailand. *Afr. J. Biotechnol*., 11(84), 14996-15001.

Bunkoed, W., Yhamsoongnern, J., & Datburum, N. (2017). Management of Fusarium wilt with fungicides and biological agent under greenhouse condition. The 55th Kasetsart University Annual Conference., 590, 258-265.

Burketova, L., Trda, L., Peter, G. O., & Valentova O. (2015). Bio-based resistance inducers for sustainable plant protection against pathogens. *J. Biotechnol. Adv*., 33(6), 994-1004.

Cambrolle, J., Vallés, S. M., Mancilla, L. J. M., & Andrades, M. L. (2014). Effects of soil physicochemical properties on plant performance of *Glaucium flavum* Crantz. *Plant Soil*., 386(1-2).

Cao, Y., Xu, Z., & Ling, N. (2012). Isolation and identification of lipopeptides produced by *B. subtilis* SQR 9 for suppressing Fusarium wilt of cucumber. *Sci. Hortic*., 135, 32–39.

Carmen, R. R., & Roncero, M I. G. (2016). Determination of intra- and extracellular glucose in mycelium of *Fusarium oxysporum*. *Bio Protoc*., 6(14), 1868.

Cavaglieri, L., Orlando, J., & Etcheverry, M. (2005). In vitro influence of bacterial mixtures on *Fusarium verticillioides* growth and fumonisin B1 production: effect of seeds treatment on maize root colonization. *Lett. Appl. Microbiol*., 41(5), 390–396.

Chakraborty, N., Sharma, P., Kanyuka, K., Pathak, R. R., Choudhury, D., & Hooley R. (2015). G-protein α-subunit (GPA1) regulates stress, nitrate and phosphate response, flavonoid biosynthesis, fruit/seed development and substantially shares GCR1 regulation in *A. thaliana*. *Plant. Mol. Biol*., 89, 559–576.

Chen, H., Wang, L., & Su, C. (2008). Isolation and characterization of lipopeptide antibiotics produced by *Bacillus subtilis*. *Lett. Appl. Microbiol*., 47(3), 180–186.

Chen, J., Young, S. M., Allen, C., Seeber, A., Péli, G. M. P., Panchaud, N., ... Sklar L. A. (2012). Identification of a small molecule yeast TORC1 inhibitor with a multiplex screen based on flow cytometry. *ACS. Chem. Biol*., 7(4), 715-22.

Chenglong, J., Meilin, Z., Zirong, K., Xue, C., Xing, W., Wei, D., ... Qiao G. (2021). Genomic analysis reveals potential mechanisms underlying promotion of tomato plant growth and antagonism of soilborne pathogens by *Bacillus amyloliquefaciens* Ba13. *Microbiol. Spectr*., 9, 3.

Choudhary, D. K., Prakash, A., & Johri, B. N. (2007). Induced systemic resistance (ISR) in plants: mechanism of action. *Indian J. Microbiol*., 47, 289-297.

Chung, W. J., Okamura, K., Martin, R., & Lai, E. C. (2008). Endogenous RNA interference provides a somatic defense against *Drosophila transposons*. *Curr Biol*., 18(11), 795--802.

Ciampi, L., Fuentes, R., Nissen, J., Schöbitz, R., Costa, M., Barahona, C., ... Alvarez, E. (2007). Biocontrol of *Rhizoctonia solani* under field conditions using a strain of *Bacillus subtilis* bioformulated in alginate beads and free liquid cell suspension. 6th International Workshop on Bioencapsulation, 4, 19.

Compant, S., & Mathieu, F. (2016). Biocontrol of major grapevine diseases: Leading Research. *Retrieved from* https://books.google.co.th/books.

Conrath, U. (2006). Systemic acquired resistance. *Plant Signal. Behav*., 1(4), 179-84.

Cook, R. J., & Baker, K.F. (1993). The nature and practice of biological control of plant pathogens. St. Paul (MN): American Phytopathological Society.

Compant, S., Duffy, B., & Nowak, J. (2005). Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol*., 71(9), 4951–4959.

Cohen, S., & Antignus, Y. (1994). Tomato yellow leaf curl virus: A white fly born Gemini virus of tomatoes. *Adv. Dis. Vect. Res.*, 10, 259-288.

Dae, S. K. & Byung, K. H. (2014). An important role of the pepper phenylalanine ammonia-lyase gene (*PAL1*) in salicylic acid-dependent signaling of the defense response to microbial pathogens. *J. Exp. Bot*., 65(9), 2295–2306.

Dalila, P-L, Borges, J. R., Gutiérrez, A., Borges, A., Cabrera, G., Ramírez, M. A., & Falcón, A. (2000). Tomato *Fusarium oxysporum* interactions: II-Chitosan and MSB induced resistance against *FOL* in young tomato plants. *Sci. agropecu*., 17-20 pp.

Dangl, J. L., & Jones, J. D. G. (2001). Plant pathogens and integrated defence responses to infection. *Nature*., 411, 826–833.

Dessalegne, L., Zewdie, Y., & Herath, E. (1992). Agronomic studies in tomato: Horticulture research and development in Ethiopia. Proceedings of the Second National Horticultural Workshops of Ethiopia, Addis Ababa, Ethiopia, p-163.

Dicklow, M. B. (2013). Powdery mildew diseases of ornamental plants. *Retrieved from* https://ag.umass.edu/greenhouse-floriculture/fact-sheets/powdery-mildew diseases-of-ornamental-plants.

Domenech, J., Reddy, M. S., Kloepper, J. W., Romos, B., & Manero, G. J. (2006). Combined application of the biological product LS213 with *Bacillus*, *Pseudomonasor*, *Chryseobacteriom* for growth promotion and biological control of soil borne diseases in pepper and tomato. *Biocontrol*., 51, 245-258.

Dongdong, N., Xiujuan, W., Yanru, w., Xiaoou, S., Jiansheng, W., Jianhau, G., & Hongwei, Z. (2015). *Bacillus cereus* AR156 activates PAMP-triggered immunity and induces a systemic acquired resistance through a NPR1-and SA-dependent signaling pathway. *Biochem. Biophys. Res. Commun.*, 469(1), 120-125.

Dry, I. B., Feechan, A., Anderson, C., Jermakow, A. M., Bouquet, A., Adam, B. A. F., & Thomas, M. R. (2010). Molecular strategies to enhance the genetic resistance of grapevines to powdery mildew. *Aust. J. Grape Wine Res*., 16, 94-105.

Dumas, V. F., Goldenberg, C. S., Martins, E., Praca, L. B., & Queiroz, P. (2009). Chitinase and cellulase activity from *Bacillus thuringiensis* strains. Universitas Ciências da Saúde, 7, 1-12.

Fazio, R. H., & Towles S. T. (1999). The MODE model of attitude-behavior processes. In S. Chaiken & Y. Trope (Eds.), Dual-process theories in social psychology. The Guilford Press, pp 97–116.

Fernando, P. A., Denise, M. G. F., Ulysses, L., & Melissa, L. E. G. (2017). Surface imaging of the filamentous fungus *Penicillium simplicissimum* growing in a solid-state fermentation system. *Micron.*, 99, 19–25.

Food and Agriculture Organization of the United Nations: FAO. (2009). Statistical Bulletin, Rome, Italy. 150, 1-2.

Fawzy, M. & Monaim, A. (2013). Improvement of biocontrol of Damping-off and Root rot/wilt of faba bean by salicylic Acid and hydrogen peroxide. *Mycobiology.*, 41(1), 47-55.

Giorgi, A., Mingozzi, M., Madeo, M., Speranaz, G., & Cocucci, M. (2009). Effect of nitrogen starvation on the phenolic metabolism and antioxidant properties of yarrow (*Achillea collina* Becker ex Rchb). *Food Chem.*, 114(2009), 204-211.

Gong, A. D., Li, H. P., & Yuan Q. S. (2015). Antagonistic mechanism of Iturin A and Plipastatin A from *Bacillus amyloliquefaciens* S76-3 from wheat spikes against *Fusarium graminearum*. *PLoS One*., 10(2), 0116871.

Gong, Q., Zhang, C., & Lu, F. (2014). Identification of bacillomycin D from *Bacillus subtilis* fmbJ and its inhibition effects against *Aspergillus flavus*. *Food Control*., 36(1), 8–14.

González, L. R., Tsitsigiannis, D. I., Ludwig, A. A., Panicot, M., Shirasu, K., & Jones, D. G. J. (2006). The U-Box protein CMPG1 is required for efficient activation of defense mechanisms triggered by multiple resistance genes in tobacco and tomato. *Plant Cell.*, 18(4), 1067–1083.

Ham, K. S., Kauffmann, S., Albersheim, P., & Darvill A. G. (1991). Host pathogen interactions. XXXIX. A soybean pathogenesis related protein with b-1,3-glucanase activity releases phytoalexin elicitor-active heat-stable fragments from fungal walls. *Mol. Plant-Microbe Interact.*, 4, 545-552.

Hammerschmidt R. (1999). Phytoalexins: what have we learned after 60 years. *Annu. Rev. Phytopathol.*, 37, 285– 306.

Hammerschmidt, R., & Smith, B. J. A. (2000). The role of salicylic acid in disease resistance. In Mechanisms of Resistance to Plant Diseases. Kluwer Academic Publisher, 37–53 pp.

Hammond, K. K. E., & Jones, J. D. G. (1996). Inducible plant defense mechanisms and resistance gene function. *Plant Cell*., 8, 1773-1791.

Hanif, A., Zhang, F., Li, P., Li, C., Xu, Y., & Gao, X. (2019). Fengycin produced by *Bacillus amyloliquefaciens* FZB42 inhibits *Fusarium graminearum* growth and mycotoxins biosynthesis. *Toxins*., 11, 295.

Heil, M., & Bostock, R. M. (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. *Ann. Bot*., 89, 503–512.

Herman, R., & Perl, T. R. (2007). Characterization and inheritance of a new source of resistance to *Fusarium oxysporum* f. sp. *melonis* Race 1.2 in Cucumis melo. *Plant Dis.*, 91(9), 1180-1186.

Hirata, F., & Axelrod, J. (1980). Phospholipid methylation and biological signal transmission. *Sci*., 209(4461), 1082– 1090.

Hoitink, H. A. J., Madden, L. V., & Dorrance, A. E. (2006). Systemic resistance induced by *Trichoderma* spp.: Interactions between the host, the pathogen, the biocontrol agent, and soil organic matter quality. *Phytopathology.*, 96(2), 186-189.

Holmes, J. M., Beck, R. W., & Repka, M. X. (2001). The amblyopia treatment study visual acuity testing protocol archives of ophthalmology. *Biostat. Epidemiol.*, 119(9), 1345-1353.

Horticulture research and development in Ethiopia (1992). Proceedings of the Second National Horticultural Workshops of Ethiopia, Addis Ababa, Ethiopia. p- 163.

Huihui, Z., Xiaobing, W., & Wei, W. (2022). *Bacillus amyloliquefaciens* SN16-1-induced resistance system of the tomato against *Rhizoctonia solani*. *Pathogens.*, 11, 35.

Hussain, S., Zheng, M., Khan, F., Khaliq, A., Fahad, S., & Peng, S. (2015). Benefits of rice seed priming are offset permanently by prolonged storage and the storage conditions. *Sci. Rep*., 5, 8101.

Iriti, M., Castorina, G., Vitalini, S., Mignani, I., Soave, C., Fico, G., & Faoro, F. (2010) Chitosan-induced ethylene-independent resistance does not reduce crop yield in bean plants. *Biol. Control.*, 54, 241-247.

Jahan, N., Naveed, S., Zeshan, M., & Tahir, M. A. (2016). How to conduct a systematic review: A narrative literature review. *Cureus.*, 8(11), 864.

Jaipin, W., & Nalumpang, S. (2014). Effect of soil actinomycetes on growth of carbendazim-resistance *Colletotrichum gloeosporioides* causing strawberry anthracnose. *J. Agric. Sci.*, 30(1), 1–10.

Jangir, M., Pathak, R., Sharma, A., & Sharma, S. (2018). Volatiles as strong markers for antifungal activity against *Fusarium oxysporum* f. sp. *Lycopersici*. *Indian. Phytopathol.*, 72, 681–687.

Jimkuntod, N. (2018). Mechanisms of induced resistance against scab in grapevine of chitosan formulation (Master's thesis), School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresour. Technol*., 199(2016), 103–112.

Karimi, R., Owuoche, J. O., & Silim, S. N. (2010). Inheritance of Fusarium wilt resistance in pigeon pea [*Cajanus cajan* (L.) Millspaugh]. *Indian. J. Genet Pl.*, 70(3), 271-276.

Karthika, S., Varghese, S., & Jisha, M. (2020). Exploring the efficacy of antagonistic rhizobacteria as native biocontrol agents against tomato plant diseases. *Biotech.*, 10(7), 3.

Kastyak, L. M. Z., Nasse, M. J., Rak, M., Hirschmugl, C., Del, B. M. R., & Albensi, B. C. (2012). Biochemical label-free tissue imaging with subcellular-resolution synchrotron FTIR with focal plane array detector. *Neuroimage.*, 60, 376–383.

Katoch, R., Mann, A. P. S., & Sohal, B. S. (2005). Enhanced enzyme activities and induction of acquired resistance in pea with elicitors. *J. Veg. Sci.*, 11, 67–83.

Ke Tong, (2017). Preparation and biosorption evaluation of *Bacillus subtilis*/alginate–chitosan microcapsule. *Nanotechnol Sci Appl.*, 10, 35–43.

Khamsap, K. (2003). Biology-Pathogenicity and chemicals control of *Sphaceloma ampelinum* de Bary causing scab of grape (Master's thesis), Science (Plant Pathology), Kasetsart University, Bangkok.

Khan, M. A., Asaf, S., Khan, A. L., Jan, R., Kang, S. M., Kim, K. M., & Lee, I. J. (2020). Extending thermotolerance to tomato seedlings by inoculation with SA1 isolate of *Bacillus cereus* and comparison with exogenous humic acid application. *PLoS ONE.*, 15(4), 0232228.

Kim, D. S., & Hwang, B. K. (2014). An important role of the pepper phenylalanine ammonia-lyase gene (*PAL1*) in salicylic acid-dependent signaling of the defence response to microbial pathogens. *J. Exp. Bot.*, 65(9), 2295–2306.

Kloepper, J., Leong, J., Teintze, M., & Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature.*, 286(5776), 885–886.

Kloepper, J. W., Ryu, C. M., & Zhang, S. (2004). Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology.*, 94(11), 1259–1266.

Kokalis, B. N., Vavrina, C. S., Rosskopf, E. N., & Shelby, R. A. (2002). Field evaluation of plant growth-promoting Rhizobacteria amended transplant mixes and soil solarization for tomato and pepper production in Florida. *Plant Soil.*, 238, 257–266.

Kongtragoul, P., & Nalumpang, S. (2010). Characterization of *Colletotrichum gloeosporioides* resistant to carbendazim. *J. Agric. Sci.*, 26(3), 203-212.

Krasaekoopt, W., Bhandari, B., & Deeth, H. (2003). Evaluation of encapsulation techniques of probiotics for yogurt. *Int. Dairy J*., 13, 3-13.

Lahlali, R., Peng, G., Gossen, B. D., McGregor, L., & Yu, F. Q. (2013). Evidence that the bio fungicide Serenade (*Bacillus subtilis*) suppresses clubroot on canola via antibiosis and induced host resistance. *Phytopathology*., 103(3), 245-54.

Larkin, R. P., & Fravel, D. R. (1998). Efficacy of various fungal and bacterial biocontrol organisms for control of Fusarium wilt of tomato. *Plant Dis*., 82(9), 1022-1028.

Lebsing, P., Wongcharoen, A., & Saepaisan, S. (2011). Control of tomato seed disease caused by *Fusarium oxysporum* f. sp. *lycopersici* using fungicides and antagonistic fungi. *Khon Kaen Agr. J*., 49(4), 956-966.

Lee, H. W., Yoon, S. R., Kim, S. J., Lee, H. M., Lee, J. Y., Lee, J. H., Kim, S. H., & Ha, J. H. (2017). Identification of microbial communities, with a focus on foodborne pathogens, during kimchi manufacturing process using culture-independent and -dependent analyses. *LWT-Food Sci Technol*., 81, 153-159.

Lemma, D., & Herath, E. (1994) Varietal development on vegetables. In: Proceeding of the Second National Horticultural Workshop (eds.), Herath and Lemma, Addis Ababa, Ethiopia, 1-3 pp.

Le Thanh, (2015). Efficacy and mechanism of inducers triggering induced resistance in rice against Xanthomonas oryzae pv. Oryzae causing bacterial leaf blight disease. (Doctoral dissertation), School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

Le Thanh, T., Thumanu, K., Wongkaew, S., Boonkerd, N., Teaumroong, N., Phansak, P., & Buensanteai, N. (2017). Salicylic acid-induced accumulation of biochemical components associated with resistance against *Xanthomonas oryzae* pv. *oryzae* in rice. *J. Plant Interact.*, 12(1), 108-120.

Lemma, D. (2002). Research experiences in tomato production, research report No. 55. Ethiopia Agricultural Research Organization, Addis Ababa.

Li, J., Yang, Q., & Zhao, L. H. (2009). Purification and characterization of a novel antifungal protein from *Bacillus subtilis* strain B29. *J. Zhejiang Univ. Sci. B.*, 10(4), 264–272.

Li, M., Guo, L., Guo, C., Wang, L., & Chen, L. (2016). Over-expression of a DUF1644 protein gene, SIDP361, enhances tolerance to salt stress in transgenic rice. *J. Plant Biol.*, 59, 62–73.

Lim, G. T. T., Wang, G.P., Hemming, M. N., Basuki, S., McGrath, D. J., Carroll, B. J., & Jones, D. A. (2006). Mapping the I-3 gene for resistance to Fusarium wilt in tomato: application of an I-3 marker in tomato improvement and progress towards the cloning of I-3. *Australas. Plant Pathol.*, 35, 671–680.

Loreto, F., Mannozzi, M., Maris, C., Nascetti, P., Ferranti, F., & Pasqualini, S. (2001). Ozone quenching properties of isoprene and its antioxidant role in leaves. *Plant Physiol.*, 126(3), 993-1000.

Luo, W., Liu, L., Qi, G., Yang, F., Shi, X., & Zhao, X. (2019). Embedding *Bacillus velezensis* NH-1 in microcapsules for biocontrol of cucumber Fusarium wilt. *Appl. Environ. Microbiol.*, 85, 9

Maget, D. R., Ptak, M., & Peypoux, F. (1985). Pore-forming properties of iturin A, a lipopeptide antibiotic. *Biochim. Biophys.*, 815(3), 405–409.

Mandal, S. (2010). Induction of phenolics, lignin, and key defense enzymes in eggplant (*Solanum melongena* L.) roots in response to elicitors. *Afr. J. Biotechnol.*, 9, 8038-8047.

Manjula, K., Kishore, G. K., Girish, A. G., & Singh, S.D. (2004). Combined application of *Pseudomonas fluorescens* and *Trichoderma viride* has an improved biocontrol activity against stem rot in groundnuts. *Plant Pathol. J.*, 20(1), 75-80.

Manjunatha, M. V., Masthana, R. B. G., Masthana, S., & Joshi, V. R. (2009). Studies on the performance of self-propelled rice transplanter and its effect on crop yield. *J. Agric. Sci.*, 22(2), 385–387.

McCann, M. C., Hammouri, N., Wilson, R., Belton, P., and Roberts, K. (1992). Fourier transform infrared microspectroscopy is a new way to look at plant cell walls. *Plant Physiol.*, 100, 1940-1947.

Mengistie, G. Y., & Awlachew, Z. T. (2022). Evaluation of the plant growth promotion effect of *Bacillus* species on different varieties of tomato (*Solanum lycopersicum* L.) seedlings. *Adv. Agric.*, 10, 1155.

Mengnan, A., Zhipeng, T., Chengsong, D., Zehao, W., Huiying, S., Zihao, X., ... Yue, L. (2019). Molecular characterization of the thaumatin-like protein PR-NP24 in tomato fruits. *J. Agric. Food Chem.*, 67, 13001−13009.

Meyer, P., Liger, D., Leulliot, N., Quevillon, C. S., Zhou, C. Z., Borel, F., ... Tilbeurgh, H. (2005) Crystal structure and conformation of the alanine: glyoxylate aminotransferase activity of the YFL030w yeast protein. *Biochimie.,* 87(12), 1041-7.

Miedes, E., Vanholme, R., Boerjan, W., & Molina, A. (2014). The role of the secondary cell wall in plant resistance to pathogens. *Front. Plant Sci.*, 5, 358.

Mihali, C. V., Buruiana, A., Turcus, V., Covaci, A., & Ardelean, A. (2012). Mophology and ultrastructure aspects in species belongs to trichophyton fenus using light and scanning electron microscopy. *Annals. of RSCB.*, 18, 1.

Mishra, S., Jagadeesh, K.S., Krishnaraj, P.U. & Prem, S. (2014). Biocontrol of tomato leaf curl virus (ToLCV) in tomato with chitosan supplemented formulations of *Pseudomonas* sp. under field conditions. *Aust. J. Crop Sci.*, 8(3), 347-355.

Musiksang, H. (2008). Screening of lactic acid bacteria as probiotics in chicken and enhancement of bacterial survival by microencapsulation (Master's Thesis). Prince of Songkla University, Songkla.

Nemutanzhela, M. E., Roets, Y., & Gardiner, N. (2014). The use and benefits of *Bacillus* based biological agents in aquaculture. In: Hernandez-Vergara MP, Perez-Rostro CI, editors. Sustainable aquaculture techniques. Rijeka (Croatia): Intech; p. 33.

Nonthapot, S. (2017). Causality between capital investment in the tourism sector and tourist arrivals in ASEAN. *J. Adv. Res. Law Econ.*, 8(8), 2504-2511.

Ohno, A., Ano, T., & Shoda, M. (1995). Effect of temperature on production of lipopeptide antibiotics, iturin A and surfactin by a dual producer, *Bacillus subtilis* RB14, in solid-state fermentation. *J. Ferment. Bioeng.*, 80(5), 517–519.

Ongena, M., Jacques, P., Touré, Y., Destain, J., Jabrane, A., & Thonart, P. (2005). Involvement of fengycin-type lipopeptides in the multifaceted biocontrol potential of *Bacillus subtilis*. *Appl. Microbiol. Biotechnol.*, 69(1), 29-38.

Oostendorp, M., Kunz, W., Dietrich, B., & Staub, T. (2001). Induced disease resistance in plants by chemicals. *Eur. J. Plant Pathol.*, 107(1), 19-28.

Ortega, O. H., Benavides, M. A., Flores, O. A., & Ledezma, P. A. (2003). Use of the interpol electrolyte complexes of poly (acrylic acid)-chitosan as inductors of tolerance against pathogenic fungi in tomato (*Lycopersicon esculentum* Mill. Var. Floradade). *Macromol. Biosci.*, 3, 566-570.

Pal, K.K., & Gardener, B. M. (2006). Biological control of plant pathogens. *Plant Health Instruct*., 2, 1117–1142.

Palmieri, M. C., Perazzolli, M., Matafora, V., Moretto, M., Bachi, A., & Pertot, I. (2012). Proteomic analysis of grapevine resistance induced by *Trichoderma harzanum* T39 reveals specific defense pathways activated against downy mildew. *J. Exp. Bot.*, 63(17), 6237-6251.

Pan, S. Q., Ye, X. S., & Kuc, J. (1991). Association of *β*-1,3- glucanase activity and isoform pattern with systemic resistance to blue mould in tobacco-induced by stem injection with *Peronospora tabacina* or leaf inoculation with tobacco mosaic virus. *Physiol. Mol. Plant Pathol.*, 39, 25-39.

Park, J. W., Balaraju, K., & Kim, J. W. (2013). Systemic resistance and growth promotion of chili pepper induced by an antibiotic producing *Bacillus vallismortis* strain BS07. *Biol. Control.*, 65(2), 246–257.

Peng, J., Bao, Z., Ren, H., Wang, J., & Dong, H. (2004). Expression of harpin *Xoo* in transgenic tobacco induces pathogen defense in the absence of hypersensitive response. *Phytopathology.*, 94, 1048–1055.

Pengnoo, A., Kanjanamaneesathian, M., & Wiwattanapatapee, R. (2007). Formulation development of *Bacillus megaterium* in tablets forms for suppression of Rice

sheath blight disease. Department of Earth Science, Faculty of Natural Resources, Prince of Songkla University, Hatyai campus, Songkhla.

Phukang, J., & Chaisuk, P. (2016). Effects of some fungicides on growth inhibition of *Colletotrichum gloeosporioides*, causal agent of onion twister disease. *J. Agric. Sci.*, 32(2), 181–189.

Ponce, D. L. I., & Montesano, M. (2013). Activation of defense mechanisms against pathogens in mosses and flowering plants. *Int. J. Mol. Sci.*, 14, 3178–3200.

Pongprayoon, W., Roytrakul, S., Pichayangkura, R., & Chadchawan, S. (2013). The role of hydrogen peroxide in chitosan-induced resistance to osmotic stress in rice (*Oryza sativa* L.). *Plant Growth Regul*., 70(2), 159-173.

Poonam, G., Indu, R., & Vinay, S. (2012). Induction of b-1,3-glucanase and chitinase activity in the defense response of Eruca sativa plants against the fungal pathogen *Alternaria brassicicola*. *J. Plant Interact.*, 2, 155-161.

Prakongkha, I. (2011). Control of scab disease in grapes caused by *Sphaceloma ampelinum* de Bary by induced resistance (Master's Thesis). School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

Prakongkha, I., Sompong, M., Wongkaew, S., Athinuwat, D., & Buensanteai, N. (2013). Foliar application of systemic acquired resistance (SAR) inducers for controlling grape anthracnose caused by *Sphaceloma ampelinum* de Bary in Thailand. *Afr. J. Biotechnol.*, 12(33), 5148-5156.

Prathuangwong, S., Preecha, C., Kasem, S., & Thowthampitak, J. (2011). Plant Bacteria for plants with plant hormone fertilizer properties and against plant pathogens. Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok.

Phiwthong, T. (2014). Application of secondary metabolites of *Bacillus subtilis* strain CaSUT007 and chitosan to induce resistance against fungal disease in grapevine (Master's Thesis). School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

Pupakdeepan, W. (2009). Formulation development of new bacterial antagonist and control efficacy against bacterial leaf blight of rice (Master's Thesis).

Department of Plant Pathology, Faculty of Agriculture, Kasetsart University. Bangkok.

Qi, G., Zhu, F., & Du, P. (2010). Lipopeptide induces apoptosis in fungal cells by a mitochondria-dependent pathway. *Peptides.*, 31(11), 1978–1986.

Raskin, I., Turner, I. M. & Melander, W. R. (1989). Regulation of heat production in the inflorescences of an Arum lily by endogenous salicylic acid. *Proc. Natl. Acad. Sci. USA.*, 86, 2214–2218.

Reissig, J., Strominger, J. L., & Lelior, L. F. (1955). A modified colorimetric method for the estimation of N-acetyl amino sugars. *J. Biol. Chem.*, 217, 959-966.

Riaz, A., Rattu, A. U. R., Tahir, M. I., & Azeem, M. (2014). Phenylalanine ammonia-lyase (PAL) and peroxidase activity in brown rust infected tissues of pakistani wheat cultivars. *Pak. J. Bot.*, 46(3), 1101-1107.

Rodprapakorn, M., & Pongket, U. (2008). Development of probiotic product for shrimp by micro-entrapment. Proceedings of 46th Kasetsart University Annual Conference: Agro-Industry, 344-351.

Ron, M., & Avni, A. (2004). The receptor for the fungal elicitor ethylene-inducing xylanase is a member of a resistance-like gene family in tomato. *Plant Cell.*, 16(6), 1604-15.

Rudrappa, T., Biedrzycki, M. L., Kunjeti, S. G., Donofrio, M. N., Czymmek, K. J., Paul, W. P., & Bais, H. P. (2010). The rhizobacterial elicitor acetoin induces systemic resistance in *Arabidopsis thaliana*. *Commun. Integr. Biol.*, 3(2), 130-138.

Ruiz, J. M., Garcia, P. C., Rivero, R. M., & Romero, L. (1999). Response of phenolic metabolism to the application of carbendazim plus boron in tobacco. *Physiol. Plant.*, 106, 151–157.

Ryu, C. M., Farag, M. A., Hu, C. H., Reddy, M. S., Kloepper, J. W., & Paré, P.W. (2004). Bacterial volatiles induced systemic resistance in arabidopsis. *Plant Physiol.*, 134, 1017–1026.

Saarakkala, S., Rieppo, L., Rieppo, J., & Jurvelin, J. (2010). Fourier transform infrared (FTIR) microspectroscopy of immature, Mature and Degenerated Articular Cartilage. *Res. Sci. Technol. Educ.,* 403-414 pp.

Saengchan, C. (2014). Control of bacterial soft rot disease caused by *Erwinia carotovora* pv. *carotovora* in chinese green mustard using resistance elicitors (Master's

Thesis). School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

Sathiyabama, M., & Balasubaramanian, R. (1998). Chitosan induces resistance components in *Arachis hypogaea* against leaf rust caused by *Puccinia arachidis* Speg. *J. Crop Prot.*, 17, 307–313.

Seyfferth, C., & Tsuda, K. (2014). Salicylic acid signal transduction: the initiation of biosynthesis, perception and transcriptional reprogramming. *Front. Plant Sci.*, 5(2), 697.

Shi, J., & Maguer, M. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Crit. Rev. Biotechnol.*, 20, 293-334.

Shirasu, K., Nakajima, H., Rajasekhar, V. K., Dixon, R. A., & Lamb, C. (1997). Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defence mechanisms. *Plant Cell.*, 9, 261–270.

Sibounnavong, P., Sibounnavong, P. S., Kanokmedhakul, S., Soytong, K. (2012). Antifungal activities of *Chaetomium brasilense* CB01 and *Chaetomium cupreum* CC03 against *Fusarium oxysporum* f.sp. *lycopersici* race 2. *J. Agric. Sci. Technol.*, 8, 1029–1038.

Singh, B., Makkar, H. P. S., & Negi, S. S. (1989). Rate and extent of digestion and potentially digestible dry matter and cell wall of various tree leaves. *J. Dairy. Sci.*, 72(12), 3233-3239.

Soithong, K. (1999). Biological control of plant disease. School of Agricultural Technology. King Mongkut's Institute of Technology Ladkrabang. Bangkok.

Sozzi, D., & Gressler, C. (1980) Fungicide (MBC) resistant mutants of *Fusarium oxysporum* f. sp. *lycopersici* and *Botrytis cinerea*: pathogenicity and fitness. *J. Phytopathol*., 97, 19-24.

Srivastava, K. M., & Dwivedi, U. N. (2000). Delayed ripening of banana fruit by salicylic acid. *J. Plant Sci.*, 158(1–2), 87–96.

Sticher, L., Mauch, M. B., & Metraux, J. P. (1997). Systemic acquired resistance. *Annu. Rev. Phytopathol.*, 35, 235–270.

Suárez, M. H., Castillo, F.D.H., Morales, G. G., Saldivar, R. H. L., Herrera, R. R., & Aguilar, C. N. (2011). Biocontrol of soil fungi in tomato with microencapsulates containing *Bacillus subtilis*. *Am. J. Agric. Biol. Sci.*, 6(2), 189-195.

Suresh, L. M. & Team of scientists. (2017). Tomato Disease - Field Guide. Publisher: Seminis Vegetable Seeds.

Suwannarat, S., Steinkellner, S., Songkumarn, P., & Sangchote S., (2017). Diversity of *Colletotrichum* spp. isolated from chili pepper fruit exhibiting symptoms of anthracnose in Thailand. *Mycol. Prog.*, 16, 677–686.

Tavallali, V., Karimi, S., Mohammadi, S., & Hojati, S. (2008). Effects of *ß*-aminobutyric Acid on the Induction of Resistance to *Penicillium italicum*. *World Appl. Sci. J.*, 5(3), 345-351.

Teaumroong, N., Meunchang, S., & Choonluchanon, S. (2012). Collection and selection of Plant Growth Promoting Rhizobacteria (PGPR). School of Biotechnology. Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

Thakur, M., & Sohal, S. B. (2013). Role of elicitors in inducing resistance in plants against pathogen infection. *ISRN Biochem.*, 1-10 p.

Thasana, N., Prapagdee, B., & Rangkadilok, N. (2010). *Bacillus subtilis* SSE4 produces subtulene A, a new lipopeptide antibiotic possessing an unusual C15 unsaturated b-amino acid. *FEBS Lett.*, 584(14), 3209–3214.

Theerakulpisut, P. (2002). Introduction to plant physiology and biotechnology. Faculty of Saculity of Science Khon Kaen University, Khon Kaen.

Thipyapong, P., Hunt, M. D., & Steffens, J. C. (1995). Systemic wound induction of potato (*Solanum tubeuosum*) polyphenol oxidase. *Phytochemistry.*, 40, 673-676.

Thumanu, K., Sompong, M., Phansak, P., Nontapot, K., & Buensanteai, N. (2015). Use of infrared microspectroscopy to determine leaf biochemical composition of cassava in response to *Bacillus subtilis* CaSUT007. *J. Plant Interact.*, 10, 270–279.

Thumanu, K., Wongchalee, D., Sompong, M., & Buensanteai, N. (2017). Synchrotron-based FTIR microspectroscopy of chili resistance induced by *Bacillus subtilis* strain D604 against anthracnose disease. *J. Plant Interact.*, 12(1), 255-263.

Trivedi, S., Mahanta, S. K., & Kundu, S. S. (2007). Evaluation of protein rich feed resources with different levels of tannin for their in vitro utilization. *Range Manage Agrofor.*, 28(2B), 233-235.

Tronsmo, A. (1992). Leaf and blossom epiphytes and endophytes as biological agents of plant diseases. *Life Sci.*, 230, 43-54.

Trouvelot, S., Héloir, M. C., Poinssot, B., Gauthier, A., Paris, F., Guillier, C., & Combier, M. (2014). Carbohydrates in plant immunity and plant protection: roles and potential application as foliar sprays. *Front. Plant Sci.*, 5, 1-14.

Tsedeke, A. (1986). A review of crop protection research in Ethiopia. Proceedings of the first Ethiopian Crop protection symposium, Addis Ababa, Ethiopia.

Tumwine, J., Frinking, H. D., & Jeger, M. J. (2002). Integrating cultural control methods for tomato late blight (*Phytophthora infestans*) in Uganda. *Ann. of appl. Biol*., 141(3), 225-236.

Vadas, P., Pruzanski, W., & Kim, J. (1989). The proinflammatory effect of intra-articular injection of soluble human and venom phospholipase A2. *J. Med. Sci.*, 134(4), 807.

Vallad, G., & Goodman, R. M. (2004). Systemic acquired resistance and induced systemic resistance in conventional agriculture. *Crop Sci.*, 44, 1920–1934.

Van, L. C., & Bakker, M. (2005). Induced systemic resistance as a mechanism of disease suppression by rhizobacteria. PGPR: Biocontrol and bio fertilization. 39–66 p.

Verhagen, B. W. M., Patricia, T. A., Michel, C., Monica, H., & Aziz, A. (2011). *Pseudomonas* spp. induced systemic resistance to *Botrytis cinereais* associated with induction and priming of defense responses in grapevine. *J. Exp. Bot.*, 61(1), 249-260.

Vickers, C. E., Possell, M., Cojocariu, C. I., Velikova, V. B., Laothawornkitkul, J., Ryan, A., Mullineaux, P. M., & Hewitt, C. N. (2009). Isoprene synthesis protects transgenic tobacco plants from oxidative stress. *Plant Cell Environ.*, 32(5), 520-531.

Vimala, R., & Suriachandraselvan, M. (2009). Induced resistance in bhendi against powdery mildew by foliar application of salicylic acid. *J. Biopestic.*, 2(1), 111–114.

Vinodkumar, S., Nakkeeran, S., Renukadevi, P., & Malathi, V. G. (2017). Biocontrol potentials of antimicrobial peptide producing *Bacillus* species: Multifaceted antagonists for the management of Stem rot of carnation caused by *Sclerotinia sclerotiorum*. *Front. Microbiol.*, 8, 446.

Vleesschauwer, D. D., Djavaheri, M., Bakker, A. H. M., & Pand, H. M. (2008). *Pseudomonas fluorescens* WCS374r- Induced systemic resistance in rice against *Magnaporthe oryzae* is based on Pseudobactin-mediated priming for a salicylic acid-repressible multifaceted defense response. *Plant Physiol.*, 148(4), 1996-2012.

Vogt, T. (2010). Phenylpropanoid biosynthesis. *Mol. Plant.*, 3(1), 2-20.

Volpon, L. & Besson, F. J. M. (2000). NMR structure of antibiotics plipastatins A and B from *Bacillus subtilis* inhibitors of phospholipase A 2. *FEBS Lett.*, 485(1), 76–80.

Walters, B., Ronnback, P., Kovacs, J., Crona, B., Hussain, S., Badola, ... Dahdouh, G. F. (2008). Ethnobiology, socio-economics and management of mangrove forests: a review. *Aquat. Bot.*, 89, 220-236.

Wanga, S., Wanga, Q., Hua, Y. M., Xub, S. N., Hea, Z. X., & Jia, H. S. (2015). Study on the synergistic co-pyrolysis behaviors of mixed rice husk and two types of seaweed by a combined TG-FTIR technique. *J. Anal. Appl. Pyrolysis.*, 114, 109-118.

Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander D. C., ... Ryals, J. A. (1991). Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell.*, 3, 1085-1094.

Weindling, R. (1934). Studies on a lethal principle effective in the parasite action of *Trichoderma lignorom*, *Rhizoctonia solani* and other soil fungi. *Phytopathology.*, 24, 1153-1179.

Weindling, R., & Fawcett, H. S. (1936). Experiment in the control of Rhizoctonia damping off of citrus seedling. *Hilgardia.*, 10, 1-16.

Wessels, J. G. H., & Sietsma, J. H. (1981). Fungal cell walls: a survey. In: Tanner W, Louewus FA, editors. Encyclopedia of plant physiology. Springer-Verlag. New Series, 352-394 pp.

Wildermuth, M. C., Dewdney, J., Wu, G., & Ausubel, F. M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. *Nature.*, 414, 562–565.

Wiwattanapatapee, R. (2011). Development of biodegradable sustained-release bacterial antagonist gel beads for control of plant diseases. Prince of Songkla University, Songkla.

Wongchalee, D. (2015). Efficacy of ancapsulated antagonistic *Bacillus* in controlling chili antracnose (Master's Thesis). School of Crop Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima.

Wongkaew, P. (1989). Plant induced resistance to develop immunity. *Thai J. Agric. Sci.*, 7, 1-3.

Xu, D., Wang, Y., & Sun, L. (2013). Inhibitory activity of a novel antibacterial peptide AMPNT-6 from *Bacillus subtilis* against Vibrio parahaemolyticus in shrimp. *Food Control*., 30(1), 58–61.

Ye, Y. F., LI, Q. Q., & Gang, F. (2012). Identification of antifungal substance (Iturin A2) produced by *Bacillus subtilis* B47 and its effect on southern corn leaf blight. *J. Integr. Agric.*, 11(1), 90–99.

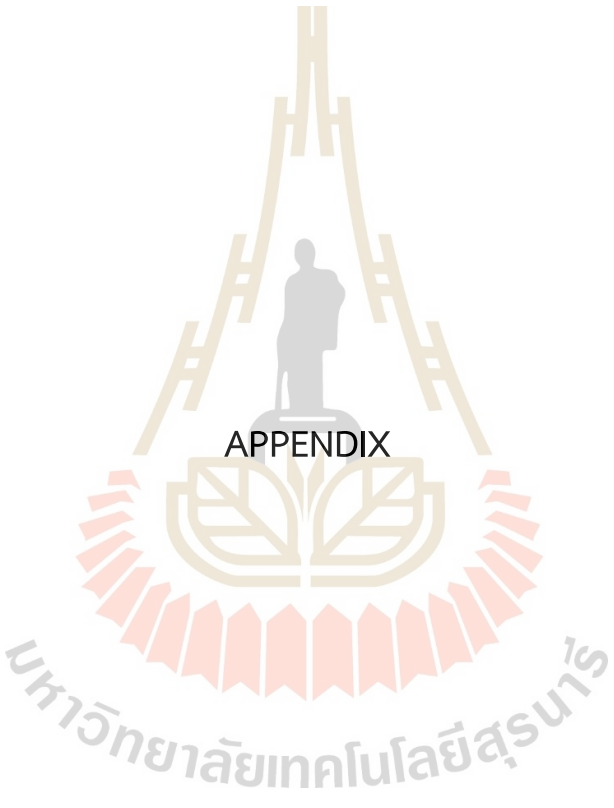
Younes, M. R., Mohamed, A. A., Hoda, M. S., Ghada, G. A., & Gamal, M. A. (2020). Synergy between endophytic *Bacillus amyloliquefaciens* GGA and arbuscular mycorrhizal fungi induces plant defense responses against white rot of garlic and improves host plant growth. *Phytopathol Mediterr.*, 59(1), 169-186.

Yuan, T., Ding, Y., Wan, C., Li, L., Xu, J., Liu, K., Slitt, A., ... Seeram, N. P. (2012). Antidiabetic ellagitannins from pomegranate flowers: inhibition of α-glucosidase and lipogenic gene expression. *Org. Lett.*, 14, 5358-5361.

Yu, P., Doiron, K., & Liu, D. (2008). Shining light on the differences in molecular structural chemical makeup and the cause of distinct degradation behavior between malting- and feed-type barley using synchrotron FTIR microspectroscopy: A Novel approach. *J. Agric. Food Chem.*, 56(9), 3417–3426.

Zehnder, G. W., Murphy, J. F., Sikora, E. J., & Kloepper, J. W. (2001). Application to rhizobacteria for induced resistance. *Eur. J. Plant Pathol.*, 14139–50.

APPENDIX



I. MEDIUMS

1.1 Water Agar (WA)

Agar	15	g
Water	1,000	ml

1.2 Potato Dextrose Agar (PDA)

Potato	200	g
Dextrose	20	g
Agar	15	g
Water	1,000	ml

1.3 Nutrient broth (NB) medium

Beef extract	3	g
Peptone	5	g
Water	1,000	ml

II. CHEMICALS

2.1 0.02 M Ferric ammonium sulfate

Ferric ammonium sulfate	4.82	g
Water	500	ml

2.2 10% Folin-ciocalteu reagent

Folin-ciocalteu reagent	10	ml
Water	100	ml

2.3 1 M Potassium chloride (KCl)

KCl	74.56	g
Water	1,000	ml

2.4 1 M Phenylmethylsulfonyl fluoride (PMSF)

PMSF	174.20	g
Isopropanol	1,000	ml

2.5 1% Triton-x 100

Triton-x 100	1	ml
Water	99	ml

2.6 Salkowski's reagent

Prepare stock solution of 0.5 M $FeCl_3$ (1.35 g in 10 ml water)

Use 1 ml of this stock to mix with 49 ml of 35% $HClO_4$

2.7 1 M Tris-HCl buffer, pH 8.0

Tris base	121.1	g
deionization water	1,000	ml
adjust pH to 8.0		

2.8 10 mM borate buffer, pH 8.8

Sodium tetraborate	0.38	g
Boric acid	62	mg
Water	100	ml
adjust pH to 8.8		

2.9 20 mM L-phenylalanine

L-phenylalanine	0.33	g
Water	100	ml

2.10 2% Sodium alginate

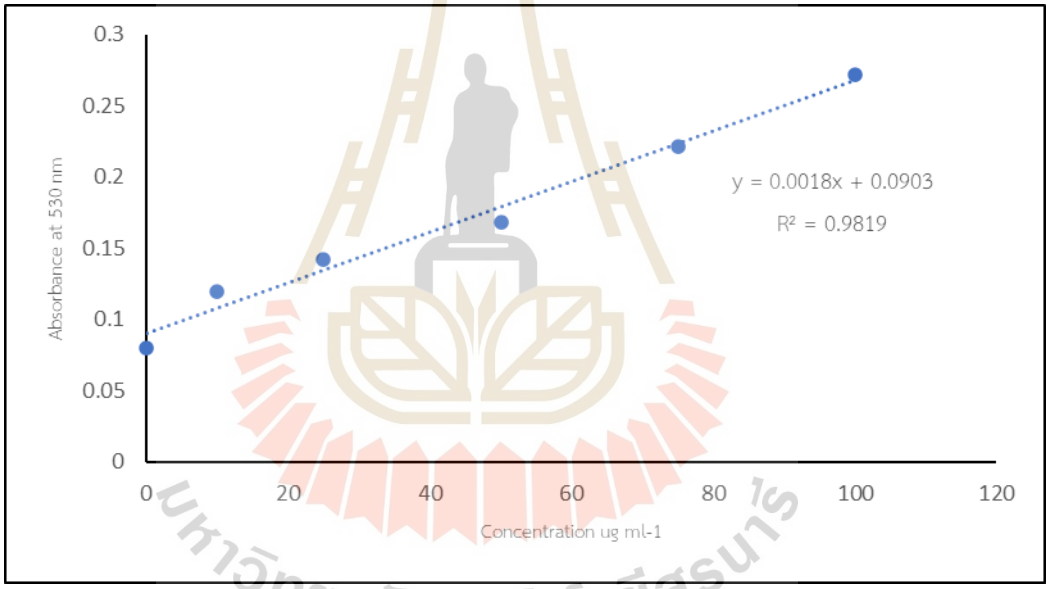
Sodium alginate	2	g
Water	100	ml

2.11 1.64 M Potassium dihydrogen phosphate (KH_2PO_4)

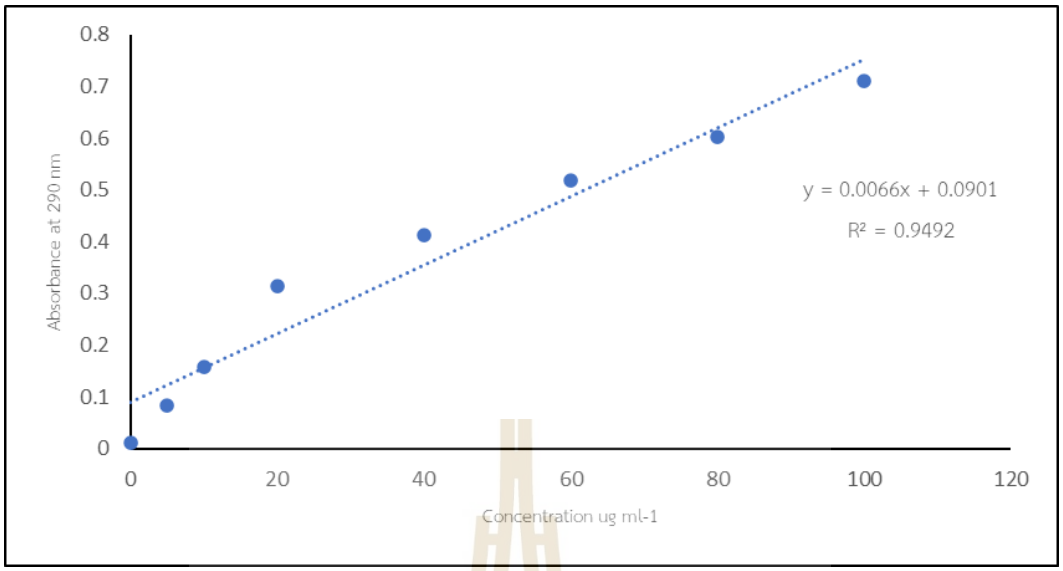
KH_2PO_4	22.31	g
Water	100	ml

2.12 0.03 M Calcium Chloride ($CaCl_2$)

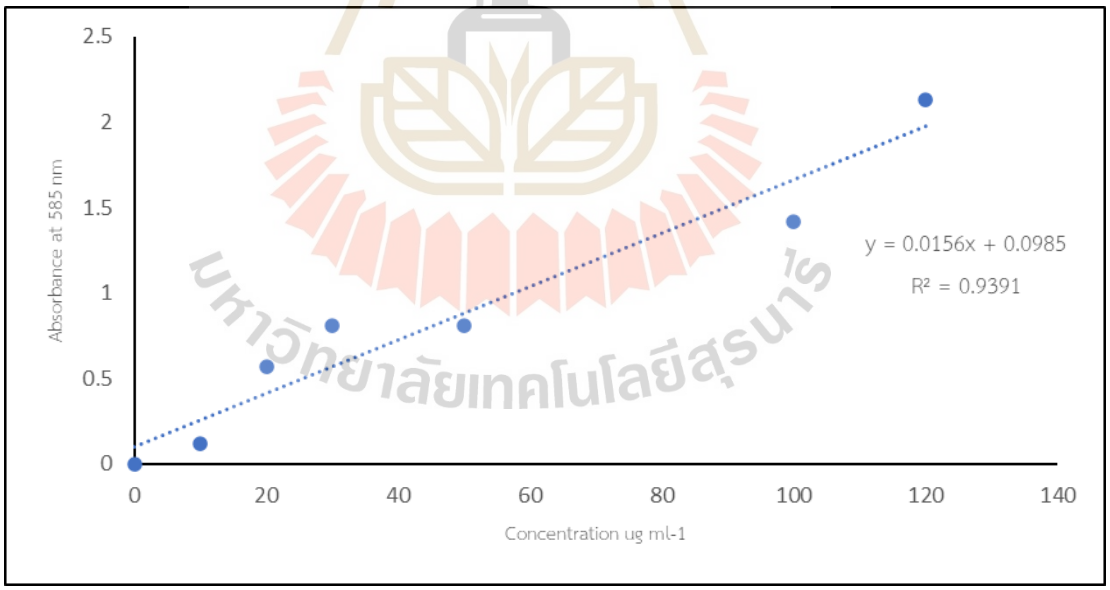
$CaCl_2$	0.44	g
Water	100	ml



Attached figure 1 Standard curve of salicylic acid (SA) at the absorbance 530 nm.



Attached figure 2 Standard curve of trans - cinnamic acid at the absorbance 290 nm.



Attached figure 3 Standard curve of N-acetyl glucosamine at the absorbance 585 nm.

BIOGRAPHY

Miss Parichat Numparditsub was born on May, 1995 in Prachuap Khiri Khan province, Thailand. She received his Bachelor of Crop Production of Technology from the Suranaree University of Technology, Thailand in 2016.

In 2017, she was admitted to study for a Master's Degree at Suranaree University of Technology, Nakhon Ratchasima, Thailand. During their study, she received a teaching assistant scholarship and research assistant funding from external grants: The Thailand Research Fund (TRF) and Bioactive Agro-Industry Company Limited under the Research and Researcher for Industry (RRi) at the Master's degree level.