EFFICACY OF *Bacillus* sp. AND INTERCROPPING TO CONTROL ROOT AND TUBER ROT DISEASES OF EDIBLE CASSAVA cv. PIRUN 2

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ประสิทธิภาพของการใช้ *Bacillus* sp. และการปลูกพืชแซมต่อการควบคุมโรครากและหัวเน่ามันสำปะหลังพันธุ์รับประทาน สายพันธุ์พิรุณ 2

นางสาวกานต์สินี แหลมเฉียบ

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คำสำคัญ: มันสำปะหลัง/สำปะหลังพันธุ์รับประทาน/โรครากและหัวเน่า/บาซิลลัส/การปลูกพืชแซม

มันสำปะหลัง (*Manihot esculenta* Crantz) เป็นพืชหัวที่มีความสำคัญ มักใช้เป็นวัตถุดิบตั้งต้นในการผลิตอาหารสัตว์และแป้งมันสำปะหลัง โดยการผลิตมันสำปะหลังมักพบปัญหาคือโรคมันสำปะหลัง ความรุนแรงของโรคที่เพิ่มขึ้นและความหลากหลายในการเข้าทำลาย โดยโรครากและหัวเน่ามันสำปะหลัง สามารถทำให้สูญเสียผลผลิตมันสำปะหลังมากกว่า 80% การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาประสิทธิภาพของ *Bacillus* sp. และการปลูกพืชแซมเพื่อควบคุมโรครากและหัวเน่ามันสำปะหลัง โดยทำการทดสอบการก่อโรคของโรครากและหัวเน่ามันสำปะหลัง (Cassava root and tuber rot disease : CRTRD) ในต้นพันธุ์มันสำปะหลังและหัวมันสำปะหลัง ผลการศึกษาพบว่า เชื้อรา *Lasiodiplodia theobromae* ทำให้เกิดแผลเน่า มีสีดำ ทั้งในต้นมันสำปะหลังและในหัวมันสำปะหลัง โดยมีระดับคะแนน 4 คะแนน เนื่องจากได้รับผลกระทบ 75% ของพื้นที่ ต่อมาศึกษาผลของ *Bacillus* sp. ในการควบคุมเชื้อรา *L. theobromae* ผลการศึกษาพบว่า *Bacillus* sp. สายพันธุ์ CaSUT008-2 สามารถยับยั้งการเจริญเติบโตของเส้นใยเชื้อรา *L. theobromae* ที่ 5 วันหลังจากการบ่ม โดยมีเปอร์เซ็นต์การยับยั้งอยู่ที่ 53.08% รองลงมาคือ *Bacillus* sp. สายพันธุ์ CaSUT007 และ D604 สามารถยับยั้งการเจริญเติบโตของเส้นใยเชื้อราอย่างมีนัยสำคัญทางสถิติที่ 50.30% และ 48.37% เมื่อเทียบกับกรรมวิธีควบคุม จากนั้นทำการทดสอบความทนทานต่ออุณหภูมิสูง ผลการศึกษาพบว่า *Bacillus* sp. สายพันธุ์ CaSUT 008-2, D604 และ CaSUT007-1 สามารถเติบโตที่อุณหภูมิสูงได้ถึง 53 °C โดยมีการรอดชีวิตที่ 5.26×10^8, 3.40×10^8, 3.33×10^8 CFU/ml ตามลำดับ จากนั้นทำการทดสอบความต้านทานรังสีอัลตราไวโอเลต (UV) ผลการศึกษาพบว่าเชื้อ *Bacillus* sp. สายพันธุ์ CaSUT 008-2 โดยมีการรอดชีวิตที่ 2.40×10^8CFU/ml หลังจากการได้รับรังสี UV ที่ 6 ชั่วโมง หลังจากนั้นจึงทำการศึกษาอัตราส่วนของเชื้อ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ในการยับยั้งการเจริญเติบโตของเชื้อรา *L. theobromae* ที่ความเข้มข้น 2%, 5% และ 10% พบว่าเชื้อ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ทั้ง 3 ความเข้มข้นไม่มีความแตกต่างกันทางสถิติในการยับยั้งการเจริญเติบโตของเส้นใยเชื้อรา ทั้งที่เลี้ยงบนเครื่องเขย่าและที่อุณภูมิห้อง จากนั้นทำการตรวจสอบลักษณะของเส้นใยโดยกล้องจุลทรรศน์ Environmental scanning electron microscope นำ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ที่

ความเข้มข้น 2% เลี้ยงในอาหารเหลวร่วมกับ *L. theobromae* เป็นเวลา 24 ชั่วโมง พบว่าในกรรมวิธีที่เลี้ยง *L. theobromae* และ *Bacillus* sp. สายพันธุ์ CaSUT008-2 เส้นใยเชื้อราที่เกิดรอยย่น บิดเบี้ยว และหดตัว ที่เส้นใยมีเซลล์แบคทีเรียเกาะอยู่บางส่วน ในกรรมวิธีควบคุมเส้นใยยังคงพื้นผิวที่เรียบ จากนั้นทำการศึกษาประสิทธิภาพของ เชื้อ*Bacillus* sp. เพื่อควบคุมโรค CRTRD ในสภาวะเรือนทดลอง ผลการทดลองพบว่าเชื้อ *Bacillus* sp. สายพันธุ์ 008-2 มีความสามารถในการกระตุ้นการเจริญเติบโตของมันสำปะหลัง โดยมีความสูงอยู่ที่ 35.20 ซม. จำนวนใบ 11.25 ความยาวใบ 8.63 ซม. เมื่อเปรียบเทียบกับกรรมวิธีควบคุม และในการทดลองลำดับสุดท้าย การทดสอบประสิทธิภาพของ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ร่วมกับการปลูกพืชแซมเพื่อควบคุมโรค CRTRD และคุณสมบัติของดินในสภาพไร่ ที่มหาวิทยาลัยเทคโนโลยีสุรนารีและอำเภอเสิงสาง ในปี 2563 (ปีแรก) และปี 2564 (ปีที่สอง) เก็บผลการทดลองการเจริญเติบโตที่อายุ 3 เดือน 6 เดือน 8 เดือนหลังปลูก พบว่ากรรมวิธีที่ใช้ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ร่วมกับปลูกพืชแซมโดยใช้ถั่วลิสงพันธุ์ Tinan 9 มีแนวโน้มการเจริญเติบโตสูงกว่าวิธีการควบคุมที่ 6 และ 8 เดือน หลังปลูก โดยมีความสูงต้น 178.43 และ 264.38 ซม. ตามลำดับ ในปีที่สอง พบว่ากรรมวิธีที่ทำการปลูกพืชแซมโดยใช้ถั่วลิสงพันธุ์ Tinan 9 และใช้ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ยังคงมีแนวโน้มการเจริญเติบโตสูงกว่ากรรมวิธีควบคุม ที่ 6 และ 8 เดือนหลังปลูก และสามารถลดความรุนแรงของการเกิดโรครากและหัวเน่ามันได้ เมื่อเปรียบเทียบกับกรรมวิธีควบคุมทั้ง 2 พื้นที่ปลูก และกรรมวิธีการปลูกพืชแซมโดยใช้ถั่วลิสงพันธุ์ Tinan 9 และ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ยังช่วยเพิ่มจำนวนหัวต่อต้นและ น้ำหนักสดของหัวต่อต้น นอกจากนี้ การปลูกพืชแซมโดยใช้ถั่วลิสงพันธุ์ Tinan 9 ร่วมกับ *Bacillus* sp. สายพันธุ์ CaSUT008-2 ยังเพิ่มปริมาณไนโตรเจน ปริมาณฟอสฟอรัส ปริมาณโพแทสเซียม ปริมาณแคลเซียม ความเป็นกรด-ด่าง และอินทรียวัตถุหลังทำการปลูก หลังจากนั้นทำการศึกษาการรอดชีวิตของ *Bacillus* sp. สายพันธุ์ CaSUT 008-2 ในวัสดุปรับปรุงดิน พบว่ามีอัตราการรอดชีวิต เท่ากับ $2.16\pm0.45\times10^{8}$, $1.98\pm0.32\times10^{8}$ และ $1.55\pm0.14\times10^{8}$ CFU/มล. ที่ 1, 2 และ 3 เดือน ตามลำดับ จากนั้นได้ทำการศึกษาความคล้ายคลึงกันระหว่างแบคทีเรียที่รอดชีวิตและ *Bacillus* sp. สายพันธุ์ CaSUT008-2 โดยใช้อาหารจำเพาะ พบว่าสัณฐานวิทยาของแบคทีเรียที่รอดชีวิตมีลักษณะเป็นโคโลนีทึบแสงล้อมรอบด้วยเมือกซึ่งคล้ายคลึงกับ *Bacillus* sp. สายพันธุ์ CaSUT008-2 การค้นพบนี้ ชี้ให้เห็นว่า *Bacillus* sp. สายพันธุ์ CaSUT008-2 สามารถเจริญเติบโตในวัสดุปรับปรุงดิน สามารถนำมาควบคุมโรครากและหัวเน่า และเพิ่มผลผลิตมันสำปะหลังได้

สาขาวิชาเทคโนโลยีการผลิตพืช ลายมือชื่อนักศึกษา .....

ปีการศึกษา 2564 ลายมือชื่ออาจารย์ที่ปรึกษา .....

KANSINEE LAEMCHIAB : EFFICACY OF *Bacillus* sp. AND INTERCROPPING TO CONTROL ROOT AND TUBER ROT DISEASES OF EDIBLE CASSAVA cv. PIRUN 2. THESIS ADVISOR : ASST. PROF. DR. NATTHIYA BUENSANTEAI, 83 PP.

Keyword : Cassava/ Edible cassava/Root and tuber rot disease/Bacillus/Intercropping

Cassava (*Manihot esculenta* Crantz) is an important tuber crop, which is often used as feed and primary starch. The increasing disease severity and pathogen diversity are the main problem in cassava production. Cassava root and tuber rot disease or CRTRD causes yield loss up to 80%. The objectives of this study were to investigate the antagonistic effects of *Bacillus* sp. and intercropping to control the CRTRD. First, pathogenicity tests of CRTRD causal agents were carried out on cassava healthy plants. The results showed that the fungi infection caused dry-rot lesions on the cassava stem, with scoring at 4 because 50 to 75% of stem area and tuber was affected. Next, the effect of *Bacillus* sp. strain CaSUT008-2 as a biological control agent against *L. theobromae* was studied. The results showed that *Bacillus* sp. strain CaSUT008-2 could inhibit mycelial growth of *L. theobromae* at 5 days after incubation. The percentage inhibition was approximately 53.08%. Moreover, *Bacillus* sp. strains CaSUT007 and D604 could significantly inhibit mycelia growth by 50.30% and 48.37% compared with the negative control, respectively. Next, the screening of *Bacillus* sp. for high temperature resistance was tested. The results showed that the *Bacillus* sp. CaSUT 008-2, *Bacillus* sp. D604 and *Bacillus* sp. CaSUT007-1 could grow up to 53 °C with survival density $5.26x10^8$, $3.40x10^8$, $3.33x10^8$ CFU/ml, respectively. Furthermore, *Bacillus* sp. for ultraviolet (UV) resistance was also tested. The results showed that the *Bacillus* sp. CaSUT 008-2 highly resisted UV by maintaining a density of 2.40×108 CFU/ml for 6 hours of exposure. Then, an assay for *Bacillus* sp. inhibiting fungal hyphae was conducted. The results showed that the ratio of *Bacillus* sp. strain CaSUT008-2 and *L. theobromae* at 2%, 5% and 10% could inhibit fungal growth in rotary shaker and room temperature conditions. Environmental scanning electron microscope observation indicated that 2% *Bacillus* sp. strain CaSUT008-2 caused the pathogen hyphae to be wrinkled, distorted, and shrunken. The hyphae had bacterial

cells attached to some portions to varying degrees, which increased in number in the treatments with increased ratio of CaSUT008-2. Conversely, in the untreated control, pathogen hyphae looked intact with a smooth surface. Then, the efficacy of *Bacillus* sp. to control CRTRD was tested in greenhouse conditions. The results showed that *Bacillus* sp. strain 008-2 had ability to stimulate growth of cassava to have the height 35.20 cm, number of leaves 11.25, leaf length 8.63 cm, while compared with sterile distilled water. These results indicated that *Bacillus* sp. strain 008-2 had high potential to stimulate growth of cassava. Finally, the efficacy of Bacillus sp. strain CaSUT008-2 and peanut intercropping to control CRTRD and improve soil properties under field conditions was tested at Suranaree University of Technology and Soeng Sang district in 2020 (first year) and 2021 (second year). The results of the experiments on growth were collected at 3, 6 and 8 months after planting (MAP). Combination of Tinan 9 and *Bacillus* strain CaSUT008-2 (CTB) treatment had a higher growth trend than the control methods at 6 and 8 MAP, with plant height at 178.43 and 264.38 cm, respectively. In the second year, the CTB treatment still had a higher growth trend than the control treatment at 6 and 8 MAP. The CRTRD severity (%) of CTB treatment could reduce CRTRD in field and this treatment also increase the number of tubers per plant and tuber fresh weight per plant. Moreover, this CTB treatment was also increased nitrogen content, phosphorus content, potassium content, calcium content, pH and organic matter after cropping. In addition, surviving *Bacillus* sp. strain CaSUT 008-2 in soil amendments was also checked. The optical density of surviving bacteria was $2.16\pm0.45\times10^8$, $1.98\pm0.32\times10^8$, and $1.55\pm0.14\times10^8$ CFU/ml, at 1, 2 and 3 months respectively. The morphology of surviving bacteria was opaque colony surrounded by mucus, similar to the bacteria mixed in the soil. This finding suggested that *Bacillus* sp. strain CaSUT 008-2 was able to grow in the soil amendments and can be used for reducing CRTRD and enhancing cassava yield.

School of Crop Production Technology Student's Signature Kansinee Laemchiab

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LIST OF ABBREVIATIONS

°C	=	Degree Celsius
µL	=	Microliter (s)
µm	=	Micrometer (s)
min	=	Minute (s)
mL	=	Milliliter (s)
nm	=	Nanometer (s)
ha	=	Hectare
mV	=	millivolt
DAI	=	Days after inoculation
DAP	=	Days after planting
DMRT	=	Duncan's multiple range tests
FAO	=	Food and Agriculture Organization
FAOSTAT	=	Food and Agriculture Organization Corporate Statistical Database
FTIR	=	Fourier-transform infrared spectroscopy
ITS	=	Internal transcribed spacer
SEM	=	Scanning electron microscope
SR	=	Synchrotron
TEM	=	Transmission electron microscopy

CHAPTER I
INTRODUCTION

1.1 Background of the selected topic

Cassava (*Manihot esculenta* Crantz) is a widely cultivated annual tuber crop in Thailand. It has been used as feed and primary starch in many countries worldwide (Roger, 1963; Sungthongwises et al., 2011; Kaewkunya et al., 2015; Dutchanee, 2015). Cassava exports are expected to increase this year, following a surge last year. by 48.41% in 2021 to 123.2 billion baht. Export quantity also rose by 45.58% to 10.38 million tones, the highest volume in the past 14 years driven by the rising prices of world cereals and demand for the production of ethanol, alcohol, and medical products (Javanmard et al., 2019; Maneechot et al., 2019; Phusadee., 2022). Cassava has been identified as the country's most important cash crop in terms of economic productivity impact among the significant native starch, modified starch, and energy crops, both for domestic use and export to other countries such as Thailand, China, and Indonesia (FAO, 2002; Tiamtaisong et al., 2011; Gao et al., 2014; Medeiros et al., 2019). Cassava, an agro-industrial crop with well-developed industry and market in Thailand, is regarded as one of the country's most important economic crops, accounting for approximately 67% of the global market. (FAO, 2002; Dutchanee, 2015; Thailand Investment Review, 2017).

At present, the planting area of cassava in Thailand was approximately 1.29 million hectares with the increasing acreage and changing cultivation practices for more than 40 years (Jakrawatana et al., 2015). The cassava plants can grow in all soil types in Thailand, but it is often cultivated in sandy loam soils with deficient nutrients and organic matter (Sangphanta et al. 2015; Timingoon et al., 2015). Under these poor soil conditions, cassava tuber yields usually decrease when the cassava crop is grown

ithe field withoutfertilizer application (Moss et al., 2009; Islam et al. Kaewkunya et al., 2015; Maneechot et al., 2016). Therefore, this cash crop is cultivated by smallholder farmers within the existing farming systems in many countries in Southeast Asia and Africa, especially in Thailand. Cassava cultivation in monoculture areas for several decades led to diseases outbreak, low organic matter, a decline in soil fertility, and soil erosion (Javanmard et al., 2009; Sungthongwises et al., 2011; DOA, 2015).

Moreover, cassava diseases have been increasing in both disease severity and pathogen diversity (Charaensatapon et al., 2014; Duchanee, 2015; Sangpueak et al., 2017). Important cassava diseases in Thailand such as Brown Leaf blight, Anthracnose, and Cassava root and tuber rot disease or CRTRD. Cassava yield loss during these recent years caused by diseases has been estimated at 80 %. Cassava root rot disease (CRTRD) caused by complex fungi has been damaged approximately 80% of annual yield loss in Thailand (Charaensatapon et al., 2014; Duchanee, 2015). In 2015, Duchanee reported that CRTRD is caused by complex fungal pathogens including *Lasiodiplodia theobromae*, *Fusarium oxysporum*, *F. solani*, *Phytophthora* sp., *Sclerotium rolfsii*, *Neosctylidium* sp. The CRTRD is a serious threat to agriculture around the world, reducing yields and negatively impacting crop survival depending on the causal agent, host susceptibility, and environmental conditions. (Krupinsky et al., 2002). For this reason, cassava yield has been reduced from 20 to 100% due to attacks by these diseases (Charaensatapon et al., 2014; Duchanee, 2015).

In these years, Traditional cassava disease control practices in Thailand have relied on cultural approaches. (DOA, 2015; Duchanee, 2015). There are many methods to control cassava diseases including resistance varieties, chemical control, biological control, and cultural practices. The alternative methods practiced to reduce the problem of the disease of growing cassava in monoculture would be biological control and intercrop cassava with a legume (Javanmard et al., 2009; Ofori et al., 2011; Piyachomkwan et al., 2011; DOA, 2015; Wang et al., 2015). Peanut is a leguminous plant that grows well in poor soils which can improve soil fertility and reduce disease

severity (Moss et al., 2009; Islam et al., 2014; Kaewkunya et al., 2015; Finley et al., 2018). Moreover, biological control using biocontrol agents (BCA) or plant growth promoting rhizobacteria (PGPR) such as *Bacillus* sp. have been widely used in a research experiments and commercial scale to control many plant pathogens and enhance crop growth (Durrant and Dong, 2004; Hukkanen et al., 2007; Buensanteai et al., 2009; Vimala and Suriachandraselvan, 2009; Cartea et al., 2011; War et al., 2011). The BCA/PGPR could have a direct effect on plant growth and development by producing plant growth regulators. *Bacillus* sp. can also colonize roots and stimulate plants to produce growth-promoting biomolecules. (Saengchan et al., 2015; Wongchalee et al., 2015). According to a previous study, the PGPR *B. amyloliquefaciens* strain KPS46 can improve the growth of several commercial crops, including corn, rice, Chinese kale, and cauliflower. (Prathuangwong and Buensanteai, 2007; Buensanteai et al., 2009; Buensanteai et al., 2012; Saengchan et al., 2015). Moreover, *B. subtilis* strain CaSUT007, and *Trichoderma virens* strain SUTTv10 can enhance plant growth in cassava and inhibit *L. theobromae*, the cassava root rot fungi pathogen (Buensanteai et al., 2012; unpublished Saengchan et al., 2015; Wongchalee et al., 2015). Nowadays, the cultural practices using intercropping in cassava are gaining importance in most developing countries where the pressure on cultivable land is ever increasing (DOA, 2015; Chen et al., 2017). In 2007, Hukkanen et al. It has been reported that cassava/peanut intercropping can increase nutrient content in peanut rhizosphere soil by improving the micro-ecological environment, and that appropriate intercropping spacing is more conducive to increasing nutrient content in peanut rhizosphere soil. Furthermore, intercropping could improve soil quality and increase the availability of phosphorus (P) in the rhizosphere of intercropped plant species. (Li et al., 1999; Li et al., 2007; Hinsinger et al., 2011). Intercropping can also improve soil resource utilization and increase crop productivity (Li et al., 2001; Zhang and Li, 2003; Javanmard et al., 2009). Furthermore, intercropping has the potential to alter the dominant microbial species and microbial communities in soils. (Li et al., 1999; Li et al., 2001; Zhang and Li, 2003; Song et al., 2007; Javanmard et al., 2009; He et al., 2013).

In previous, research has not proved of the effectiveness of intercropping and *Bacillus* sp. for controlling economic crop diseases. Hence, the aim of this study was a focus on the investigation of the antagonistic effects of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping to control the CRTRD.

1.2 Research objectives of the study

To investigate the antagonistic effects of *Bacillus* sp. and intercropping to control the CRTRD.

1.3 Research hypothesis

Intercropping and *Bacillus* sp. could be controlled CRTRD in the greenhouse and field conditions.

1.4 Scope of the study

This research would be scope to study the efficacy of cassava cv. Pirun 2 was treated with Bacillus sp. strain 008-2 and intercropped with peanuts to control cassava root rot caused by *L. theobromae* in the greenhouse and field conditions. The experiments were conducted in the 1 location field experiment at Suranaree University of Technology and 1 location farmer field at Soeng Sang District, Nakhon Ratchasima province.

CHAPTER II
LITERATURE REVIEW

2.1 Cassava

2.1.1 Importance of cassava

Cassava (*Manihot esculenta* Crantz) is the most important tropical root crop. It is grown in 105 countries and is the fourth most important crop on the planet (Olsen and Schaal, 1999; Latif and Müller, 2014). More than 500 million people rely on its starchy roots for dietary energy, protein, vitamins (A and C), and a variety of mineral salts (FAO, 2002; Ravindran et al., 1988). They are widely grown in tropical and subtropical countries in Africa, Asia, and Latin America, with an estimated production of 276.7 million tons. Furthermore, it is an important agro-industrial crop with well-developed industry and market in Thailand, and it is regarded as one of the country's most important economic crops. Thailand's cassava processing industry has progressed from chips and pellets as primary products to starch, which is then processed into high-value-added starch derivative products. Thailand exported over 4.26 million tons of starch and starch derivatives, earning the country 62 THB billion (1.86 billion USD) (Sriroth and Piyachomkwan 2008; Tanticharoen, 2009). Cassava is grown in two varieties in Thailand:

1) Bitter cassava with a high hydrocyanic acid content, which must be processed before being used, includes 9 cassava varieties of the Department of Agriculture comprising Rayong 1, Rayong 3, Rayong 60, Rayong 90, Rayong 5, Rayong 72, Rayong 7, Rayong 9 and Rayong 11; another 3 varieties from Kasetsart University including Kasetsart 50, Huai Bong 60 and Huai Bong 80 (Thai Tapioca Production Promotion Center, 2010); and Pirun 1, developed by the Department of Agriculture, National Science and Technology Development Agency and Mahidol University National Science and Technology Development Agency: NSTDA,2016).

2) Sweet cassava is used for direct consumption because there is a low amount of hydrocyanic acid, no bitter taste and it can be used to cook directly. The varieties include Rayong 2, 5 natee, Pirun 2, and Pirun 4 (Wirasilp, 1999; Phuti, 1983). This sweet cassava can be used as human food under the policy of the United Nations Food and Agriculture Organization (FAO, 2002) that the amount of cyanide is less than 10 mg per kilogram of dry weight (FAO / WHO, 2009). In addition, the free gluten sweet cassava flour has been used in various bakery products to replace wheat flour which led to the development of the high value bakery products (Chotineeranat et al., 2013; Boonseng et al., 2015). The leading target group of sweet cassava flour consumers is people who are allergic to wheat-sensitive patients and patients with celiac disease or an autoimmune disease caused by an autoimmune disease.

Cassava is a viable cash crop for millions of farmers as well as an industrial crop with a high-value chain. As a result, the crop has played an important role in the country's socioeconomic development (Pomkam, 2009; Kunkeaw at el., 2010; Wongcharoen, 2013). Many stakeholders, including the government, academic, and private sectors, as well as farmer smallholders, have recognized the cassava industry in Thailand. The industry has continued to develop and improve its ability to effectively utilize the cassava crop, not only the roots but also other biomass and waste, which bodes well for cassava-based bio-refineries and bio-based products in the future.

Cassava cv. Pirun 2: since 2006, the tapioca cultivar "Pirun 2" has been developed by the Department of Agriculture Institute of Molecular Biosciences Mahidol University and the National Center for Genetic Engineering and Biotechnology with research funding from the Office of Science and Technology Development National from the first generation of cassava hybrids between Huai Bong 60 and 5 minutes. Characteristics include light green shoots, red petioles, erect shape, average yield of 5.8 tons per rai, starch content in fresh tubers of 24.7 percent, cone-shaped heads or lotus bud, short stalk, easy to cut off, dark brown head bark, head flesh white, less fiber. Advantages are the higher yield of fresh tubers five-minute varieties when grown in a field that relies only on rainwater, it has a beautiful shape with a

short stem. Make it easy to cut off the head suitable for growing in the reddest clay, followed by clay loam and black clay. When the tubers are steamed or welded to make them white. Delicious taste and soft texture without burrs, this variety of cassava can be Food and industrial varieties (National Science and Technology Development Agency: NSTDA, 2016).

2.2 Important diseases of cassava

Regarding, the cassava crop cultivated by smallholder farmers within the existing farming systems in many countries in Southeast Asia and Africa, especially in Thailand. At present, the planting area of cassava in Thailand was increasing acreage and changing cultivation practices for more than 40 years (Jakrawatana et al., 2015). Cassava cultivation in monoculture areas for several decades led to diseases outbreak, low organic matter, a decline in soil fertility, and soil erosion (Javanmard et al., 2009; Sungthongwises et al., 2011; DOA, 2015). Cassava diseases have increased in both disease severity and pathogen diversity (Charaensatapon et al., 2014; Duchanee, 2015; Sangpueak et al., 2017). For this reason, cassava yield has been reduced from 20 to 100% due to attacks by these diseases (Msikita et al., 2005; Salami and Akintokun, 2008; Banito et al., 2010; Javanmard et al., 2009; Charaensatapon et al., 2014; Duchanee, 2015).

2.2.1 Cassava bacterial blight (CBB)

Cassava bacterial blight caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*), is a destructive disease in South America and Africa that reduces yield by affecting both yield and planting material. International phytosanitary quarantine efforts are now focusing on CBB. Symptoms of the disease include angular leaf spot and blight, wilt, gum exudation, stem necrosis, and die-back. (Charaensataporn and Kitjiadeaw, 2010; André, 2016).

2.2.2 Brown leaf spot (BLS) disease

Brown leaf spot caused by *Cercosporidium henningsii*. It is one of the most serious cassava fungal diseases. BLS disease appears on the upper leaf surface as small brown spots with dark borders. The brown spots form between the leaf veins, limiting their size and shape. The brown spots' centers may fall out, leaving a hole in

the leaf. During a severe attack, infected leaves turn yellow and dry, and they may die prematurely. The disease has the potential to significantly reduce yield (Karyeija, 2012; Charaensataporn and Kitjiadeaw, 2010).

2.2.3 Cassava anthracnose disease (CAD)

Cassava anthracnose disease is caused by the fungus *Colletotrichum gloeosporioides* f.sp. Cassava is a serious economic disease of cassava in the tropics. In some high-rainfall areas of Africa, the condition has reached epidemic proportions. CAD is distinguished by specific symptoms (cankers on stems, branches, and fruits, leaf spots, and tip dieback) on diseased plants' aerial parts (Muimba, 1982). The disease's appearance is determined by the cassava variety and the infected plant. Cassava is grown on large fields or plantations on an industrial scale. The leaves grow and spread, forming a closed canopy that creates very humid conditions conducive to the development and spread of CAD in the area.

2.2.4 Cassava root and tuber rot disease (CRTRD)

CRTRD is an increasing problem in Africa and Thailand where yield losses of approximately 80% have been recorded (Onyeka et al., 2005; Duchanee, 2015). CRTRD is a complex of soilborne pathogens such as *Botryodiplodia theobromae*, *Fusarium* sp., *Sclerotium rolfsii*, *Neoscytalidium* sp., *Pythium* sp., and *Phytophthora* spp. (Onyeka et al., 2005; Bandyopadhyay et al., 2006; Aigbe and Remisin, 2009; Banito et al., 2010; Duchanee, 2015). Plants were examined for visible symptoms of root and stem rots during the cassava disease survey. Infection was indicated by fungal mycelium or fruiting bodies on the plants stem base, as well as wilting. Rot disease is distinguished by a breakdown in the tissue of mature tuberous roots, which is usually accompanied by a foul odor and color changes. (Bandyopadhyay et al., 2006; Banito et al., 2010; Duchanee, 2015; DOA, 2002; DOA, 2015). CRTRD is caused by numerous fungi that live on or in the soil. The fungus is found primarily in poorly draining soils and recently removed forest fallow land. When cassava plants are infected with root rot disease, the leaves turn dark, and wilt, and the plant seems burnt. The cassava leaves may or may not remain attached to the plant, but the plant suffers from water loss and eventually dies. Cassava feeder and store roots are both killed by root rot pathogens. If the roots fracture in the soil or are sliced open, the storage roots may

enlarge unnaturally and have a pale brown hue. As the beginnings decompose, they may emit a foul odor. (Msikita et al., 2000). Root rot pathogens cause some of the most crucial plant diseases worldwide impacting several crops (Owolade et al., 2005; Charaensataporn and Kitjiadeaw, 2010; Gonzalez et al. 2011, Eliane Thaines Bodah, 2017). Root rot symptoms pose a serious hazard since the damage begins beneath the earth, where the earliest symptoms are not visible. When symptoms appear on the plant's surface, the yield is already impaired, and the plant's existence is risked. Studies on root rot causative pathogens have revealed these agents (Nzungize J et al., 2011). The most prevalent root rot pathogens are oomycetes and fungi. Aphanomyces spp., Pythium spp., and Phytophthora spp. have all been identified as oomycetes. Several fungi, including Rhizoctonia spp., Fusarium spp., Phoma spp., Aphanomyceseuteiches, and Thielaviopsisbasicola, have been documented to cause root rot. Many root rot infections illnesses are greatly influenced by the environment, with a diverse variety of hosts, hidden subsurface symptoms, and overwintering structures. (Msikita et al., 2005; Banito et al., 2010; Eliane Thaines Bodah, 2017).

Root and tuber rot diseases can kill both feeder and storage roots of cassava. The storage roots may swell and develop light brown discoloration. The storage roots may swell and develop light brown discoloration. The roots may give out a bad smell as they rot. Root rots can occur on young or old cassava plants and can be caused by members of one or several fungal genera. Disease incidence usually is higher during the rainy season (Msikita et al., 2005; Banito et al., 2010). The main symptom of rot disease is a breakdown in the tissue of the mature tuberous roots, usually associated with a foul odor and color changes, which may be useful in distinguishing the pathogens involved. For example, storage tissues of tuberous roots primarily infected by a *Fusarium* species often are pink or yellow, whereas tissues infected with *Botryodiplodia theobromae* often is a dark blue or grayish black (Akinyele and Ikotun, 1989; Owolade et al., 2005). The research of Duchanee (2015) found that *Lasiodiplodia* is the most common species causing CRTRD in Thailand with 54% and followed by *Fusarium* spp with approximately 29% of cases. Symptoms of CRTRD vary according to the causal agent and can be divided into soft rot/wet rot, dry rot, damping-off, and black rot is characterized by dark lesions (blackened) in the roots

and stems and may evolve into soft rot but without the unpleasant odor and dry rot is characterized by the appearance of dark brown streaks in the roots with no aqueous aspect (Duchanee, 2015).

Figure 2.1 Symptoms of cassava root rot diseases (A) Soft rot/wet rot (B) Dry rot (C) Damping-off (D) Black rot (Duchanee, 2015).

2.3 Control of Cassava root rot disease

The sources of cassava root rot pathogens are soils, cassava root, and stem debris contaminated with the fungal. The fungal infected cassava plants through wounds caused by pests or farming tools or by infecting the roots by themselves. Similarly, cassava plant debris in farms with the disease serves as a source of root rot fungal and should be destroyed by antagonistic microorganisms (Onyeka et al., 2005; Bandyopadhyay et al., 2006; Aigbe and Remisin, 2009; Banito et al., 2010; Chatchai, 2014; Duchanee, 2015). CRTRD management concentrates on reducing the initial inoculum and subsequently reduce of the pathogen on cassava plants. These targets can be accomplished using chemicals, cultural practices, disease-resistant cultivars, biological controls, and induced resistance.

2.3.1 Disease resistance cultivars

Emerging cassava diseases are outbreaking damage to cassava around the world, leading to an encouraging action for developing crops that can resist drought and climate change. In the recent year, higher yield and productivity of cassava varieties with resistance or tolerance to diseases and pests have been developed by several Agricultural Institutions in Thailand such as the International Institute of Tropical Agriculture (IITA) and The International Center for Tropical Agriculture (CIAT) (CIAT,1981; Ravindran et al., 1988; FAO, 2002; Javanmard et al., 2009; Sungthongwises et al., 2011; DOA, 2015). However, developing new varieties has been proven effective. In Thailand and Vietnam, 60-75% of cassava farmers are planting the highly productive cassava variety Kasetsart 50 (KU 50). KU 50 is the world's widely grown cassava variety. It is cultivated on more than one million hectares in Thailand, Vietnam, Cambodia, Indonesia, and Laos. It has high starch content, wide adaptation, and high root yield. Moreover, in Thailand, also the recommended varieties that are resistant to several cassava diseases are Rayong 9 and Rayong 72 (Chatchai, 2014).

2.3.2 Chemical control

Cassava is the most cultivated food crop and is planted in backyards even in the cities in South Africa as such the use of chemicals in the control of cassava diseases is not recommended. CRTRD is challenging to manage because it is caused by a complex of soil-borne fungi/oomycete species, which makes chemical control infeasible due to environmental damage and its high treatment costs for producers. In Thailand, plant pathologists of the department of agriculture have found that *Phytophthora* sp. caused the CRTRD. The application of chemicals fosetyl-aluminum (50 g/ 20 L water) as dipping before planting and foliar spraying at one-month intervals until three months gave the best results to control the disease (Chatchai, 2014)

2.3.3 Cultural methods

The cultural methods of plant disease control are practiced on a planting crop. In addition to land tillage practices, they include inter-cropping, crop rotation, row, and plant spacing, plant canopy, timing of fertilizer and soil amendment application, and other planting techniques (Bandyopadhyay et al., 2006; Banito et al., 2010; DOA,

2002; DOA, 2015). At present, cultural control approaches find their considerable value in huge planting areas and low unit value economic crops such as cassava, sugarcane, and rice. Cultural control practices could be also considered a form of biological control, contribute to being preventative and indirect in their mode of action against several plant pathogens (Li et al., 1999; Li et al., 2001; Zhang and Li, 2003; Song et al., 2007; Javanmard et al., 2009; He et al., 2013). The success of cultural control practices to plant disease management depends on the understanding of plant-pathogen life cycle biology and the response of the plant host to pathogen attack.

Intercropping systems are one of the cultural methods that involve two or more crop species or genotypes growing together and coexisting for a time. This distinguishes intercropping from mixed monocropping and rotation cropping (Vandermeer, 1989; Kunkeaw at el., 2010; Wongcharoen, 2013). Cassava intercropping with short-duration crops is a common practice among smallholder farmers in many tropical countries. These intercrops are useful because they supply either food or additional income, especially at a time when the cassava crop cannot yet be harvested; they may fix N and supply other nutrients to the topsoil; they may protect the soil from the direct impact of rainfall; reduce the speed of runoff water when the cassava canopy is not yet closed to reduce soil erosion, and reduce weed growth during the early stages of cassava development. However, intercrops need light, water, and nutrients to be carefully managed to minimize the competition with cassava. This is usually done through modifications of the plant spacing or planting pattern of both crops, by adjusting the relative time of planting, and adequately fertilizing each crop to maximize yields.

As intercropping is a traditional cropping system that can be defined as the growing of two or more crops simultaneously in the same area of land. The crops are not necessarily sown at a similarly precise time and their harvest times may be quite different, but they are usually simultaneous for a significant part of their growing periods (Willey, 1979; FAO, 1983). The forms of these advantages are yield stability, low input, higher and better use of growth resources, and better control of weeds, pests, and disease. When two or more crops are grown together, each must have

adequate space to maximize cooperation and minimize competition between the crops to accomplish this, four things need to be considered (Sullivan, 2001).

1. Spatial arrangement: Intercropping includes four important special methods:
    1.1 Row intercropping: the simultaneous cultivation of two or more crops, at least one of which is planted in rows.
    1.2 Strip intercropping: growing two or more crops together in strips wide enough to allow independent crop production with machines but close enough to allow the crops to interact.
    1.3 Mixed intercropping: growing two or more crops together with no defined row arrangement.
    1.4 Relay intercropping: planting a second crop in a standing crop during the reproductive stage but before harvesting.

2. Plant density: to optimize plant density; the seeding rate of each crop in the mixture is adjusted below its maximum price.

3. Maturity dates: a selection of crops or varieties with different maturity dates can reduce the competition between the two crops. It can also assist in harvesting and separating grain commodities.

4. Plant architecture: is commonly and strategically used to allow one member of the mixture to capture sunlight that would not otherwise be available to the others.

Furthermore, intercropping is seen as a means of increasing income in the cassava field (Asokan et al., 1987). Even though intercrops lowered cassava output, the reduction was offset by intercrop yield, increasing the farmer's net profit (Mohankumar, 1974). Intercropping resulted in higher gross returns and dietary requirements than mono-cropping (Andrews et al., 1976; Okigbo et al., 1976). The economic examination of various intercropping techniques in tapioca found that groundnut as an intercrop performed the best (Singh, 1970; Sintuprama et al., 1973; Sheela, 1981; Anilkumar and Sasidhar, 1987; CTCRI, 1988). Maximum economic gains were realized under the intercropping strategy when root crops and legumes were planted in the same period.Cowpea may be the most profitable intercrop in cassava and legume intercropping (Kerala. Ghosh et al. 1987) obtained the highest net return from the cassava + french bean or cowpea crop combination. Asokan and Sreedharan

(1987) discovered that cassava, groundnut, and red gram (BCR-1.94) produced the maximum income, followed by cassava and groundnut (BCR-1.86) and cassava and cowpea (BCR-1.87). Intercrops such as green gram, cowpea, black gram, or groundnut provided additional income without reducing primary crop yield and yielded a larger return than single cassava (Varughese et al., 1988). Ghosh and colleagues (1989) Cassava in combination with seasonal intercrops offered the highest net returns than cassava as a pure crop; cowpea as a ground tier crop is more profitable than groundnut. Sheela and Kunju (1990) discovered that groundnut intercrop was significantly superior to cowpea, with a mean net return of 11,63 ha-1. Furthermore, according to Bai et al. (1992), paired row planting of cassava and cowpea yielded the highest net income of Rs11,335 ha^{-1}, followed by uniform planting of cassava and cowpea (10,433 ha^{-1}). The cost-benefit ratios were 1.65 and 1.60, respectively. Furthermore, per rupee invested suggested that cassava cowpea intercropping was more efficient than cassava groundnut association. Interplanting cassava with other crops such as upland rice, maize, and legumes increased the gross income by 33% compared to monoculture cassava (Wargiono et al., 1992; Prabhakar and Nair; 1992).

Intercropping in cassava is becoming more popular in most developing countries, as the demand for scarce cultivable land is increasing. Aside from boosting output, there were numerous other motivations for intercropping systems to be used in various parts of the world (Andrews and Kassam, 1976; Willey, 1979). Intercropping cassava with short-term crops such as onion and french bean was discovered to be feasible. Cassava has also been grown in conjunction with other leguminous crops such as groundnut, cowpea, and soybean, as well as non-leguminous crops such as maize, sunflower, and upland rice crops. (Okigbo and Greenland, 1976; Prabhakar and Nair, 1984). According to Kawano and Thung (1982), Cassava can be planted in conjunction with short-duration crops such as faba beans, cowpeas, and so on, and cassava genotypes with lower vegetative vigor may be favored for such relationships. According to Leihner (1984), approximately 40% of cassava grown in Latin America and 50% in Africa is intercropped. In Africa, mixed cropping accounts for around 50% of cassava production (Okigbo and Greenland, 1976). Following a survey of two cassava-growing states in India, it was discovered that intercropping in cassava was a

widespread practice in Tamil Nadu, but mixed cropping comprising tuber crops and crop rotation is extensively practiced in Kerala. (Ramanathan et al., 1990).

The most popular and prolific bi-specific mixture planted in Tropical Africa, Asia, and Latin America was cassava intercropped with maize. In Nigeria, Ikeorgu and Odu-rukwe (1990) discovered that cassava/maize intercrops would be extremely productive, which might be boosted further by the addition of groundnut. Hartojo and Widodo reported in 1991 that hybrid maize may be intercropped with cassava in Indonesia without hurting cassava yield. Furthermore, Olasantan et al. (1994) concluded that intercropping cassava with early maturing maize under optimal soil nutrient availability, particularly N, maintains high combination productivity. In recent years, several grain legumes could be intercropping with cassava such as cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), soybean (*Glycine max*), groundnut *(Arachis hypogaea*), and pigeon pea (*Cajanus cajan*) (Sasidhar., 1976; Pillai et al., 1986). The major aspects led to the recommendation of the legume in multiple cropping systems with cassava. For starters, because it is a legume crop, it may fix atmospheric N and hence enhance soil fertility. Second, the cassava tuber, which is poor in protein and includes a pulse crop, is important for balanced nutrition. (Sasidhar, 1976).

Another cultural practice for plant root rot disease control is soil amendment mixed with antagonistic microorganisms. Many farmers in the world and also in Thailand are becoming increasingly aware of the importance of organic production. Challenges for organic production are the management of nutrients, diseases, and insects. Biofertilizers and soil amendment are keeping the soil environment rich in all kinds of macro and micronutrients via nitrogen fixation, phosphate, and potassium solubilization or mineralization, the release of plant growth regulating substances, production of antibiotics, and biodegradation of organic matter in the soil (Sinha et al., 2014). Biofertilizers soil amendment, generally 60% to 90% of the total applied fertilizer is lost and the remaining 10% - 40% is taken up by plants (Vessey et al., 2003). Hence biofertilizers soil amendment can be an essential component of integrated nutrient management systems to sustain agricultural productivity and a healthy environment (Adesemoye et al., 2009). In organic systems soil management involves, the use of tilled cover crops, animal manures, composts, and the application

of organic fertilizers can increase soil-organic matter a steady release of nutrients to the crops as the organic matter breaks down (Barakat et al., 2012; Diacono et al., 2015; Alcantara et al., 2016).

Soil amendments are the practices used to improve mine soil quality in terms of its structure and biochemical function (Sayed et al., 2014; Sangphanta et al, 2015; Timingoon et al., 2015). The management of adverse soil conditions on post-mining land has a negative impact on the physical and biochemical properties of the soil (Liu et al., 2016; Kunkeaw at el., 2010; Wongcharoen, 2013). In 2018, Tahir et al. reported that the combined application of bio-organic phosphate and phosphorus solubilizing bacteria as *Bacillus* sp. strain MWT 14 improved the performance of bread wheat with low fertilizer input under an arid climate. The research reported that the soil fertility, growth, and productivity of bread wheat cultivars were significantly increased with the application of bio-organic phosphate (BOP) or phosphate solubilizing bacteria (PSB) the effects being more pronounced with the combined application of both. Indeed, the application of BOP and PSB enhanced the soil organic matter (SOM) soil phosphorous which worked as a soil conditioner thus improving the growth of both bread wheat cultivars which was visible through improvement in the tree. Also in 2006, Finckh et al. found that the *Bacillus amyloliquefaciens* strain LH23 and *Bacillus subtilis* LH36 were derived bio-organic fertilizers (BIO23 and BIO36) as potential biocontrol agents against potato bacterial wilt. The BIO23 and BIO36 could be decreased the incidence of bacterial wilt disease and increase potato yields. In pot experiments, the disease incidence of BIO23 and BIO36 was 8.9% and 11.1% respectively, lower than the negative control of the disease severity of 57.7%. This is also consistent with Ha et al. (2009) report that the soil amendment mixed with shrimp and crab shell powder at 0.5-1% (w/w) effectively reduced the population of *F. oxysporum* f. sp. *tracheiphilum* and increased populations of antagonistic rhizosphere microorganisms including fungi, bacteria, and actinomycetes; thereby reduced severity of *Fusarium* wilt of asparagus bean and promoted growth and nodule formation of this crop.

Regarding, organic agriculture is rapidly developing today, which developed in at least 170 countries to produce organic food commercially. The world's organic

producers are in Asia (36%), followed by Africa (29%) and Europe (17%). The objectives of environmental, social, and economic sustainability are the basics of organic farming. The key characteristics are protecting the long-term fertility of soils by maintaining organic matter levels, fostering soil biological activity, intervening with careful mechanical involvement, having nitrogen self-sufficiency through the use of legumes and biological nitrogen fixation, and effectively recycling organic materials including crop residues and livestock, weed, controlling diseases and pest based primarily on crop rotations, natural predators, diversity, organic manuring, and resistant varieties.

In 2006, field experiments by Amanullah et al. were carried out to determine the impact of intercropping and organic manures on nutrient uptake and soil fertility in cassava intercropping systems. The study found that single cassava had the highest nutritional uptake, followed by cassava intercropped with cowpea. Composted poultry manure exhibited the highest absorption among the organic manures. Soil nutrient depletion was lower in cassava grown alone, followed by cassava intercropped with cowpea. In 2017, Chen et al. The impact of monoculture peanut and cassava/peanut intercropping on physical and chemical qualities in peanut rhizosphere soil was studied using biochar and straw mulching. The results showed that biochar application enhanced organic matter in the rhizosphere soil of the plots by 29.53% (monoculture peanut) and 32.97% (cassava/peanut intercropping) compared to the control (P0.05). Because root exudates are more abundant under intercropping, and these secretions can activate soil nutrients, cassava/peanut intercropping can boost accessible physical and chemical characteristics in peanut rhizosphere soil. Furthermore, disease severity on farms can be decreased by destroying trash that carries spores into the next planting season. Farm implements like cutlasses, hoes, and plows used on fields with a history of root rot must be cleaned immediately after use before being used on another farm. This prevents disease transmission from farm to farm (Moses et al., 2007). Continuous cassava planting on the same plot of land leads to the pathogen population growing and becoming more severe year after year. Rotate cassava with grains or cereals on a three-year cycle to help decrease the impacts of root rot disease (Lozano, 1992).

2.3.4 Biological control

Biological control or biocontrol is a method of controlling pests such as insects, weeds, and plant diseases using other antagonistic organisms or microorganisms such as fungi, bacteria, nematodes, or predators. The severity of losses caused by the CRTRD disease mandates the development of eco-friendly and cost-effective methods. Bacterial biocontrol agents were studied on root rot disease of cassava such as *Bacillus* sp.; *B. lentus*, *B. cereus*, *B. circulans* and *P. fluorescens* Vasudevan; Velusamy and Gnanamanickam, 2003), *B. polymyxa, B. subtilus, Burkholderia glumae, Pseudomonas aeruginosa, Pseudomonas fluorescens, Stenotrophomonas maltophilia, Streptomyces mutabilis* (Janse, 2005), *Trichoderma virens* (Mukherjee et al., 2012). The bacterial population size introduced by bacterial or fungal treatments and sustained throughout the crucial phases of cassava growth appears to be the secret to the consistently great performance of helpful bacteria in suppressing complicated diseases.Hridya et al. (2012) reveal that the effect of *Trichoderma* spp. and *Pseudomonas fluorescens* (biocontrol agents) and *Azospirillum*, vesicular-arbuscular mycorrhizal fungi, and phosphorus-solubilizing bacteria (biofertilizers) on root rot, yield, harvest index and nutrient uptake of cassava at two NPK rates. According to Silva et al. (2016), the antagonist capacity of *T. aureoviride* URM 5158 against cassava root rot caused by *F. solani* showed 88.91% of the highest inhibition of pathogen growth. All isolates have shown chitinase activity, but *Trichoderma aureoviride* URM 5158 produced the highest amount of chitinase. *T. hamatum* URM 6656 and *T. aureoviride* URM 5158 were selected to be applied in *vivo*. The two *Trichoderma* strains reduced 64 and 60% of the disease severity in the shoot and 82 and 84% in the root, respectively. Cassava plants infected with *Trichoderma* have shown the highest peroxidase and ascorbate peroxidase production, because they showed competitive antagonist capability in vitro, highest chitinase production, and reduced the severity of cassava root rot in shoot and root of cassava plants. The application of the selected antagonists led to maximum enzyme activities of the ROSs group in cassava plants. *Bacillus* spp. can control fungal pathogens by competition, direct antibiosis, and induced resistance. In the rhizosphere, competition takes place for space at the root surface and nutrients, noticeably those released as root exudates.

The popular antagonistic bacteria is *Bacillus*, a gram-positive aerobic organism that can resist environmental stress by forming endospores (Prathuangwong and Buensanteai, 2007; Buensanteai et al., 2009; Kumar et al., 2011; Buensanteai et al., 2012; Saengchan et al., 2015). *Lactobacillus* and *Paenibacillus* are known to stimulate plant growth and induced plant defense mechanisms. The beneficial bacteria, *Bacillus* spp. are widely used as commercial bacteria to control plant pathogens and enhance plant growth promotion. Emmert and Handelsman (1999) highlighted that the endospore-forming character of *Bacillus* sp. is necessary for a potential biocontrol inoculant. This is because the spore can endure heat and desiccant for ensuring the formulation are stable over time. These 3 biocontrol genus is considered non-rhizosphere competent, unlike *Pseudomonas*; but given that rhizospheric competency is strain-dependent, some strains of *Bacillus* may be rhizosphere bacteria (Buensanteai et al., 2009; Kumar et al., 2011; Palaniyandi et. al., 2011; Saengchan et al., 2015; Wongchalee et al., 2015).

Nowadays, *Bacillus* spp. are globally dispersed bacteria producing numerous bioactive compounds in an industrial scale as the bio-pesticide products with a broad spectrum of activities towards pathogens or inducing host systemic resistance (Wei *et al*., 2016). *Bacillus* spp. are widely used commercially to control plant pathogens and to enhance plant growth (Adesemoye et al., 2009; Barakat et al., 2012; Diacono et al., 2015; Alcantara et al., 2016). In addition, *Bacillus* sp. could enhance directly affecting on plant growth through the production of plant growth regulators, which colonize roots and trigger plants to produce growth-promoting biomolecules (Pal and McSpadden Gardener, 2006; Buensanteai et al., 2008; Dicklow, 2013; Saengchan et al., 2015; Wongchalee et al., 2015). The plant growth promoting rhizobacterium (PGPR), *B. amyloliquefaciens* strain KPS46, can enhance growth in several economic crops such as soybean, vegetable soybean, corn, rice, Chinese kale, and cauliflower (Prathuangwong and Buensanteai, 2007; Buensanteai et al., 2009; Buensanteai et al., 2012; Saengchan et al., 2015).

Bacillus sp. strains KPS46, D604, CaSUT007, and CaSUT008 promote plant development in part by excreting phytohormones such as auxin and indole-3-acetic acid (IAA), lipopeptides, and extracellular proteins (Buensanteai et al., 2009;

Saengchan et al., 2015; Wongchalee et al., 2015). Similar to endogenous plant growth regulators, bacterial production of phytohormones promotes the earliest phases of lateral and adventitious root development and elongation (Buensanteai et al., 2009; Saengchan et al., 2015; Wongchalee et al., 2015). Wongchalee et al. reported in 2015 that *Bacillus* sp. D604 was used to control anthracnose on chili spur pepper seeds using a normal blotter plate. *Bacillus* sp. strain D604 significantly reduced the severity of anthracnose on seeds by 41.90% when compared to the control. Furthermore, Melnicket al. (2008) *B. cereus* isolates BT8 and BP24 were found to be largely epiphytic, with endophytic populations accounting for 5-15% of total foliar bacteria, resulting in considerable reductions in disease severity. Meng et al. (2015) claimed that dry flowable formulations of *B. subtilis* strain T429 at 50 and 75 g/667 m2 concentrations would be as effective as a commercial fungicide in controlling rice blast, with control efficiencies of up to 77.6% and 78.5%, respectively. There was no significant difference in disease control performance between the formulations and the chemical pesticide tricyclazole (79.5%), and it had a long shelf life and high viability after 12 months of storage at room temperature. Shternshis et al. (2016) reported on the efficacy of two microbial formulations based on *Bacillus subtilis* Cohn in 2016. and *Pseudomonas fluorescens* Mig. on the fungus Didymella applanata (Niessl.) Sacc., the causative agent of red raspberry spur blight (*Rubus idaeus* L.). The findings demonstrated that antagonistic activity against *D. applanata* varied according to red raspberry cultivar and meteorological circumstances. Furthermore, *B. subtilis* outperformed *P. fluorescens* in biocontrol of raspberry spur blight. *Bacillus* sp. has been studied for its biological activity in the management of cassava root disease. (Becker et al. 1994; Buensanteai et al., 2009; Saengchan et al., 2015; Wongchalee et al., 2015). Although *B. amyloliquefaciens* has been demonstrated to selectively increase iron buildup in cassava (Freitas et al. 2015). Plant development, on the other hand, is heavily controlled by the nutrients present in the soil. Plants receive phosphorus (P) and nitrogen (N) from the soil via root transporters, but the accessible forms of P and N in rhizospheres are limited (De-Willigen, 1986; Robinson, 2001; Bidondo et al., 2012). *Bacillus* spp. have a favorable effect because they convert the complicated form of vital nutrients such as P and N to a simple form that plant roots

can utilize during uptake (Kang et al., 2015; Kuan et al., 2016). Phosphate is involved in the metabolism of nucleic acids, phospholipids, and adenosine triphosphate (ATP) in plant cells, among other metabolic pathways (Theodorou and Plaxton, 1993). *Bacillus* sp. secretion of phosphatases and organic acids acidifies the surrounding environment, facilitating the conversion of inorganic phosphate to free phosphate (Kang et al., 2014). Furthermore, N is a necessary component of proteins, nucleic acids, and other organic molecules in plants, and the available form of N in soil is restricted, slowing plant growth in natural settings (Barker et al., 1974; Willigen, 1986). Some *Bacillus* species produce ammonia from nitrogenous organic materials (Hayat et al., 2010).Ding et al. (2005) The *Bacillus* sp. has the *nifH* gene and generates nitrogenase (EC 1.18.6.1), which can fix atmospheric N2 and supply it to plants to improve plant growth and yield by delaying senescence (Kuan et al., 2016). *Bacillus* species have high potential as microbial agents for the biocontrol of *Fusarium* cf. *incarnatum*-caused ginseng root rot. When compared to the untreated control, root dipping treatment with *Bacillus subtilis* formulations resulted in a significant increase in root length with *B. subtilis* (33 cm) and chlorothalonil (28.5 cm) (15 cm). Root dip treatment improved growth promotion while seed application improved disease control. Soil and seed application approaches reduced disease incitation by 66% and 84%, respectively (Narasimhan).

2.3.5 Integrated management

Disease management that is integrated. The adoption of varietal resistance and/or cultural measures is part of the integrated management of root rots. Cultural customs. The following are the best cultural practices for integrated root rot management: Choosing a suitable, well-drained, and somewhat deep soil. Planting should be done on ridges if the ground is flat and the soil is clayey. If the incidence of rot rises to 3%, the cassava crop should be alternated with grasses at least once a year. Eradicating unhealthy plants by removing and burning infected roots from the field. Choosing healthy plants to obtain pure seed. Stakes should be treated with metalaxyl at 0.3 g/L a.i. if the farming area is contaminated. Treating stakes in hot water at 49 degrees Celsius for 49 minutes is an alternative to chemical treatment. (Álvarez et al. 2003) The use and application of biological control agents, such as

Bacillus spp., helps to prevent the negative effects of pathogen attack on crops, making them an appealing option for sustainable agriculture due to their stimulating effects on plant growth, biomass production, and the potential to increase plant production. As proof of lower disease incidence and severity, this chapter mentions clear and efficient biocontrol of plant pathogenic fungi by *Bacillus* strains. As a result, it is proposed that *B. subtilis* be introduced into integrated disease management, where these strains might be employed as both a biocontrol agent and a biofertilizer (Hernández F.D. Castillo et al., 2014).

2.2.6 Induced resistance

Based on the induced resistance principle, resistance elicitors have been widely evaluated to control plant diseases (Buensanteai et al., 2009; 2010). Induced resistance is a component of the plant's natural defensive mechanism, which provides long-term protection against a wide range of plant diseases (Heil, 2002; Heil and Silva Bueno, 2007; Mandal et al., 2009; Buensanteai et al., 2010; Graham and Myers, 2011). Induced systemic resistance (ISR) and systemic acquired resistance (SAR) are two essential plant defense mechanisms (SAR). Salicylic acid signaling and systemic expression of pathogenesis-related protein genes are also required for SAR (Sticher et al., 1997; Hammerschmidt, 1999). Certain strains of plant growth promoting rhizobacteria (PGPR) induce ISR (Van Loon et al., 1998). Unlike SAR, ISR is not associated with local necrotic lesion formation. ISR also differs in that it depends on the perception of ethylene and jasmonic acid, and is not associated with the expression of PR genes.

CHAPTER III
MATERIALS AND METHODS

3.1 CRTRD causal agent and culture conditions

L. theobromae – a causal agent of CRTRD was obtained from stock culture stored in nutrient broth with 10% glycerol at - 80°C at Plant Molecular Biology Laboratory, Suranaree University of Technology, Thailand. The fungi were transferred from potato dextrose broth to potato dextrose agar (PDA) (potato 200 g/L, dextrose 15 g/L, agar 15 g/L) plate, which was incubated at 28 ± 2°C for 7 days and was used in this the research (slightly modified from Duchanee, 2015; Sangpueak et al., 2017).

3.2 Pathogenicity tests of CRTRD causal agents on cassava

3.2.1 Cassava stem inoculation

The pathogenicity of the *L. theobromae* was evaluated on stems of cassava cv. Pirun 2. The experiment was carried out in CRD with 4 replications. The four cassava stalks with 10-cm-long stem portions were cut from healthy cassava plants, then surface-disinfected in 1% sodium hypochlorite (NaOCl) solution for 3 minutes, rinsed twice with sterile distilled water. For *L. theobromae* pathogen inoculation, one representative virulent isolate was grown in a petri dish with PDA for 7 days at 25 °C. The healthy cassava bark was peeled off at the collar region and then replaced with 6 mm-diameter discs containing *L. theobromae* mycelia from the margins of the growing culture. A portion of moistened cotton was placed just below each disk and subsequently covered with parafilm. The negative control treatments consisted of cutting stems inoculated with sterile PDA plugs. These inoculated stems were kept moist by sprinkling the moistened cotton with sterile distilled water every day. Evaluation for pathogen invasion was measured after one week of incubation by measuring the spread of the pathogen along the cassava stem. For the pathogenicity level, the length percentage of the cutting colonized by the pathogen was estimated

(slightly modified from Onyeka et al., 2005; Banito et al., 2010; Duchanee, 2015). From the symptomatic inoculated cassava plants, the fungi were re-isolated in PDA. The virulence of fungal isolate was evaluated based on its severity. The score is as follows: 1 = no symptoms, 2 = less than 25% of stem area was affected, 3 = 25-less than 50% of stem area was affected, 4 = 50-less than 75% of stem area was affected, and 5 = more than 75% of stem area was affected (Onyeka et al., 2008; Wokocha et al., 2010; Sompong et al., 2012; Duchanee, 2015).

3.2.2 Cassava tuber inoculation

L. theobromae was grown in a petri dish with PDA for 7 days at 25°C. Cassava roots of cv. Pirun 2 was washed in 1% NaOCl and rinsed three times with sterilized distilled water. Each cassava root of approximately 20 cm × 10 cm in size was wounded superficially with a scalpel on the inoculation site. Fungal culture discs 6 mm in diameter in size were obtained from the margins of the growing culture and then placed on the wounds of cassava root. The wounded cassava root with PDA disc served as the negative control. The four cassava roots were inoculated with each isolate and placed in plastic boxes containing a portion of moistened cotton wool and were maintained in a moist chamber at approximately 25 °C for one week (Ismail et al., 2012; Trakunyingcharoen et al., 2013, 2015; Li et al., 2015; Duchanee; 2015; Chen et al., 2016). The virulence of fungal isolate was evaluated based on its severity score. The scoring is as follows: 1 = no symptoms, 2 = less than 25% of root area was affected, 3 = 25-less than 50% of root area was affected, 4 = 50-less than 75% of root area was affected, and 5 = more than 75% of root area was affected (Onyeka et al., 2008; Wokocha et al., 2010; Sompong et al., 2012; Duchanee, 2015).

3.3 Effect of *Bacillus sp.* against *L. theobromae* under greenhouse conditions

3.3.1 Dual culture

The antagonistic effect of 14 strains of *Bacillus* sp. was investigated for capacity against *L. theobromae* (Huang and Hoes, 1976). The experiment was carried out in CRD with 4 replications. The 14 strains of *Bacillus* sp.(SUBIN1,SUBIN2,BS38-4,BS38-5,BS37-

6,BS111,N501,N501-1,D604,D604-1,CaSUT007,CaSUT007-1,CaSUT008 and CaSUT008-2,) were obtained from the stock culture of Plant Pathology and Biopesticide Laboratory, Suranaree University of Technology, Thailand. One 5-mm disc of an *L. theobromae* pure culture was placed at the center of a petri dish containing PDA. The tested *Bacillus* sp. was inoculated at the opposing corners. For the negative controls, distilled water was used instead of *Bacillus* sp. Chemical fungicide, PCNB (200 ug/ml) was used as a positive control. All Petri dishes were incubated at 28 °C, for 72 h. The growth diameter of the pathogen was measured and compared to negative and positive control. The % Inhibition of Radial Growth (PIRG) was calculated by the following formula:

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

Where R1 = Radial growth of fungus in control.

R2 = Radial growth of fungus in dual culture.

3.3.2 Screening of Bacillus sp. for resistance to high temperature.

The *Bacillus* sp 5 strains. The suspension of spores of the given strain, Characterized by the number of spores per 1 ml counted by plating on PCA, was diluted before experiment 1:10 using sterile water, subjected to temperatures of 37, 46 °C, and 153 °, respectively. 24 h Initial concentrations of spores in suspensions varied from 10^4 to $10^7 \cdot ml^{-1}$. After cooled, we determined the number of surviving spores using the Plate Count Agar (PCA) (Jantova et al.,2001).

3.3.3 Screening of 3 strains of *Bacillus sp.* ultraviolet (UV) resistance

The *Bacillus* sp 3 strains (10^7 spores/ml) were subjected to UV light from a mercury low-pressure lamp with a major emission line at 253.65 nm (NN 8/15, Heraeus, Berlin, Germany) at 1 h, 3 h, and 6 h. Following UV illumination at defined fluences, 100 ml of the aqueous solution was extracted for further investigation. After overnight growth on PCA medium at 37 C, survival was assessed using suitable dilutions in distilled water as colony forming ability (CFA). The surviving fraction was calculated as the quotient N/N0, where N = the number of colony formers in the irradiated sample and N0 the number of colony formers in the non-irradiated controls. Survival curves

were created by plotting the logarithm of N/N0 as a function of fluence. To calculate the curve parameters,the following relationship was used: $\ln N/N_0 = -IC \cdot F + n$ within N = colony formers after UV-irradiation; N0 = colony formers without UV-irradiation; IC = inactivation constant (m2/J); n = extrapolation number, i.e.the intercept with the ordinate of the extrapolated semi-log straight-line.

3.3.4 Assays for *Bacillus* sp. inhibit fungal hyphae

L. theobromae was prepared in spore suspension at the concentration of $1x10^6$ spore/ml. The 1 ml of fungal suspension was inoculated into 100 ml of potato dextrose broth (PDB) in a 250 mL conical flask. The *Bacillus* sp. strain CaSUT008-2 cell suspension was prepared from single colonies of *Bacillus* sp. strain CaSUT008-2 growth on NGB media for 24 hr. The culture of *Bacillus* sp. strain CaSUT008-2 was incubated on a rotary shaker at 120 rpm, 30°C, then adjust cell suspension to the concentration of 1×10^8 CFU/ml. After that, add *Bacillus* sp. strain CaSUT008-2 and *L. theobromae* suspension at the ratio of 2% 5%, and 10% into PDB media. Incubate the co-inoculation of *Bacillus* sp. strain CaSUT008-2 and *L. theobromae* at 30°c for four days in a rotary shaker at room temperature conditions. The mass reduction in shaking conditions was measured and compared with the control. The effective culture sample was analyzed under the ESEM (Kumar et al., 2016).

3.4 The efficacy of *Bacillus* sp. to control CRTRD under greenhouse conditions

The experiment was conducted in a randomized complete block design (RCBD) with seven treatments and three replications. The treatments *Bacillus* sp. was applied as a soil amendment (cassava residue, phosphate rock, chicken residue) containing 2% *Bacillus* sp. (Dapaah, Asafu-Agyei, et al. 2003). The cell suspension of *Bacillus* sp. was mixed with soil amendment 1 month before use. Then, the soil amendment was mixed with sandy loam soil at the ratio of 1:2 in the big bag size 2x2 m at the amount of 500 Kg/big bag (Duchanee, 2015). Cassava stalks cv. Pirun 2 was surface-disinfected with 1% NaOCl for 2 min, followed by washing with sterile distilled water three times, after that let dry for 5 min at room temperature. The cassava stalks were soaked with *Bacillus*

sp. strain CaSUT008-2 or sterile distilled water for 10 minutes before planting. After planting for 1 month, the soil was inoculated with a spore suspension of *L. theobromae* at the concentration of $1x10^6$ spores/ml. The disease severity score of cassava plants was collected at 1, 2, 3, and 8 months after being planted. The evaluation is based on its severity score from slightly modified by Onyeka et al. (2008), Wokocha et al. (2010), Sompong et al. (2012), Duchanee (2015). Disease severity (%) was calculated using the formula slightly modified by Le Thanh et al. (2017). The population of *Bacillus* sp. strain CaSUT008-2 was also checked at 1, 3and 6 months after planting using the serial dilution plate method (Martin, 1950; Gams et al., 1987). % of cassava germination, root length, shoot length, and lateral roots were recorded 1 month after planting compared with negative control as distilled water and positive control as chemical PCNB (Anwar et al., 2013).

3.5 The efficacy of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on control CRTRD under field conditions

The experiment was conducted in a Split-plot design with three replications. The experiment was conducted for 2 crops in 2 locations including the 1 location field at the Suranaree University of Technology and 1 location field at Soeng Sang District, Nakhon Ratchasima province. Each plot consisted of 5 cassava planting rows with the size of 10×1 m. The planting distance was 1 x 0.80 m and 2 varieties of peanuts including Khon Kaen 60-2 and Tainan 9 were planted between cassava rows with a spacing of 40 cm (Awang, Ismail, et al., 2015). Land preparation was done by plowing to a depth of 30 cm, followed by harrowing to create ridges at the heights of 30-60 cm, and then mixed with the soil amendment containing 1% *Bacillus* sp. strain CaSUT008-2 with the amount 200 kg/rai (Dapaah, Asafu-Agyei, et al. 2003; Naren et al. 2010).

T1: Tinan 9+*Bacillus* strain CaSUT008-2(CTB)

T2: Tinan 9(CT)

T3: Tinan 9+chemical (CTc)

T4: Khonkan 60-2+*Bacillus* strain CaSUT008-2(CKB)

T5: Khonkan 60-2(CK)

T6: Khonkan 60-2 +chemical (CKc)

T7: *Bacillus* strain CaSUT008-2(CB)

T8: Control (water)

T9: Chemical (PCNB concentration of 200 ug/ml) (Positive control)

Cassava stalks cv. Pirun 2 aged 8 months old with 15 cm in length was planted vertically in a space of 1×0.8 m. Before planting, cassava stalks cv. Pirun 2 was soaked with *Bacillus* sp. strain CaSUT008-2 at the concentration of 10^6cfu/ml (Ayoola and Makinde, 2007). A carbendazim chemical fungicide at the concentration of 200 ug/ml was used as a positive control. The application of *Bacillus* sp. strain CaSUT008-2 was foliar sprayed 2 times at 1 and 3 months after planting. The root rot disease was used as natural infection. The 2 varieties of peanuts were harvested at 90-105 days depending on the varieties (Sirichumpan, 2015). After the harvest, the peanut was performed biodegradable to use as a plant nutrient. The cassava plants were recorded for root rot severity at 3, 4, 6, and 8 months after planting (MAP) using the disease scores of Onyeka et al. (2008), Wokocha et al. (2010), Sompong et al. (2012), and Duchanee (2015). % of Disease severity (%) was calculated using the formula slightly modified from Le Thanh et al. (2017). Moreover, tuber root number, fresh tuber yields, and starch contents were collected at 8 MAP (Terry and Hahn, 2009; Subekti et al., 2013; Polthanee et al., 2014; Romkhambut, 2015). The *Bacillus* sp. strain CaSUT008- 2 population.. (Terry and Hahn, 2009; Subekti et al., 2013; Polthanee et al., 2014; Romkhambut, 2015).

3.5.1 Soil sampling and preparation for laboratory analysis

The soil samples were collected from 2 locations and labeled according to the site of the collection as SS for Sang Sang District and SUT for the Suranaree University of Technology. In the collected size, vertical samples were taken from 10 cm depths with a disinfected spatula which the spatula was applied perpendicular to the vertical surface of the profile. The sample was collected from the 10 points to distribute throughout the experiment plots, then combined into 1 sample for analysis. The samples were stored in a bag sterilized until they reached the laboratory (Dilek, 2002). All samples were prepared for serial dilution by adding 1 g of a soil sample to 9 ml of sterile distilled water (SDW) in a sterilized test tube and shaking well. The serial dilution of up to (10^{-3}) of 1 ml was poured into each petri dish for three replications. The positive control was 1 ml of

Bacillus CaSUT 008-2 at the concentration of 10^{-3} CFU/ml poured in each petri dish of NA+ PCNB+ ampicillin medium that NA supplement with 1ml/100ml of PCNB and 1ml/100ml of ampicillin for three replications. All samples were incubated at 25 °C for 24 hours. The large plates and the plates with less than 10 colonies should not be counted. The cultural characteristics of the colonies were attended to and described (Peper et al., 2006).

3.6 Data analysis

The data was analyzed and subjected to Analysis of Variance (ANOVA) using SPSS software, version 16. The significance of treatments was determined by the magnitude of the F value (P = 0.05). Treatment means were separated by Duncan's Multiple Range Test (DMRT).

CHAPTER IV

RESULTS

4.1 CRTRD causal agent and culture condition

L. theobromae causal agent of CRTRD was obtained from the stock culture of Plant Pathology & Biopesticide Laboratory, Suranaree University of Technology, Thailand, stored in Potato Dextrose Broth with 10% glycerol at - 80°C. The fungi pathogen was cultured by needle from their Potato Dextrose Brothonto potato dextrose agar (PDA) plate (potato 200 g/L, dextrose 15 g/L, agar 15 g/L) at 28 ± 2°C for 7 days and was used in this the research (slightly modified from Duchanee, 2015; Sangpueak et al., 2017).

4.2 Pathogenicity tests of CRTRD causal agents on cassava healthy plant

4.2.1 Cassava stem inoculation

The pathogenicity tests of CRTRD caused by *L. theobromae* 7 days after inoculation were shown in Figure 1. Figure 1(A) showed the cassava healthy plant and Figure 1(B) showed the rot symptoms of pathogenicity test in stem caused by *L. theobromae* at 7 days after inoculation. The fungi infection caused dry-rot lesion on the cassava stem, with scoring at 4 as 50 to 75% of the stem area was affected.

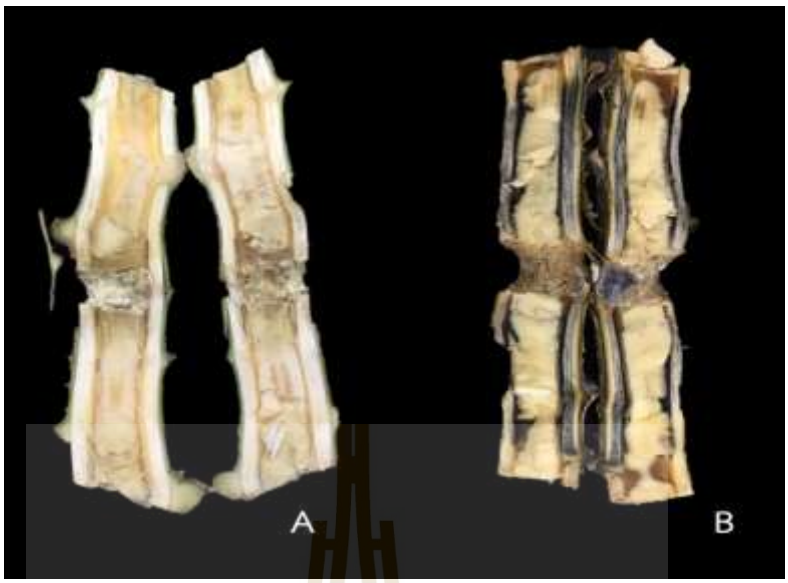

Figure 4.1 The symptoms of pathogenicity test in stem caused by *L. theobromae* at 7 days after inoculation.

4.2.2 Cassava root inoculation

The pathogenicity tests of CRTRD caused by *L. theobromae* 3 days after inoculation were shown in Figure 2. Figure 2(A) showed the cassava healthy root and Figure 2 (B) showed the rot symptom of pathogenicity test in root caused by *L. theobromae* at 3 days after inoculation. The fungi infection caused dry-rot lesion with a rotten smell on the cassava root tissue bark of trees Brown discoloration in the vicinity of the inoculated area when longitudinally dissecting Tissues were discovered in the trunk's core was destroyed, eventually turning brown with black with scoring at 4 as 50 to 75% of root area was affected.

Figure 4.2 The symptoms of pathogenicity test in root caused by *L. theobromae* at 3 days after inoculation.

4.3 Effect of *Bacillus* sp. as a biological control agent against *L.theobromae*

4.3.1 Effect of *Bacillus* sp. to control *L. theobromae in vitro*

The effect of *Bacillus* sp. on inhibition *L. theobromae* growth was evaluated by a dual culture test. The results of the anti-fungal activity showed that *Bacillus* sp. strain CaSUT008-2 can inhibit mycelial growth of *L. theobromae* at 5 days after putting fungal slice (DAPFS) (Table1). The percentage inhibition was approximately 53.08%. Moreover, *Bacillus* sp. strain CaSUT007 and D604 had significantly inhibited mycelial growth by 50.30% and 48.37% compared with negative control, respectively. However, the fungicide PCNB (Pentachloronitrobenzene) inhibited *L. theobromae* growth at approximately 1.67%, non-significantly compared with the negative control at 0.00 % (Table 1; Figure 3). Choose 5 strains were used for further experimentation.

Table 4.1 Effect of *Bacillus* sp. as a biological control agent against *L. theobromae*

Treatments[1/]	Mean of mycelium radius[1/] (Cm)	PIRG[1/] (%)
SUBIN1	1.89±0.47^{cd}	39.13^{b}
SUBIN2	1.89±0.12^{cd}	39.91^{b}
BS38-4	3.02±0.64^{a}	3.61^{f}
BS38-5	2.79±0.21^{c}	19.18^{d}
BS37-6	2.33±0.17^{c}	21.01^{d}
BS111	3.02±0.40^{a}	3.63^{f}
N501	1.70±0.40^{cd}	45.32^{ab}
N501-1	3.01±0.51^{a}	18.33^{d}
D604	1.67±0.12^{ab}	48.37^{ab}
D604-1	2.08±0.32^{c}	30.49^{c}
CaSUT007	1.64±0.15^{d}	50.30^{a}
CaSUT007-1	1.68±0.17^{d}	47.44^{ab}
CaSUT008	1.86±0.21^{cd}	40.13^{b}
CaSUT008-2	1.61±0.25^{d}	53.08^{a}
Bacillus commercial	3.02±0.23^{a}	3.14^{d}
Chemical(PCNB)	3.06±0.04^{a}	1.67^{fe}
Control	3.08±0.01^{a}	0.00^{e}
F-test	*	**
CV (%)	14.10	18.38

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

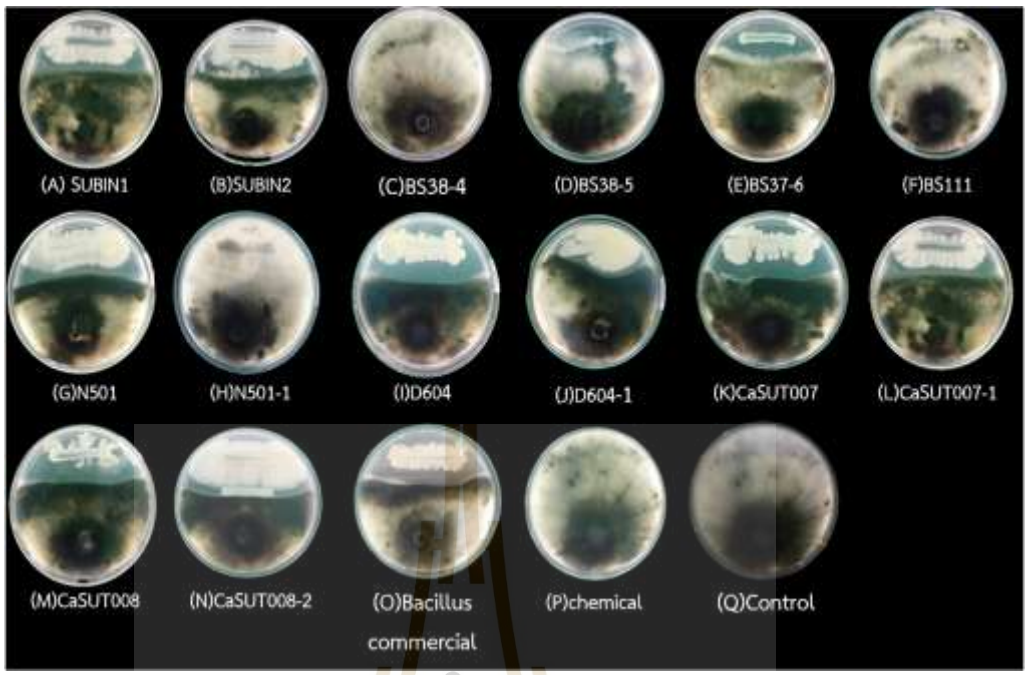

Figure 4.3 Screening of *Bacillus* sp. isolates as a biological control agent against *L. theobromae*

4.3.2 Screening of *Bacillus* sp. for resistance to high temperature

The screening of *Bacillus* sp. for resistance to high temperature was shown in Table 2 and Figure 4. The *Bacillus* sp. strain CaSUT 008-2, D604 and CaSUT007-1 can grow up to 53 °C with survival density 5.26×10^8, 3.40×10^8, 3.33×10^8 CFU/ml, respectively. Choose 3 strains were used for further experimentation.

Table 4.2 Screening of *Bacillus* sp. 5 strains for high-temperature resistance test

Treatment	37°C CFU/ml	46°C CFU/ml	53°C CFU/ml
N501	$10.26x10^{8}$	$2.13x10^{8}$	< 20000
D604	$18.49x10^{8}$	$7.56x10^{8}$	$3.33x10^{8}$
CaSUT007	$9.53x10^{8}$	$6.56x10^{8}$	< 20000
CaSUT007-1	$9.86x10^{8}$	$8.26x10^{8}$	$3.40x10^{8}$
CaSUT008-2	$19.16x10^{8}$	$10.10x10^{8}$	$5.26x10^{8}$
F-test	**	**	**
CV (%)	6.10	9.38	10.12

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

4.3.3 Screening of 3 strains of *Bacillus* sp. for ultraviolet (UV) resistance

The screening of 3 strains of *Bacillus* sp. for ultraviolet (UV) and ultraviolet (UV) resistance were shown in Table 3 and Figure 5. The table 3, it shows that the *Bacillus* sp. strain CaSUT 008-2 can grow up to UV exposure at 6 hours with a survival density 2.40 $x10^{8}$ CFU/ml,. *Bacillus* sp. strain CaSUT 008-2 and *Bacillus* sp. CaSUT007-1 can grow up to UV exposure at 3 hours with a survival density of 5.06x108 and $3.61x10^{8}$ CFU/ml, respectively. At UV exposure at 3 hours *Bacillus* sp. strain CaSUT 008-2, *Bacillus* sp. CaSUT007-1 and Bacillus sp. D604 can grow up with survival density $9.10x10^{8}$, $8.20x10^{8}$, $3.60x10^{8}$ CFU/ml, respectively. Choose 1 strain was used for further experimentation.

Table 4.3 Screening of *Bacillus* sp. 3 strains ultraviolet (UV) resistance.

Treatment	1 h CFU/ml	3 h CFU/ml	6 h CFU/ml
CaSUT008-2	9.10×10^{8} a	5.06×10^{8} a	2.40×10^{8} a
CaSUT007-1	8.20×10^{8} b	3.61×10^{8} b	< 20000
D604	3.60×10^{8} c	< 20000	< 20000
F-test	*	**	*
CV (%)	12.15	8.92	10.19

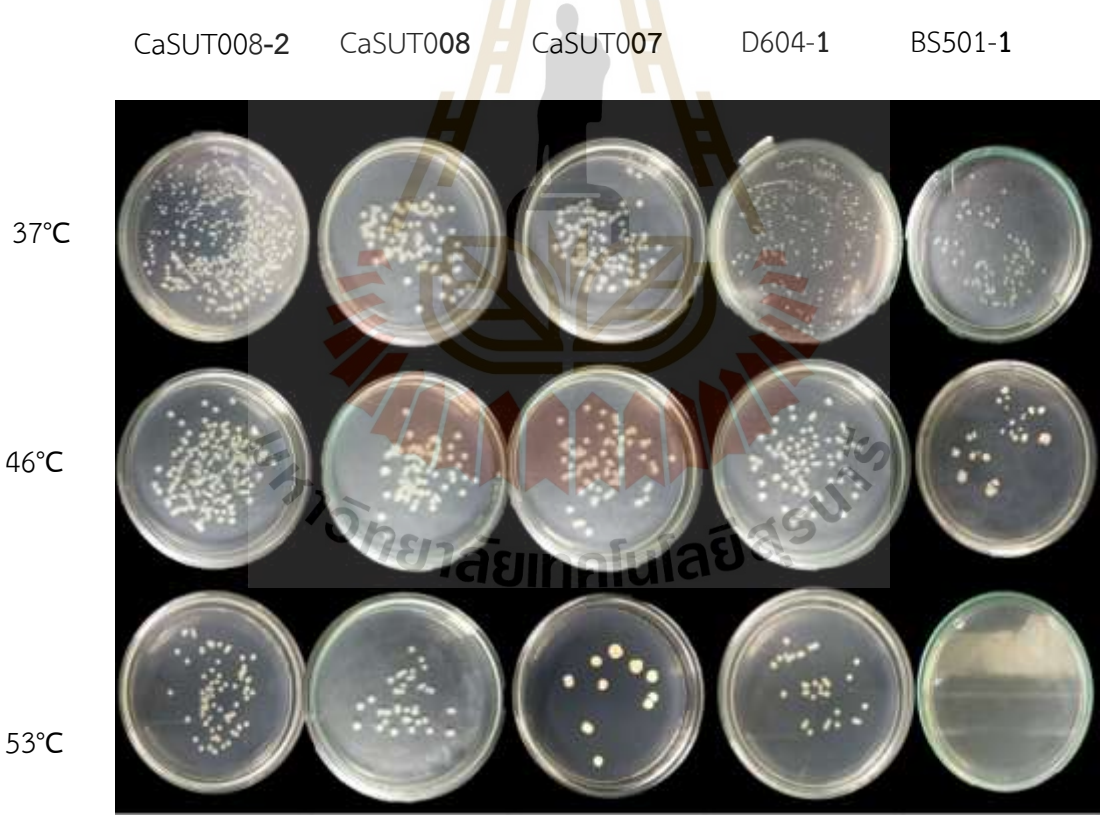

Figure 4.4 Screening of 5 strains of *B*acillus sp. for high-temperature resistance test at 37 °C, 45 °C, and 53 °C

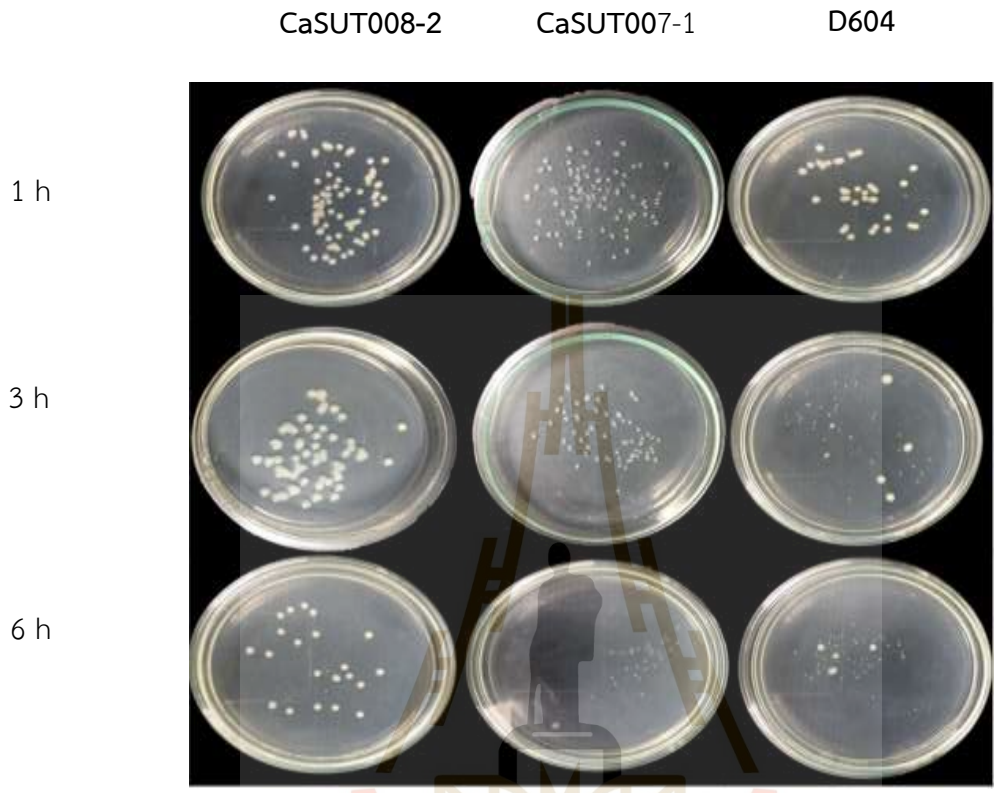

Figure 4.5 Screening of 3 strains of *Bacillus sp.* ultraviolet (UV) resistance

4.3.4 Assays for *Bacillus* sp. inhibit fungal hyphae

The ratio of *Bacillus* sp. strain CaSUT008-2 and *L. theobromae* at 2%, 5%, and 10% and keep in different conditions 1) 30°c for four days in a rotary shaker 2) Room temperature for four days don't shake. The result show that the fungal growth inhibited 30°c for four days in a rotary shaker show that at 10, 5,2 % of *Bacillus* sp. strain CaSUT008-2 can inhibit fungal hyphae at 70.75 %,71.46%,73.46 % went compared with control which is non-statistically different Moreover, the Room temperature condition show at 10,5,2 % of *Bacillus* sp. strain CaSUT008-2 can inhibit fungal hyphae at 71.40 %,71.50 %,72.34% went compared with control which are non-statistically different. The best method is to grow the *Bacillus* sp. strain CaSUT008-2 at room

temperature. The growth of fungal fibers was not statistically different at a concentration of 2% because it was the least expensive process. (Table 4.4).

Table 4.4 Antifungal activity of *Bacillus* CaSUT008-2 against *L. theobromae*

Treatments[2/]	Disease reduction (%)[1/]	
	30°C in Rotary shaker	Room temperature
2 %	70.75	71.40
5 %	71.46	71.50
10%	73.46	72.34
Control (PDB)	0.00	0.00
F-Test	ns	ns
%CV	1.26	13.31

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

[2]/Concentration of *L. theobromae*: *Bacillus* sp. CaSUT 008-2)

4.3.5 ESEM observation of *L. theobromae* treated by the *Bacillus* sp. CaSUT008-2

Bacillus sp. strain CaSUT008-2 at concentrations of 10^8 CFU/mL caused the pathogen hyphae to be wrinkled, distorted, and shrunken. The hyphae had bacterial cells adhering on some portions to varying degrees, which increased in number in the treatments with an increased ratio of CaSUT008-2. Conversely, in the untreated control pathogen hyphae looked intact with a smooth surface, sometimes showing a contour of the septum with no bacterial cells present (Figure 4.6).

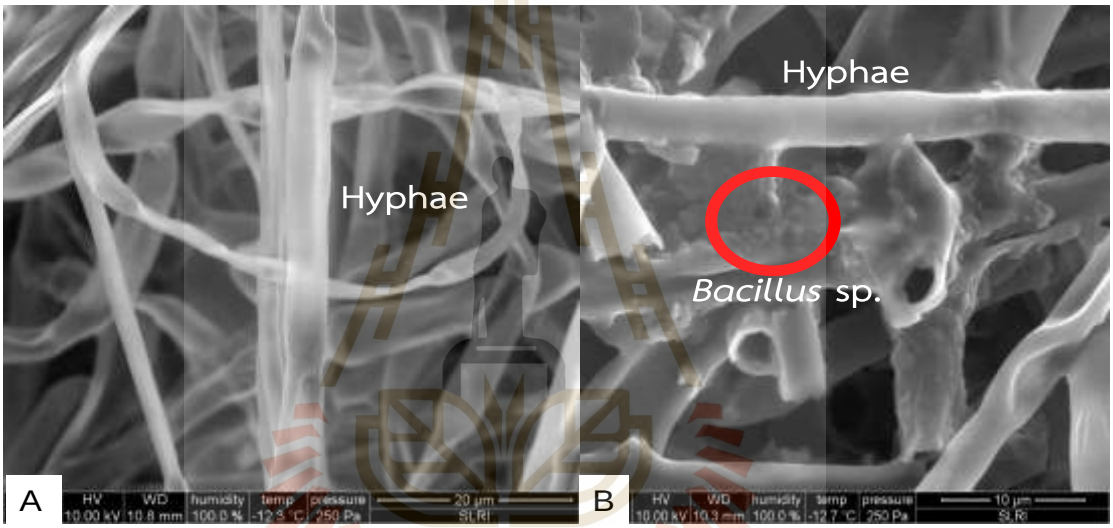

Figure 4.6 Scanning electron micrographs of *L. theobromae* with no bacterial treatment (A), and treated with the bacterial isolate *Bacillus* CaSUT 008-2 strain CaSUT008-2 at inoculum concentrations of 10^8 CFU/mL(B), showing wrinkled, distorted, and shrunken pathogen hyphae adhered with bacterial cells, compared to intact hyphae with smooth surface showing a contour of the septum in the untreated control.

4.4 The efficacy of *Bacillus* sp. to control CRTRD under greenhouse conditions

The cassava plants were pretreated with *Bacillus* sp. was applied as a soil amendment (cassava residue, phosphate rock, chicken residue). Then, the cassava plants were inoculated with CRTRD for 1 month after planting. Foliar spraying at 30, 45, and 65 days after the plant. And record data 90 days after plant. The results showed that *Bacillus* sp. strain 008-2 was the ability to stimulate the growth of cassava with a height of 35.20 cm, number of leaves of 11.25, and leaf length of 8.63 cm, while compared with sterile distilled water these results indicated that *Bacillus* sp. strain 008-2 have high potential stimulate growth on cassava. The *Bacillus* sp. CaSUT008-2 was applied as a soil amendment and foliar spraying could reduce CRTRD by 74.13% at 60 days after inoculation high than fungicide treatment (PCNB) at 51.13%. The efficacy of Bacillus sp. CaSUT008-2 was significantly higher. (Table 4.5)

Table 4.5 Growth rate of cassava cv. Pirun 2 at three month after planting.

Treatment	Height[1/] (cm)	No. leaf[1/]	Leaf length[1/] (cm)	No. root[1/]	Root length[1/] (cm)	Disease reduction (%)
CaSUT008-2	35.20[a]	11.25 [a]	8.63[a]	65.5	10.75	74.13[a]
CaSUT007-1	33.75[a]	8.00 [c]	6.64bc	69.0	12.75	68.12[ab]
CaSUT007	21.20 [f]	2.00d	3.24 [d]	67.3	11.07	68.89[ab]
D604	29.52 [bc]	10.25ab	8.04 [ab]	64.3	12.54	53.12[c]
BSN501	26.50 [e]	8.25b	7.33 [abc]	70.3	12.58	49.55[cd]
Water	21.85 [f]	8.50b	5.90 [c]	56.5	12.14	69.12[ab]
PCNB	31.10 [ab]	9.00 [ab]	3.97 [d]	58.5	12.69	51.13[c]
lamina	30.30[b]	8.00 [b]	7.21 [abc]	62.0	13.46	72.56[a]
F-test	**	**	**	ns	ns	**
CV%	21.56%	14.77%	4.33%	15.60%	15.75%	18.23

[1/] Mean in the column followed by the same letter are not significantly different according to the LSD test (α= 0)

4.5 The efficacy of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping to control CRTRD and soil properties under field conditions

The effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping was evaluated for enhancing cassava growth and controlling CRTRD under field conditions, at Suranaree University of Technology and Soeng Sang district in 2020 (first year) and 2021 (second year). The results of the growth experiments were collected at 3 months, 6 months, and 8 months after planting. The combination of Tinan 9 and *Bacillus* strain CaSUT008-2 treatment has a high growth trend than control methods at 6 and 8 months after planting with plant heights was 178.43 and 264.38 cm, respectively. In the second year, the combination of Tinan 9 and *Bacillus* sp. strain CaSUT008-2 treatment still was a high growth trend than the control treatment at 6 and 8 months after planting with plant heights was 178.36 and 254.36 cm. respectively. (Table 4.6) At Soeng Sang district in the first year, the combination Tinan 9 and *Bacillus* sp. strain CaSUT008-2 treatment was a high growth trend than control methods at 6 and 8 months after planting with plant heights was 162.39 and 270.88 cm, respectively. In the second year, the combination of Tinan 9 and *Bacillus* sp. strain CaSUT008-2 treatment still was a high growth trend than the control methods at 6 and 8 months after planting with plant heights was 164.349 and 269.78 cm, respectively. (Table 4.7) The results from Table 4.8 CRTRD disease severity (%) at Suranaree University of Technology in 2020-2021 show that a combination of Tinan 9 and Bacillus strain CaSUT008-2 treatment has could reduce. It was found that the disease severity (%) in 2020 at 43.48% and in 2021 at 42.68 % which less than Control (water) in 2020 at 69.58% and in 2021 at 68.14% and Chemical (Positive control) in 2020 at 57.82% and in 2021 at 56.89%, respectively. The results from Table 4.9 at Soeng Sang district in 2020-2021 show that the combination of Tinan 9 and *Bacillus* strain CaSUT008-2 treatment has could reduce. It was found that the disease severity (%) in 2020 at 51.66% and in 2021 at 49.63% which was less than

Control (water) in 2020 at 77.32% and in 2021 at 75.66% and Chemical (Positive control) in 2020 at 67.72% and in 2021 at 53.48% , respectively. The results from table 4.10 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on yield in cassava under field conditions at Suranaree University of Technology in 2020-2021 in 2020 Starch content (%) in treatment casaava+Tinan9 show the hight at 24.12% the second was the combination of Tinan 9 and *Bacillus* strain CaSUT008-2 23.43% and the third wasthe treatment of cassava + *Bacillus* strain CaSUT008-2 at 22.83%. For the second year, 2021 the treatment combination of Tinan 9 and *Bacillus* strain CaSUT008-2 treatment shows the high of starch content (%) at 23.13% the second was treatment casaava+Tinan9 23.00% and the third is the treatment of Cassava+*Bacillus* strain CaSUT008-2 at 22.84%. In 2020 Number of tubers per plant (tubers/plant) in treatment Cassava+ Khonkan 60-2 shows the hight of several tubers per plant at 35.67 tuber/plant the second was the combination of Tinan 9 and *Bacillus* strain CaSUT008-2 32.56% and the third was Control at 32.67%. For the second year 2021 Number of tubers per plant (tubers/plant) in the treatment combination of Tinan 9 and *Bacillus* strain CaSUT008-2 treatment show the hight of Number of tubers per plant (tubers/plant) at 36.67% the second was treatment casaava+*Bacillus* strain CaSUT008-2 36.23% and the third is the treatment of Cassava+ Khonkan 60-2 at 32.40%. In 2020 Tuber Fresh Weight per plant (kg/plant) in treatment combination of Tinan 9 and *Bacillus* strain CaSUT008-2 show the hight of Tuber Fresh Weight per plant (kg/plant) at 3.40 the second was Cassava+ Khonkan 60-2 3.10 kg/plant and the third was Control at 3.01 kg/plant. For the second year 2021 Tuber Fresh Weight per plant (kg/plant) in treatment combination of Tinan 9 and *Bacillus* strain CaSUT008-2 treatment show the hight of Tuber Fresh Weight per plant (kg/plant) at 3.20% the second was treatment Cassava+ Khonkan 60-2 3.09% and the third is the treatment of Cassava+ Khonkan 60-2 at 3.04%. The results from Table 4.12 and 4.13 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on soil properties before and after cropping at Suranaree University of Technology and Soeng Sang district

the show that combination of Tinan 9 and *Bacillus* strain CaSUT008-2 treatment has could increase of pH Organic matter (%) Total nitrogen (ppm) Total phosphorus (ppm) Total potassium (ppm) Total Calcium (ppm) went compare with control and PCNB (positive control) in 2020 and 2021

Table 4.6 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on cassava growth rate under field conditions at Suranaree University of Technology in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Plant height (cm.)			Plant height (cm.)		
	3 months	6 months	8 months	3 months	6 months	8 months
T1:CTB	96.31±2.10	178.43±9.45^{a}	264.38±9.85^{a}	97.16±3.49	178.36±9.65^{a}	254.36±9.65^{a}
T2:CT	95.25±2.12	119.38±1.21^{c}	230.81± 4.42^{b}	95.45±2.18	122.36±1.41^{c}	225.8± 4.32^{b}
T3:CTc	90.11±4.35	147.43±2.13^{bc}	194.46±4.17^{cd}	93.25±4.34	142.43±2.13^{bc}	184.46±4.77^{c}
T4:CKB	96.32±2.20	146.36±3.57^{bc}	269.83±4.46^{a}	96.32±2.20	146.96±3.57^{bc}	253.83±4.36^{a}
T5:CK	89.36±7.65	110.84±1.07^{e}	200.42±5.04^{cd}	89.36±7.65	109.4±1.07^{e}	199.4±5.06^{cd}
T6:CKc	89.28± 5.32	148.8± 4.32^{bc}	206.56±6.80^{c}	89.28± 5.32	144.8± 4.72^{bc}	202.56±6.80^{c}
T7:CB	92.46±4.07	164.46±4.57^{b}	235.36±4.45^{b}	92.46±4.07	164.46±4.57^{b}	233.36±9.45^{b}
T8:Control (water)	90.31±4.45	130.8± 4.32^{c}	195.84± 4.12^{cd}	90.31±4.45	129.8± 4.32^{c}	194.8± 4.32^{cd}
T9:Chemical (Positive control)	89.14±3.72	125.4±2.83^{c}	162.46±4.37^{e}	89.14±3.72	122.4±2.83^{c}	164.46±4.17^{e}

Table 4.6 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on cassava growth rate under field conditions at Suranaree University of Technology in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Plant height (cm.)			Plant height (cm.)		
	3 months	6 months	8 months	3 months	6 months	8 months
F-Test	ns	**	*	ns	**	*
CV%	19.33	20.77	18.89	19.33	20.77	18.89

[1/]T1:Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT) T3:Cassava+Tinan 9+chemical(CTc) ,T4:Cassava+Khonkan 60-2+*Bacillus* sp. strain CaSUT008-2(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+ Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

/ Mean in the column followed by the same letter are not significantly different according to the LSD test (α= 0.05).

Table 4.7 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on cassava growth rate under field conditions at Soeng Sang district in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Plant height (cm.)			Plant height (cm.)		
	3 months	6 months	8 months	3 months	6 months	8 months
T1:CTB	110.86±3.35	162.39±2.33^{a}	270.88±9.65^{a}	109.96±3.49	164.49±2.13^{a}	269.78±9.65^{a}
T2:CT	109.72±2.14	122.43±1.31^{d}	195.47± 4.22^{e}	105.25±2.18	124.45±1.01^{c}	194.87± 4.32^{ce}
T3:CTc	111.35±4.62	159.97±3.57^{b}	247.36±4.17^{b}	111.25±4.32	156.96±3.57^{b}	244.46±4.07^{b}
T4:CKB	109.36±3.30	161.28±1.74^{a}	271.83±2.43^{a}	108.36± 3.29	160.20±1.73^{a}	273.83±2.36^{a}
T5:CK	107.62±9.45	147.36±9.45^{cd}	251.37±5.16 ^{b}	107.36±9.65	148.36±9.65^{bc}	249.47±5.06^{b}
T6:CKc	108.81± 4.42	144.81±4.42^{cd}	223.46±6.49^{bc}	102.8± 5.32	144.89± 4.72^{bc}	226.56±6.89^{bc}
T7:CB	108.46±3.21	164.44±4.17^{a}	209.46±9.15^{c}	104.46±4.41	164.43±4.07^{a}	208.36±9.65^{c}
T8:Control (water)	105.31±2.19	130±6.34^{d}	199.87± 4.32^{ce}	107.31±4.19	158.30±6.74^{b}	198.87± 4.32^{ce}
T9:Chemical (Positive control)	104.14±0.62	129.40±2.43^{d}	202.46±4.31^{c}	103.14±0.72	112.4±2.83^{c}	204.46±4.07^{c}

Table 4.7 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on cassava growth rate under field conditions at Soeng Sang district in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Plant height (cm.)			Plant height (cm.)		
	3 months	6 months	8 months	3 months	6 months	8 months
F-Test	ns	*	*	ns	*	*
CV%	19.28	22.77	22.83	19.58	19.75	20.08

[1/]T1: Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT) T3:Cassava+Tinan 9+chemical(CTc) ,T4:Cassava+Khonkan 60-2+*Bacillus* sp. strain CaSUT008-2(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

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

Table 4.8 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on root rot disease severity in cassava under field conditions at Suranaree University of Technology in 2020-2021

Treatments1/	1st year (March-November 2020)			2nd year (March-November 2021)		
	Disease severity (%)			Disease severity (%)		
	3 months	6 months	8 months	3 months	6 months	8 months
T1:CTB	7.33	22.38^{c}	43.48^{c}	7.46	20.38^{c}	42.68^{c}
T2:CT	8.75	31.42^{a}	53.66^{bc}	8.54	31.32^{a}	51.16^{bc}
T3:CTc	9.32	22.21^{c}	69.85^{a}	9.05	22.22^{c}	69.35^{a}
T4:CKB	9.55	19.75^{c}	58.25^{b}	9.58	20.75^{c}	58.75^{b}
T5:CK	7.58	25.23^{ab}	56.67^{b}	7.85	24.33^{ab}	55.63^{b}
T6:CKc	10.35	26.25^{ab}	67.36^{a}	10.25	26.85^{a}	61.36^{bc}
T7:CB	8.25	19.57^{c}	59.83^{b}	8.33	20.57^{c}	56.83^{b}
T8:Control (water)	9.78	25.89^{ab}	69.58^{a}	8.95	24.89^{ab}	68.14^{a}
T9:Chemical (Positive control)	7.47	18.48^{c}	57.82^{b}	7.35	19.45^{c}	56.89^{b}

Table 4.8 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on root rot disease severity in cassava under field conditions at Suranaree University of Technology in 2020-2021

Treatments1/	1st year (March-November 2020)			2nd year (March-November 2021)		
	Disease severity (%)			Disease severity (%)		
	3 months	6 months	8 months	3 months	6 months	8 months
F-Test	ns	*	*	ns	*	*
CV%	20.01	17.03	17.36	19.78	18.13	19.23

[1/]T1:Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT) T3:Cassava+Tinan 9+chemical(CTc) ,T4:Cassava+Khonkan 60-2+*Bacillus* sp. strain CaSUT008-2(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+ Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

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

Table 4.9 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on root rot disease severity in cassava under field conditions at Soeng Sang district in 2020-2021

Treatments[1/]	1st year(March-November 2020)			2nd year(March-November 2021)		
	Disease severity (%)			Disease severity (%)		
	3 months	6 months	8 months	3 months	6 months	8 months
T1:CTB	7.35	13.66	51.66^{ce}	5.31	16.51	49.63^{e}
T2:CT	9.82	15.66	63.66^{bc}	7.62	16.54	61.38^{bc}
T3:CTc	9.12	13.78	69.16^{b}	7.45	16.38	68.23^{b}
T4:CKB	8.56	15.36	56.15^{c}	6.35	14.36	56.52^{c}
T5:CK	6.66	17.59	76.63^{a}	4.32	16.59	75.98^{a}
T6:CKc	8.16	17.83	66.56^{b}	6.15	17.13	67.58^{b}
T7:CB	7.56	19.25	58.73^{c}	5.23	18.12	53.62^{c}
T8:Control (water)	9.91	18.54	77.32^{a}	8.46	16.23	75.66^{a}
T9:Chemical (Positive control)	7.32	14.43	67.72^{b}	7.56	17.18	53.48^{c}
F-Test	ns	ns	*	ns	ns	*

Table 4.9 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on root rot disease severity in cassava under field conditions at Soeng Sang district in 2020-2021

Treatments[1/]	1st year(March-November 2020)			2nd year(March-November 2021)		
	Disease severity (%)			Disease severity (%)		
	3 months	6 months	8 months	3 months	6 months	8 months
CV%	20.58	19.10	18.89	19.54	20.10	18.33

[1/]T1:Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT) T3:Cassava+Tinan 9+chemical(CTc) ,T4:Cassava+Khonkan 60-2+*Bacillus* sp. strain CaSUT008-2(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

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

Table 4.10 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on yield in cassava under field conditions at Suranaree University of Technology in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)
T1:CTB	23.43	32.56b	3.40^{a}	23.13	36.67^{a}	3.20^{a}
T2:CT	24.12	26.57^{c}	2.64^{b}	23.00	27.67^{c}	2.84^{b}
T3:CTc	22.3	31.45^{b}	2.56^{b}	22.43	32.33^{b}	2.91^{b}
T4:CKB	21.29	25.35^{c}	1.81^{d}	20.96	24.33^{c}	1.61^{d}
T5:CK	20.66	35.67^{ab}	2.20^{c}	20.56	35.27^{ab}	3.04^{ab}
T6:CKc	22.23	32.00^{b}	1.92^{cd}	22.29	32.40^{b}	1.92^{cd}
T7:CB	22.83	36.33^{a}	3.10^{a}	22.84	36.23^{a}	3.09^{a}
T8:Control (water)	22.63	32.47^{b}	3.01^{ab}	22.71	32.49^{b}	2.20^{c}
T9:Chemical	23.00	27.33^{c}	2.98^{b}	23.00	27.33^{c}	2.98^{b}

Table 4.10 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on yield in cassava under field conditions at Suranaree University of Technology in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)
(Positive control)						
F-Test	ns	**	**	ns	**	**
CV%	20.48	22.26	18.13	20.48	22.26	18.13

[1/]T1:Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT) T3:Cassava+Tinan 9+chemical(CTc) ,T4:Cassava+Khonkan 60-2+*Bacillus* sp. strain CaSUT008-2(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+ Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

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

Table 4.11 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on yield in cassava under field conditions at Soeng Sang district in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)
T1:CTB	16.19	9.73^{a}	3.46^{a}	16.39	9.00^{a}	3.42^{a}
T2:CT	14.66	8.26^{a}	2.98^{b}	13.64	8.35^{a}	2.75^{b}
T3:CTc	13.26	6.26^{bc}	1.93^{bc}	12.67	6.76^{bc}	1.33^{bc}
T4:CKB	11.00	8.16^{a}	1.30^{c}	11.15	8.12^{a}	1.20^{c}
T5:CK	12.20	7.06^{b}	2.57^{b}	12.24	6.15^{b}	2.59^{b}
T6:CKc	13.76	4.93^{c}	0.93^{c}	14.73	5.43^{c}	0.95^{c}
T7:CB	15.73	8.13^{a}	0.71^{c}	16.11	8.62^{a}	0.69^{c}
T8:Control (water)	13.73	7.13^{b}	0.82^{c}	12.35	7.13^{b}	0.82^{c}

Table 4.11 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on yield in cassava under field conditions at Soeng Sang district in 2020-2021

Treatments[1/]	1st year (March-November 2020)			2nd year (March-November 2021)		
	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)	Starch content (%)	Number of tubers per plant (tubers/plant)	Tuber Fresh Weight per plant (kg/plant)
T9:Chemical (Positive control)	14.60	8.46[a]	0.37[c]	12.61	8.46[a]	0.62[c]
F-Test	ns	*	*	ns	*	*
CV%	20.12	21.36	19.17	18.37	19.15	18.16

[1/]T1:Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT) T3:Cassava+Tinan 9+chemical(CTc) ,T4:*Cassava+Khonkan 60-2+Bacillus sp. strain CaSUT008-2*(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+ Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

/ Mean in the column followed by the same letter are not significantly different according to the LSD test (α= 0.05).

Table 4.12 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on soil properties before and after cropping at Suranaree University of Technology

Treatment[1/]	1st year (March-November 2020)						2nd year (March-November 2021)					
	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)
Before	6.26	2.25	0.20	26.12^{c}	140.09^{e}	151.23^{e}	6.13	2.20	0.21^{e}	26.10^{e}	139.04^{e}	151.42^{e}
T1:CTB	6.29	2.22	0.23	33.62^{a}	160.20^{c}	193.33^{a}	6.13	2.58	1.41^{a}	40.53^{a}	197.21^{a}	198.61^{a}
T2:CT	6.27	2.47	0.23	32.29^{b}	160.13^{c}	175.61^{b}	6.24	2.47	0.76^{b}	33.31^{b}	156.35^{c}	176.53^{b}
T3:CTc	6.31	2.22	0.23	33.16^{a}	143.55^{e}	175.46^{b}	6.81	2.38	0.61^{b}	29.32^{c}	121.54^{c}	174.33^{b}
T4:CKB	6.24	2.31	0.21	31.48^{bc}	198.32^{a}	168.31^{c}	6.41	2.32	1.09^{a}	38.32^{a}	198.73^{a}	169.32^{c}
T5:CK	6.26	2.58	0.21	31.29^{bc}	171.58^{b}	160.24^{c}	6.61	2.23	0.81^{b}	35.20^{b}	182.85^{b}	163.31^{c}
T6:CKc	6.31	2.20	0.20	31.54^{bc}	173.28^{b}	197.65^{a}	6.75	2.21	0.69^{bc}	31.16^{c}	183.78^{b}	198.63^{a}
T7:CB	6.54	2.31	0.23	31.87^{bc}	172.46^{b}	173.21^{b}	6.92	2.54	0.85^{b}	36.81^{ab}	193.95^{a}	175.21^{b}
T8:Control (water)	6.37	2.32	0.22	33.42^{a}	140.53^{e}	169.51^{c}	6.09	2.36	0.63^{c}	24.41^{d}	120.33^{c}	168.32^{c}
T9: Chemical	6.33	2.27	0.22	20.37^{cd}	143.51^{e}	194.38^{a}	6.17	2.27	0.63^{c}	19.21^{d}	119.88^{c}	194.24^{a}

Table 4.12 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on soil properties before and after cropping at Suranaree University of Technology

Treatment[1/]	1st year (March-November 2020)						2nd year (March-November 2021)					
	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)
(Positive control)												
F-Test	ns	ns	ns	**	**	*	ns	ns	**	**	**	*
CV%	6.83	12.33	11.33	20.83	19.13	20.10	9.85	12.57	18.21	20.13	19.57	20.21

[1/]T1:Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT) T3:Cassava+Tinan 9+chemical(CTc) ,T4:Cassava+Khonkan 60-2+*Bacillus* sp. strain CaSUT008-2(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+ Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

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

Table 4.13 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on soil properties before and after cropping at Soeng Sang district

Treatment [1/]	1st year (March-November,2020)						2nd year (March-November 2021)					
	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)
Before	5.23	2.34	0.41	23.12^{e}	140.05^{e}	150.40^{e}	6.26	2.250	0.20^{e}	26.12^{e}	140.05^{e}	150.40^{e}
T1:CTB	5.85	2.68	0.43	32.64^{a}	161.20^{c}	196.37^{a}	6.02	2.582	1.03^{a}	41.64^{a}	198.2^{a}	198.37^{a}
T2:CT	5.16	2.43	0.42	31.40^{b}	160.36^{c}	175.62^{b}	6.22	2.427	0.75^{b}	34.30^{b}	157.36^{c}	177.62^{b}
T3:CTc	5.45	2.33	0.43	30.12^{c}	145.56^{e}	175.23^{b}	6.08	2.328	0.71^{b}	30.12^{c}	122.56^{c}	175.23^{b}
T4:CKB	5.36	2.44	0.41	32.42^{a}	198.41^{a}	169.37^{c}	6.14	2.312	1.01^{a}	39.42^{a}	199.21^{a}	169.48^{c}
T5:CK	5.49	2.57	0.41	31.75^{b}	173.56^{b}	161.14^{c}	6.16	2.293	0.79^{b}	36.25^{b}	183.56^{b}	162.54^{c}
T6:CKc	5.55	2.35	0.41	31.56^{c}	174.21^{b}	197.33^{a}	6.25	2.201	0.69^{bc}	32.56^{c}	184.21^{b}	198.33^{a}
T7:CB	5.35	2.32	0.42	31.89^{ab}	172.51^{b}	175.25^{b}	6.19	2.534	0.84^{b}	37.89^{ab}	194.51^{a}	176.65^{b}
T8:Control (water)	5.38	2.34	0.43	33.47^{d}	141.57 ^{e}	169.53^{c}	6.07	2.326	0.62^{c}	25.47^{d}	121.54^{c}	169.53^{c}
T9:Chemical (Positive control)	5.48	2.37	0.40	20.25d	144.57^{e}	195.34^{a}	6.13	2.217	0.64^{c}	20.25^{d}	120.87^{c}	195.34^{a}

Table 4.13 Effect of *Bacillus* sp. strain CaSUT008-2 and peanut intercropping on soil properties before and after cropping at Soeng Sang district

Treatment [1/]	1st year (March-November,2020)						2nd year (March-November 2021)					
	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)	pH	Organic matter (%)	Total nitrogen (ppm)	Total phosphorus (ppm)	Total potassium (ppm)	Total Calcium (ppm)
F-Test	ns	ns	ns	ns	**	*	ns	ns	**	**	**	*
CV%	7.93	2.57	18.21	20.83	19.57	19.21	6.83	9.57	8.21	18.83	19.57	17.21

1/T1:Cassava+Tinan 9+*Bacillus* sp. strain CaSUT008-2(CTB) ,T2:Cassava+Tinan 9(CT)

T3:Cassava+Tinan 9+chemical(CTc) ,T4:Cassava+Khonkan 60-2+*Bacillus* sp. strain CaSUT008-2(CKB) ,T5:Cassava+Khonkan 60-2(CK) ,T6:Cassava+ Khonkan 60-2 +chemical(CKc),T7:Cassava+*Bacillus* sp. strain CaSUT008-2(CB) , T8:Control (water), T9:Chemical (Positive control)

/ Mean in the column followed by the same letter are not significantly different according to the LSD test (**α**= 0.0

4.5.1 Survival *Bacillus* sp. strain CaSUT 008-2 in soil amendments

The survival *Bacillus* sp. strain CaSUT 008-2 in soil amendments was tested by the serial dilution method. The soil amendment samples were collected at 5 points and mixed. The survival bacteria density at 1, 2, and 3 months was $2.16\pm0.45\times10^8$, $1.98\pm0.32\times10^8$, and $1.55\pm0.14\times10^8$ Cfu/ml, respectively (Table 11). After that, experiments were performed to confirm the similarity between survival bacteria and *Bacillus* sp. strain CaSUT 008-2 by using selective media by spread plate method. The morphology of surviving bacteria was an opaque colony surrounded by mucus, similar to the bacteria mixed in the soil that suggested it was able to grow in the soil.

Table 4.14 The number of survival *Bacillus* sp. strain CaSUT 008-2 in soil amendments.

Treatments (months)[1/]	The survival of *Bacillus* strain CaSUT 008-2 Cfu/ml[2/]
0	$3.62\pm0.31\times10^8$
1	$2.16\pm0.45\times10^8$
2	$1.98\pm0.32\times10^8$
3	$1.55\pm0.14\times10^8$

[1/] Storage period for *Bacillus* sp. Strain CaSUT 008-2 in soil amendments.

[2/] The number of survival bacteria.

CHAPTER V
CONCLUSION AND DISCUSSION

The research was performed to investigate the antagonistic effects of *Bacillus* sp. and intercropping to control the CRTRD under greenhouse and field conditions. First, the pathogenicity tests of *L. theobromae* were confirmed to cause CRTRD. Then, the isolations of *Bacillus* sp. were screened for the antifungal test, high-temperature resistance test, and ultraviolet (UV) resistance test. The *Bacillus* sp. strain CaSUT008-2 was selected for assay antifungal effect by ESEM observation, and evaluation of their efficacy to control CRTRD under greenhouse and field conditions. Lastly, the survival *Bacillus* sp. strain CaSUT008-2 in soil amendments was exanimated. That the all of results were summarized as follows:

5.1 The pathogenicity tests of CRTRD

In the research of Suttisa (2015), the five genera of fungi causing CRTRD were studied for morphology. The *Lasiodiplodia* spp. was the most common with 54 percent, followed by *Fusarium* spp., *Neoscytalidium* sp., *Phytophthora* spp., *Sclerotium* sp. and other fungi were 29, 7, 4, 1, and 5 %, respectively. This is consistent with foreign reports that the disease has a name different in each country and has more than one type of fungal pathogen.

5.2 Antifungal of *Bacillus* sp. against *Lasiodiplodia theobromae* under *in-vitro* conditions

The efficacy of *Bacillus* sp. on fungal growth inhibition was different depending on the isolate. Bacillus sp. strain CaSUT008 showed the most effective in inhibiting *L. theobromae* under *in-vitro* conditions. Similarly, Sajitha et al. (2014) reported that the

efficacy of the antagonistic *B. subtilis* reduced *L. theobromae*. The *Bacillus* sp. could act as inhibition agents to inhibit phytopathogen activity. An antagonistic bacterium inhibits fungal growth by plasmolysis, shrinkage, and lysis of the fungal mycelium caused by active molecules produced by the bacterial biocontrol agent (Hashem and Alamri, 2009; Ashwini and Srividya, 2014). *Bacillus subtilis* (isolate B246), *Bacillus cereus* (isolate B247 and B249) and *Bacillus licheniformis* (isolate B248) inhibited to *L. theobromae*. Moreover, the mode of action was determined by using an *in vitro* spore-germination assay. The antagonist effectively reduced the percentage of spore germination of *L. theobromae*. In addition, bulb formation and lysis of hyphae were observed, as well as bacterial movement towards germinating spores and subsequent attachment to the spores.

5.3 Screening of *Bacillus* sp. for high-temperature resistance, and ultraviolet (UV) resistance test.

The high temperature and ultraviolet resistance tests of bacillus showed that *Bacillus* sp. can withstand high temperatures as 53 ºC and can grow under UV conditions. Berendsen et al. (2016) reported that bacterial endospore formers can produce spores that are heat resistant. It was recently discovered that a spoVA operon called spoVA2mob, found on a Tn1546 transposon in *Bacillus subtilis*, leads to significantly increased wet heat resistance in *B. subtilis* spores. Tn1546 transposon elements, including the spoVA2mob operon, were found in several strains of *Bacillus amyloliquefaciens* and *Bacillus licheniformis*, and these strains produced spores with significantly higher resistance to wet heat than their counterparts lacking this transposon. And as expected, a quick drop in both cell turbidity and colony forming units (~10^4) along with spores were observed after 12–24 h of the incubation period, when cells were grown at 54 °C in both Luria–Bertani and nutrient broth and agar. The critical temperature (the temperature above which it is no longer possible to survive) of *Bacillus* spp. SUBB01 was estimated to be 53 °C. Furthermore, a positive impact was observed on the inhibited *E. coli* SUBE01 growth at 45 and 47 °C, upon the supplementation of the extracellular fractions of *Bacillus* species into the growing culture (Munna, 2015)

5.4 Efficacy of *Bacillus* sp. to control CRTRD under greenhouse conditions.

Methods using *Bacillus* sp. were evaluated for reducing CRTRD and promoting growth under greenhouse conditions. The *Bacillus* sp. CaSUT008-2 showed the highest effective reducing disease by 74.13%, which was similar to fungicide (PCNB) treatment (72.56%). Latha et al. (2011) report that the fungal and bacterial biocontrol agents were tested individually and in combinations with oil cakes, organic manures, and micronutrients for their efficacy against the collar and root rot pathogen, *Lasiodiplodia theobromae* under *in vitro*, greenhouse, and field conditions. Sriwaura et al. (2015) report that the organisms of 977 isolates were screened for their antagonistic towards *Lasiodiplodia theobromae*. Among these, 25 isolates expressed their inhibitory action towards the mycelial growth of *L. theobromae*. Of these, 10 isolates were distinctive because of their vigorous antagonism towards *L. theobromae* conidium germination, 7 of them caused the formation of swollen and vacuolated germ tubes and the remained 3 caused conidium abortion with 63 % conidium germination while 82% germination occurred for the control. The potential of antagonistic yeasts and bacteria was demonstrated on detached rambutan fruit. The significant effectiveness with disease reduction between 52-78 % was demonstrated when protection occurred before the inoculation with the pathogen. This is consistent with Chukeatirote et al. (2018) Rhizobacteria capable of inhibiting *Lasiodiplodia theobromae* were screened. In total, 890 rhizobacterial, isolate named JN15 showed maximum inhibition of the fungus *L. theobromae* (approximately 60%). The antifungal activity of the JN15 culture supernatant was stable in the pH range 4e10 (29e42% inhibition) and remained active at 40 C. The bacterial strain JN15 isolated from Senna siamea was then characterized in terms of its phenotypic and genotypic properties including morphology, biochemical profiles, and 16S rRNA gene sequence. Based on this analysis, the bacterium JN15 was identified as *Bacillus amyloliquefaciens.* Among the fungal (*Trichoderma*) and bacterial (*Pseudomonas* and *Bacillus*) antagonists tested against *L. theobromae in vitro*,

Trichoderma viride (Tv1), *Pseudomonas fluorescens* (Pf1), and *Bacillus subtilis* (Bs16) isolate showed the greatest inhibition. Among the oil cakes, organic manures, and micronutrients tested *in vitro* against the pathogen, neem cake, farmyard manure (FYM), and zinc sulphate was the most effective in inhibiting pathogen growth. *Bacillus* can also support cassava growth. According to reports plant growth promoting rhizobacteria has long been studied to promote plant benefits from disease control to plant development and increased nutrient uptake in the absence of the pathogen (Lugtenberg and Kamilova, 2009). In addition, *Bacillus* spp. could transfer phosphorus to a 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). And it also enhances yield compared with the control or fungicide treatment.

5.5 Efficacy of *Bacillus* sp. to control CRTRD under field conditions

Methods using *Bacillus* sp. CaSUT008-2 and intercropping were evaluated for reducing CRTRD and promoting growth under field conditions. The *Bacillus* sp. CaSUT008-2 and peanut intercropping showed the highest effective reducing disease at Suranaree University of Technology and Soeng Sang district in 2020-2021. Combination Tinan 9 and *Bacillus* sp. strain CaSUT008-2 treatment have a high growth trend than control methods at 6 and 8 months with the plant height was 178.43, and 264.38 cm in the first year, and 178.36 and 254.36 cm in the second year respectively. In addition, this treatment also enhanced plant growth with the plant height at 6 and 8 months after planting was 162.39 and 270.88 cm in the first year, and 164.349 and 269.78 cm in the second year in Soeng Sang district. On the other hand, the combination of Tinan 9 and *Bacillus* sp. strain CaSUT008-2 treatment could reduce CRTRD by 33.18-38.66%, and enhance the number of tubers per plant by 12.24-15.84%, tuber fresh weight per plant by 6.31-21.95 %. Moreover, this treatment also increased nitrogen content by 50.23-66.12%, phosphorus content by 49.47-63.48%, potassium content by 50.47-63.07%, calcium content by 12.35-17.01%, pH by 1.38-2.47 % and organic matter by 9.85-11.00% after cropping. And under field conditions, the PGPRs,

such as *Bacillus amyloliquefaciens* strain KPS46, can boost growth in a variety of economic crops, including soybean, vegetable soybean, corn, rice, Chinese kale, and cauliflower. This process is mediated in part by the excretion of phytohormones such as auxin and indole-3-acetic acid (IAA), lipopeptides, and extracellular proteins (Prathuangwong and Kasem, 2004; Prathuangwong et al., 2005; Prathuangwong and Buensanteai, 2007; Buensanteai et al., 2008a). This could explain how the *Bacillus* group interacts with plants by synthesizing phytohormones similar to the plant endogenous growth regulator and increasing those levels in plants that are involved in the initial processes of lateral and adventitious root formation and elongation (Buensanteai et al., 2008a, 2008b; Erturk et al., 2010). Tang et al. (2020) report that when compared to monocropping cassava (MC) and monocropping peanut (MP) systems, both intercropping cassava/peanut (IP) and intercropping peanut/cassava (IC) systems significantly increased available N, available K, pH value, and urease activity. The total N, total P, total K, available P, organic matter, protease activity, catalase activity, sucrose activity, and acid phosphatase activity, on the other hand, showed little variation. IP and MP soils had more bacteria and fungi than IC and MC soils, which were mostly made up of Proteobacteria and Actinobacteria. Intercropping significantly increased the number of Nitrospirae in IP and IC soils when compared to MC and MP soils. The abundances of DA101, Pilimelia, and Ramlibacter were found to be positively correlated with soil quality using redundancy analysis (RDA). These findings suggest that intercropping increases soil available nitrogen content by increasing the number of rhizospheric microbes, particularly DA101 and Pilimelia. The potential benefits of intercropping are manifold and have been repeatedly demonstrated. Intercropping has the potential to create more productive and resilient agroecosystems, by improving land utilization, yield and yield stability, soil quality, and pest, disease, and weed suppression. One advantage of this practice could be disease control. Intercropping reduced disease in 73 percent of more than 200 studies comparing disease in monocrops and intercrops, primarily due to foliar fungi. The primary pathogen for which disease increases are reported is fungi, but disease impacts can vary greatly between studies for all types of diseases. Intercrops influence disease dynamics through a variety of mechanisms, including changes in wind, rain, and vector dispersal;

microclimate modification, particularly temperature and moisture; changes in host morphology and physiology; and direct pathogen inhibition.

5.6 Survival *Bacillus* sp. strain CaSUT 008-2 in soil amendments.

The *Bacillus* sp. strain CaSUT008-2 was shown stable at 1 month after storage. But there was a decline that remained steady at 2 and 3 months after storage. Wongchalee (2015), report that 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. Radhakrishnan et al. (2017) report that soil moisture has a significant impact on crop productivity in arid and semiarid areas Drought stress is caused by low moisture content in the soil as a result of low annual precipitation. Plant productivity under drought conditions can be improved by regulating nutrient uptake and distribution, water transport, and the accumulation of compatible solutes and antioxidants in plant tissues (Boomsma and Vyn, 2008). Applying drought-tolerant Bacillus spp. to the soil boosts bacterial populations on the roots and stimulates root exuviation, promoting both bacterial and plant growth (Sandhya et al., 2011). Bacillus spp. colonized plants absorb more water, which is an important mechanism for plant protection against drought-induced damage (Marulanda et al., 2009). Bacillus-induced physiological changes and their mitigating effects in plants.

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APPENDIX

1. MEDIUMS

1.1 Water Agar (WA)

Agar	15g
Water	1,000 ml

1.2 Potato Dextrose Agar (PDA)

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

1.3 Nutrient broth (NB) medium

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

1.4. Potato Dextrose broth (PDB)

Potato	200g
Dextrose	20g
Water	1,000ml

1.5 Selective medium

Streptomycin	1g
Water	1,000ml

BIOGRAPHY

Miss Kansinee Laemchiab was born on January, 1995 in Nakhon Sawan province,Thailand. She received his Bachelor of Crop Production of Technology from the Suranaree University of Technology, Thailand in 2017.

2017, she was admitted to study for a Master's Degree at Suranaree University of Technology, Nakhon Ratchasima, Thailand. During their study, she received she won a scholarship from the External Grants and Scholarships for Graduate Students (OROG) Scholarships 2017-2017 a teaching assistant scholarship and research assistant funding from external grants: The Thailand Research Fund (TRF) and CS Tapioca Research & Innovation Co., Ltd. Under the Research and Researcher for Industry (RRi) at the Master's degree level.