INNOVATIVE PREPARATION METHOD OF CHITOSAN OLIGOMER, MONOMER, DROSS AND ITS APPLICATION IN AGRICULTURE FIELD

PAKPOOM VATCHARAKAJON

DOCTOR OF PHILOSOPHY IN ORGANIC AGRICULTURE MANAGEMENT
(INTERNATIONAL PROGRAM)
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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
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บทคัดย่อ

โรครากเน่าเป็นปัญหาที่ทำให้ผลผลิตทุเรียนลดลง การใช้สารเคมี เช่น เมทาแลกซิล เป็นอันตรายต่อมนุษย์และสิ่งแวดล้อม และทำให้เกิดการดื้อยาฆ่าเชื้อรา สารไคโตโอลิโกเมอร์และโมโนเมอร์ และจุลินทรีย์ เป็นวิธีการใหม่ในการรักษาโรครากเน่าของทุเรียน การศึกษานี้พบว่า สารไคโตโอลิโกเมอร์และโมโนเมอร์ และจุลินทรีย์ สามารถรักษาโรครากเน่าของทุเรียนได้ดีกว่าการใช้เมทาแลกซิลอย่างมีนัยสำคัญ อาจเป็นทางเลือกแทนสารเคมีเพื่อรักษาโรคเชื้อรา

กากอะลูมิเนียมเป็นผลพลอยได้จากกระบวนการผลิตอะลูมิเนียมที่เป็นอันตรายต่อสิ่งแวดล้อม ในการศึกษาปัจจุบัน กากอะลูมิเนียมที่ปรับสภาพให้เป็นกลางแสดงให้เห็นว่าสามารถส่งเสริมให้น้ำหนัก ส่วนสูง และการเจริญเติบโตของผักเพิ่มขึ้น สามารถใช้เป็นปุ๋ยที่เป็นมิตรต่อสิ่งแวดล้อมได้เนื่องจากไม่มีธาตุโลหะหนักที่เป็นพิษในดิน

โรคกรีนนิ่งของส้ม ทำให้เกิดความเสียหายอย่างรุนแรงและทำให้ผลผลิตของสวนส้มลดลง การใช้แอมพิซิลลินในการรักษาโรคกรีนนิ่ง ทำให้เกิดการดื้อยาปฏิชีวนะและพบการตกค้างในสิ่งแวดล้อม และผลไม้รสเปรี้ยว การศึกษาครั้งนี้ได้พัฒนาสารไฮบริดของซิลเวอร์นาโนที่มีสารไคโตโอลิโกเมอร์และโมโนเมอร์ ซึ่งพิสูจน์แล้วว่ามีประสิทธิภาพในการรักษาโรคกรีนนิ่ง มีความคุ้มค่ามากกว่าอย่างเห็นได้ชัด เพิ่มผลผลิต และไม่มีการสะสมเมื่อเทียบกับแอมพิซิลิน สามารถใช้เป็นทางเลือกแทนแอมพิซิลินในการรักษาโรคกรีนนิ่งในส้มได้

คำสำคัญ : กากอะลูมิเนียม, ไคโตซานโอลิโกเมอร์, โรคส้มเขียว, สารละลายไฮบริด, โรครากเน่า, อนุภาคซิลเวอร์นาโน

Title	INNOVATIVE PREPARATION METHOD OF CHITOSAN OLIGOMER, MONOMER, DROSS AND ITS APPLICATION IN AGRICULTURE FIELD
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ABSTRACT

Root rot disease is a problem that reduces durian productivity. The usage of chemicals such as metalaxyl is hazardous to humans and the environment and causes fungicide resistance. Chito oligomers and monomers (COAMs) and Integrated microorganisms (IMO) are novel methods for the treatment of durian root rot disease. This study found that COAMs and IMO can cure durian root rot disease significantly better than using metalaxyl. It could be an alternative to chemical fungicides to treat fungal diseases.

Aluminium dross is a byproduct of the aluminium manufacturing process that is hazardous to the environment. In the current study, neutralized dross showed enhancement in the height weight, and growth of vegetables. It can be used as an environmentally friendly fertilizer as there are no toxic heavy metal elements in the soil.

Citrus greening disease (CGD) causes severe damage and decreases the yield of citrus farms. Ampicillin usage in the treatment of CGD has led to antibiotic resistance and the discovery of residues in the environment and citrus fruits. This study effectively developed a hybrid solution of silver nano with COAMs that proved effective in the treatment of CGD. It was significantly more cost-effective, increased productivity, and had no accumulation compared to Ampicillin. It can be used as an

alternative to ampicillin in CGD treatment.

Keywords : Aluminium dross, Chitosan oligomers, Citrus greening disease, Hybrid solution, Root rot disease, silver nanoparticles

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Finally, I sincerely apologize for any inaccuracies in my research and hope that it will be valuable to attract the best talent who are interested in managing plant diseases through organic innovation.

Pakpoom Vatcharakajon

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ABBREVIATIONS AND SYMBOLS

ACP	Asian Citrus Psyllid
AgCSs	Synthesized chitosan-stabilized silver nanoparticles
AgNPs	Silver Nanoparticles
ATP	Adenosine triphosphate
BCAs	Biological Control Agents
CGD	Citrus Greening Disease
COAMs	Chitooligomers and Monomers
COS	Chitooligosaccharide
DA	Degree of Acetylation
GDP	Gross Domestic Product
GI	Germination Index
DNA	Deoxyribonucleic acid
DP	Degree of Polymerization
GlcNAc	N-acetyl-D-glucosamine
HLB	Huanglongbing
IC 50	The half-maximal (50%) inhibitory concentration
ICMS	Integrated Crop Management System
IMOs	Integrated Microorganisms
IPM	Integrated Pest Management
kDa	Kilodalton
MW	Molecular Weight
PA	Pattern of Acetylation
RNA	Ribonucleic acid
UV	Ultraviolet

CHAPTER I
RESEARCH BACKGROUND

Agriculture has long been a major source of revenue for the Thai people. It is important to Thailand's economy and culture. It accounts for 10% of the total gross domestic product (GDP). According to data from the Office of Agricultural Economics, the value of agricultural outputs and goods exported in 2019 is approximately 7.6 trillion baht. Thailand has an agricultural area of approximately 149 million rai, 46% of which is rice farms, 25% is fruit orchards and perennials, 21% is field crops such as sugarcane, maize, and cassava, and the remaining is flowering crops and livestock. (Office of Agricultural Economics, 2019)

Plant diseases are serious issues in agriculture, causing enormous economic harm. Plant disease is an abnormal symptom of a plant that can appear in any section of the plant or throughout its life cycle, up to and including the death of the entire tree. Farmers must therefore employ a variety of techniques to manage plant diseases, such as foliar spraying and injecting chemicals into the trunk, scraping the trunk bark around the rotting wound, and then applying chemicals, etc. However, it is only a transient cure for the disease or simply delays the deterioration of the diseased plant. (Leksomboon, 2014)

In the long term, the continued use of chemicals to control the disease has caused problems for production, such as economic issues resulting in high production costs, problems with diseases and pests that frequently cause resistance to chemicals, health problems of farmers who use, and the problem of pesticide residues in goods that affect consumers and the problem of pesticide residues or contaminate to the environment. (Ungsoongnern, 2015)

Thailand is the world’s largest exporter of durian accounting for 77.33% of the world’s export value of fresh durian. In 2021, the volume of fresh durian exported was 620,892.72 tons, valued at 2,072.79 million US dollars. (Department of Trade Negotiations, Ministry of Commerce, 2022) Durian (*Durizio zibethinus*) has been cultivated for commercial locally in southeast Asia for more than a century. Durian care practices in the pre-production period are important for durians to grow rapidly and provide faster productivity. The biggest problem with durian cultivation is problems related to plant diseases including root rot disease. (Klubnuam and Pudmetej, n.d.)

Root rot is a problem that destroys durian trees in tropical areas. Root rot disease in durian is the most important disease that causes crop loss, and limits production and durian trees can be damaged to death. The root rot disease is caused by several Phytophthora species, including P. palmivora, Fusarium spp., and

Lasiodiplodia pseudotheobromae (Chantarasiri and Boontanom, 2021). Farmers have applied various fungicide groups such as phenylamide, the quinone outside inhibitors, and carboxylic acid amides to control these pathogens. Metalaxyl of the phenylamide group is the most commonly applied as a fungicide. The side effects of fungicides may come with risks, such as serious hazards to farmers, consumers, and the environment. Fungicide-resistant Phytophthora strains have been reported in many countries including Thailand. This becomes increasingly a more important issue to manage root rot disease in durian efficiently and safely. (Kongtragoul et al., 2021)

Citrus is a genus of flowering trees and shrubs in the rue family, Rutaceae includes essential crops like oranges, lemons, grapefruit, and limes. They rank among Thailand's major export crops roughly 128,046 rai are under cultivation, mostly in the north 95.3 percent were accountable. Citrus worth 1,138.26 million Baht were exported in 2021, totaling 47,318.94 tons. (Office of Agricultural Economics, 2021)

Citrus Greening Disease (CGD), also known as Huanglongbing (HLB), is important in citrus farming because of its severe damage, decreased yield, poor quality, and frequent disappearance before harvest. Asymmetrical, blotchy mottling patterns on leaves are one of the disease's main symptoms. *Candidatus* Liberibacter species of bacteria are primarily accountable for the disease and cannot be grown on a culture medium (Poonyapitak et al., 2016). One of the primary vectors for transmitting the disease by feeding on citrus leaves is the Asian citrus psyllid. It can carry a bacterium for many days and has the ability to transmit to an uninfected plant when feeding on it. The phloem tissues of the roots and leaves are severely obstructed because of bacterial migration inside these two parts of the plant. As a result, obstruction to the circulation of nutrients and sugars in the interior tissues leads to leaf loss, uneven fruit size, which can impair the flavor and texture of fruits, premature fruit drop, and eventually the death of the tree. (Poonyapitak et al., 2016; Bendix and Lewis, 2018; Food Agriculture Organization, 2013; Jantasorn et al., 2007; Tipu et al., 2021)

The excessive accumulation of starch in the remaining parenchyma cells of the aerial parts and photosynthetic cells is one of the most obvious symptoms of CGD in citrus trees. These can be detected by an iodine test. By observing how the leaves change color, one can tell whether or not a citrus tree is infected with the CGD by determining if the leaves turn blue or purple (positive test). If not, the leaves are healthy. (Whitaker et al., 2014)

Since the beginning of the disease, a variety of techniques have been applied to treat CGD including eradicating the insect vector, administering insecticides, and using broad-spectrum antibiotics to treat the symptoms in infected trees. The use of antibiotics in field crops is, undoubtedly, constrained by the emergence of microbial resistance, which also poses a significant risk to human health due to its indirect effects. (Stephano-Honedo et al., 2020) The research from the Faculty of Pharmacy, Chiang Mai University, Thailand found the accumulation of antibiotics in citrus fruit and remains for up to 90 days. They are seriously concerned about the citrus orchard's ecology, where antibiotic residues have begun to accumulate in the water, soil, and citrus plants. These might impact the food chain and result in bacterial resistance that can be transmitted to humans. In actuality, the disease cannot currently be treated with chemicals or any other types of controls. Therefore, it is obvious that a long-term cure for CGD infection in plants is required immediately (Stephano-Honedo et al., 2020).

Shrimp shells are waste from the seafood industry that is dumped by millions of tons each year. Their structure consists of chitin polymer, a polysaccharide chain of N-acetyl-D-glucosamine (GlcNAc) bound together with β-1,4 glycosidic linkage. Thailand, the rich in raw materials to produce chitin and chitosan from crab shells and shrimp shells, then, chitosan oligomers and monomers (COAMs) are biological organic substances. Their interesting biological properties are biocompatible, non-toxic, and biodegradable. They can dissolve in an aqueous solution as well making plants absorb better than chitosan and with a short chain and small molecule allowing plants can absorb and use immediately (Winkler et al., 2017; Lopez-Moya et al., 2019).

Chitosan is a chitin derivative obtained by deacetylation of the chitin chain (Schmitz et al., 2019). Chitosan is an acetylated derivative of chitin defined by the degree of acetylation (DA). The properties also depend on the degree of polymerization (DP). DA and DP determined general properties such as solubility and mode of chemical interaction but another important property is the pattern of acetylation (PA), which can be defined as the sequence of glucosamine and N-acetyl glucosamine units along the molecular backbone (Schmitz et al., 2019).

Chitin and chitosan have been used in agriculture to control plant diseases. Both of them have demonstrated antiviral, antibacterial, and antifungal properties. Thus, they have been researched for many agricultural uses. They are also used to control disease or reduce the spread of plant diseases, chelate nutrients, and minerals, prevent pathogens from accessing them or enhance plant innate defenses (Hadarami et al., 2010).

Chitooligomers also known as chitooligosaccharides (COS) or chitosan oligomers produced by the degradation of chitosan. Their degrees of polymerization (DPs) are less than 20, and their average molecular weight is less than 3900 Da (generally 0.2-3.0 kDa). (Lodhi et al., 2014) It also has a greater solubility in addition to having outstanding qualities including biodegradability, biocompatibility, adsorptive capacities, and non-toxicity like chitin and chitosan. (Liang et al., 2018) According to several studies, applying chitosan and its derivatives on plants can inhibit pathogens, particularly fungi, boost the immune system, and promote plant growth. (Hadwiger, 2017; Winker et al., 2017; Schmitz et al., 2019) At present, the preparation method of chitosan oligomer mainly uses chemical, physical, enzymatic, and electrochemical degradation but they have different advantages and disadvantages (Lopez-Moya et al., 2019).

COAMs are applied in agriculture due to their properties as an immune stimulant, antimicrobials such as antifungal and antibacterial, plant growth regulators, and fertilizer additives (Schmitz et al., 2019). Due to their biological properties and activity, COAMs can be suitable solutions to reduce chemical use and improve farmers' and consumers' quality of life.

There are numerous studies of silver nanoparticles (AgNPs) that demonstrate potential anti-phytopathogenic effects and plant disease management including fungi, bacteria, and viruses. (Tariq et al., 2022) The application of AgNPs in the production of various hybrid products is being reconsidered to their outstanding characteristics (Daniel et al., 2017). There are some studies for nanocomposite production of silver nanoparticles with chitosan and its derivative in order to produce nanocomposites with improved characteristics that might potentially be used as an antibacterial agent, silver was nano-mediated with chitosan as a reducing and stabilizing agent. (Sonseca et al. 2020)

L. Mei et al. (2020) have found the synergistic antibacterial effect of nanocomposite of COS and AgNPs. It is possible to enhance the surface charge of AgNPs by conjugating COS to them. This intensifies their electrostatic contacts and improves their capacity to cling to negatively charged bacterial cytoplasmic membranes. By functioning as an antibacterial agent in multivalent binding, COS can also make it easier for bacteria to absorb AgNPs. (Mei et al., 2020)

Aluminium dross is an unavoidable by-product obtained from the aluminium smelting process which consists of metallic aluminium (60-75), oxides (20-30%), and salts (5-10%). The smelting process of 1000 kg of molten aluminium produced ca.20kg of dross (Sultana et al., 2013). Normally this dross is treated in rotary kilns to recuperate the remaining aluminium, and the resultant waste materials (salts) may still contain a small fraction of dross disposed of by landfills. When disposed of landfills the aluminium dross may come in contact with water and emits harmful gases such as NH_3, H_2, CH_4, and H_2S and contaminate the environment (Das et al., 2007). New environmental regulations have forced the aluminium industries across the world to recycle dross rather than landfill for the prevention of waste and environmental conservation (Capuzzi et al., 2018). The recycling of aluminium dross is becoming increasingly important because of environmental issues and high landfill costs. The process that could convert dross to value-added aluminium products is necessary for the profitability of dross recycling (Hong et al., 2010; Meshram et al., 2018).

Mostly, the dross treatment comprises crushing, grinding, and sieving, followed by water leaching to remove the salt. Later the salt is recovered by filtering and evaporation and is useful for applications such as mortar components, inert filling, etc. The produced alumina and alloying element can be used in various industries after washing (Yoshimura et al, 2008; Liu et al. 2013). The main element composition in dross raw material was aluminium oxide reacting with moisture and releasing ammonia gas about 47 %wt of dross.

Numerous studies have been reported on the conversion of aluminium dross into a useful resource. Gireesh et al. (Mailar et al., 2016) reported that the mortar with 20% of aluminium dross yields optimum strength and durability for the concrete. In an important study, Dash et al. (Dash et al., 2008) leached out 85% of alumina from aluminium dross by acid dissolution in sulphuric acid. Pratumma et al (Pratumma et al., 2016) applied the aluminium dross as a plant fertilizer by the acid treatment (HCl and H_3PO_4) process and found that acid treatment of aluminium dross provided a strong effect on the germination index (GI) of the seed. The efficiency of nitrogen trapping forming as ammonium ion ($NH4^+$) depended on the types of acid. HCl treatment caused an irreversible reaction equation; however, H_3PO_4 treatment provided a reversible reaction.

Research Objectives

The objectives of these studies are as follows.

1. To produce COAMs by innovative methods including cold plasma and ultrasonic treatments.
2. To analyze the physical properties of COAMs including molecular weight and particle size.
3. To analyze the antimicrobial effect of COAMs against the fungal pathogens and isolated pathogens from infected durian trees.
4. To analyze the efficacy of innovative methods including COAMs and integrated microorganisms (IMO) in the treatment of durian trees' root rot diseases caused by fungal pathogens.
5. To examine the chemical composition of primary and secondary nutrients of dross fertilizer.

6. To analyze the effect on plant growth of treated and untreated aluminium dross fertilizer by determining the height and weight of the plant along with the control plant.

7. To analyze residue chemical composition of planting soil which apply aluminium dross.

8. To produce the hybrid solution of silver nanoparticles and Chitooligomer (COAMs) as anti-phytopathogenic substance.

9. To analyze the physical properties of the hybrid solution including particle size.

10.To analyze the efficacy of the hybrid solution in the treatment of CGD.

11.To evaluate the productivity and cost of treatment of the hybrid solution and ampicillin treatment.

Scope and Limitation of the Research

According to fungi pathogens are the main problem of root rot diseases in durian cultivation. Now a day farmers apply chemical fungicides to treat that problem, but they cause harmful side effects to farmers and destroy the environment.

In this study, COAMs, which are produced utilizing a novel technique combining cold plasma and ultrasonic treatment, and IMO, which comprise important bacteria including *Trichoderma harzianum* and *Bacillus subtilis*, were utilized as the treatment.

The scope of this study is to recruit durian crops that have problems with root rot diseases in the eastern area of Thailand including Chanthaburi, and Trat provinces. The treatment of COAMs and IMO apply to the infected durian trees. The outcome is determined by improving the score, which defines how the phloem tissues of durian are observed under a digital microscope.

Dross ADC12 and 6063 grade were used to prepare the fertilizer and examine the component of primary nutrient and secondary nutrient. The effect of HCl and H_3PO_4 treated and untreated aluminium dross fertilizer were analyzed by determining the height and weight of plant along with the control plant.

Chinese cabbage, sweet corn, basil, and spring onion seeds were planted in soil under controlled temperature, light intensity, and humidity. Treated and untreated aluminium dross fertilizer were applied to testing plant. Height and weight were recorded along 20 days of planting compared to control (applied deionized, DI water).

ADC12 treated with HCl and H_3PO_4 of soil was compared with the original soil. The chemical element composition of soil was measured to analyze the effect of dross on soil pollution.

CGD is important in citrus farming because of its severe damage, decreased yield, poor quality of fruit, and frequent disappearance before harvest. The excessive accumulation of starch in the remaining parenchyma cells of the aerial parts and photosynthetic cells is one of the most obvious symptoms of CGD in citrus trees. These can be detected by an iodine test. By observing how the leaves change color, one can tell whether a citrus tree is infected with the CGD by determining if the leaves turn blue or purple (positive test). If not, the leaves are healthy.

A citrus plantation in Fang district, Chiang Mai province, Thailand was selected for 50 citrus trees that had CGD which was confirmed by iodine test kits. An experiment with a completely randomized design (CRD) was designed. Three different substances-ampicillin, silver nanoparticles (AgNPs), and AgNPs- COAMs or hybrid solution -were used in five different ways, three times a repetition every month for each plant.

The liquid and litmus iodine test kits and physical changes in citrus leaves are observed as the criteria for disease improvement. The data was collected during the period of January-March 2022 and March-May 2022. The statistical F-test will be used to verify the impact of physical changes at 99% confidence, $p < 0.01$.

Expected Results

The expected results are as follows.

1. COAMs which are produced by an innovative method using cold plasma and ultrasonic treatment can produce antimicrobial activity and plant growth stimulation.

2. COAMs should inhibit fungi pathogens equal to or better than chemical carbendazim.

3. The innovative method employing COAMs, and IMO might decrease the prevalence of root rot diseases in durian plants by improving the exterior and internal appearance of the phloem tissues.

4. Aluminium dross treated with acid was non-toxic to plants which can be clearly seen that the germination index (GI) of plants with dross fertilizer showed better than the GI of the control.

5. The dross fertilizer shows enhancement in the height and weight of the plant when compared with the control.

6. It can be observed that there is no existence of residual chemical composition in soil from using aluminium dross fertilizer. Aluminium dross fertilizer is not harmful to the environment.

7. Hybrid compound production that is efficient. It can be injected into the plant's stem or branches or sprayed on the leaves. The particulate size is lower than that of plant stomata.

8. The hybrid solution should inhibit bacterial pathogens of CGD which can be confirm by iodine test kits.

9. The hybrid solution can both prevent and treat CGD while increasing productivity and cost effectiveness in comparison to ampicillin.

Knowledge improving

This study will improve the knowledge in the field of research as follow.

1. Improve the knowledge and understanding of chitosan oligomer and monomer including their preparation method and mechanism of action as an immune stimulator, inhibition of plant pathogens, and growth regulator.

2. The optimal mix ratio of oligomer and monomer would provide more efficacy on plant immune stimulator, anti-plant pathogens, and plant growth stimulator.

3. Learning and understanding to manage plant disease with organic items.

4. Improve the knowledge and understanding the preparation of aluminium dross fertilizer.

5. Learning and understanding the mechanism of dross fertilizer entrapping the nitrogen to its structure and release the nutrition to enhance plant growth.

6. The optimal amount of dross fertilizer which provide best efficacy on plant growth.

7. Learning and understanding to manage plant disease with organic items.

8. Improve the knowledge and understanding of the hybrid solution of AgNPs-COAMs including their preparation method and mechanism of action as anti-plant pathogens.

9. Learning and understanding to manage plant disease with organic items.

CHAPTER II

LITERATURE REVIEW AND RELATED STUDY

Plant Diseases

Plant diseases are serious issues in agriculture, causing enormous economic harm. Plant disease is an abnormal symptom of a plant that can appear in any section of the plant or throughout its life cycle, up to and including the death of the entire tree. Plant disease has two causes: pathogenic diseases caused by fungi, viruses, and bacteria, and nonpathogenic diseases caused by soil acidity, chemical pollution, nutritional shortages, etc. (Leksomboon, 2014)

Farmers must find solutions when crops are harmed, particularly by infectious diseases. There are numerous methods, but farmers frequently use chemical spraying methods and overuse them, causing damage to both the farmers and those who purchase the product for consumption.

In the long term, the continued use of chemicals to control the disease has caused problems for production, such as economic issues resulting in high production costs, problems with diseases and pests that frequently cause resistance to chemicals, health problems of farmers who use, and the problem of pesticide residues in goods that affect consumers and the problem of pesticide residues or contaminate to the environment. (Ungsoongnern, 2015)

Durian diseases

Root and foot rot disease

The scientific name for durian is *Durio zibethinus* L., and it belongs to the Bombacaceae family. It is a tropical fruit that is cultivated extensively in Thailand's eastern and southern areas. Durian farming is not only produced for local consumption; it is also produced for export to sell overseas, both as fresh fruits and processed in different ways. (Klubnuam and Pudmetej, n.d.)

Durian has been cultivated for commercial locally in southeast Asia for more than a century. Durian care practices in the pre-production period are important for durians to grow rapidly and provide faster productivity. Thailand has a durian planting area of 791,165 rai in 2020, which is distributed across all areas of the nation as follows: the South, East, North, and Northeast. with productive regions of 437,993, 299,184, 47,636, and 6,352 rai respectively. The southern region, particularly Chumphon, Nakhon Si Thammarat, Yala, and Surat Thani, has the most durian-growing regions. and the eastern area, which includes Chanthaburi, Rayong, and Trat. (Office of Agriculture Economics, 2020)

Thailand is the world's largest exporter of durian accounting for 77.33% of the world's export value of fresh durian. In 2021, the volume of fresh durian exported was 620,892.72 tons, valued at 2,072.79 million US dollars. (Department of Trade Negotiations, Ministry of Commerce, 2022)

Durian is a fruit tree with numerous diseases and insect pests. Farmers confront disease and insect pest issues that always cause harm from the beginning of development until harvest. The most prevalent durian diseases are root and foot rot, fruit rot, leaf blight, algal leaf spot, pink disease, and powdery mildew. (Chuebundit et al, 2020)

Root rot is a problem that destroys durian trees in tropical areas. Root rot disease in durian is the most important disease that causes crop loss, and limits production and durian trees can be damaged to death. The root rot disease is caused by several *Phytophthora* species, including *P. palmivora*, *Fusarium* spp., and *Lasiodiplodia pseudotheobromae* (Suksiri et al., 2018; Chantarasiri and Boontanom, 2021; Kongtragoul et al., 2021).

S. Suksiri et al. (2018) examined pathogens by collecting soil samples from durian trees showing root and stem rot disease in durian orchards in Chumphon province, Southern Thailand. The reports demonstrate that *P. palmivora* and *P. cucurbitacearum* were highly pathogenic isolates (Suksiri et al., 2018). A. Chantarasiri & P. Boontanom (2021) evaluated fungal pathogens causing stem rot disease on durian trees by collecting symptomatic bark and wood samples from different durian orchards in Rayong and Chanthaburi Provinces in Eastern Thailand. The report provides evidence that *Fusarium solani* and *Lasiodiplodia pseudotheobromae* are the fungal pathogens causing stem rot disease on durian trees (Chantarasiri and Boontanom, 2021).

Root and foot rot is caused by a fungus that develops and destroys durian at the base, stem, branches, and roots. It is common to see rough, non-glossy foliage on diseased trees, and the color gradually fades to yellow before falling off (Figure 1). Rot and wilting of the foliage are symptoms of diseased plants. Wounds on stems or branches will rot and turn into succulent spots. The bark rots turn brown and produce mucus (Figure 2), which is noticeable in the morning or during humid weather. When the bark is pulled away, the inner bark is reddish brown or dark brown, and when the roots are dug up, the taproot and fibrous roots are injured and brown rot (Figure 3), causing the durian tree to deteriorate and eventually perish. (Klubnuam and Pudmetej, n.d.; Department of Agriculture [DOA], 2022)

Figure 1 The foliage at the tips of the branches is pale, non-glossy, and wilting at first. The foliage turns yellow and drops off as the symptoms worsen.

Source DOA (n.d.)

Figure 2 The bark rot turns brown and produced mucus. Brown lesions of bark and wood tissue are discovered when scraping that area with a knife.

Source DOA (n.d.)

Figure 3 The color of the root rot symptoms is a dark color,
and when the bark is peeled, it is discovered
that the bark is brown rot.

Source DOA (n.d.)

The researcher used a digital microscope to study the phloem tissues of durian bark and discovered different features related to the severity of root and foot rot diseases. The healthy tissue is yellow. The tissues change slightly brown, dark brown, and reddish brown depending on the severity of the disease. (Figure 4)

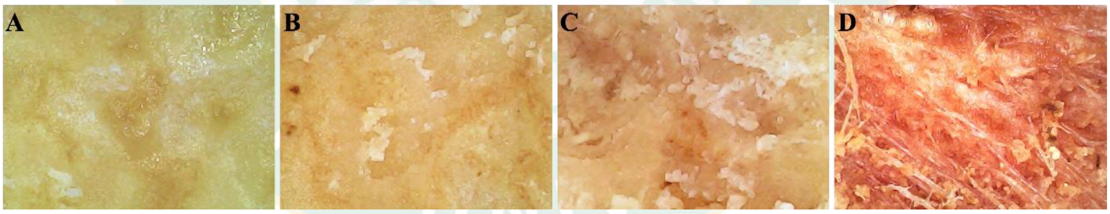


Figure 4 Phloem tissues of durian

Remark: healthy (A) mild infection (B), moderate infection (C), and severe infection (D)

Phytophthora fungus can survive on the soil for many years as chlamydospores. It can germinate into hyphae and develop sporangium when the environment is appropriate, which is the origin of zoospores that have tails moving through the water to destroy plant roots. Furthermore, the disease is transmitted by windstorms and floods, as well as by infected breeding stems and growing soil.

Durian disease control

The following techniques are used in combination to prevent and eradicate root rot disease, as suggested by the DOA:

1. The crop area should have good drainage and should not overflow.
2. Improve the soil by adding manure or compost and adjusting the soil acid-base condition or pH to around 6.5.
3. Avoid damaging the roots or stems. This will provide an easy route for the fungi that cause the disease to destroy the plant.
4. The durian tree that is severely infected or the entire tree that is desiccated and deceased should be pulled and cut down outside of the planting field. The soil should then be allowed to dry before planting a new tree to replace it.
5. Inspect the field on a regular basis, and if diseased sections, leaves, blossoms, or fruit are discovered, cut them, and eliminate them outside the planting field prior to spraying fungicide.
6. Pruning tools used on infected plants should not be used on healthy plants, and tools should be cleaned before each use.
7. After harvesting, prune the diseased dry branches and cut off the leftover fruit stalks outside the planting area to minimize pathogen accumulation.
8. Control the number of pathogens in the soil by using the fungus antagonist *Trichoderma*.

Farmers have applied various fungicide groups such as phenylamide, the quinone outside inhibitors, and carboxylic acid amides to control these pathogens. Metalaxyl of the phenylamide group is the most commonly applied as a fungicide. It is applied daily in the durian plantations of southern Thailand, typically 2-3 times per month or more frequently during the rainy season from May to October, causing the development of resistance to this fungicide. The discovery of *P. palmivora* isolates resistant to metalaxyl suggested that this fungicide may be ineffective in suppressing *Phytophthora* infections in durians. Metalaxyl was sprayed, but its effectiveness was poor, resulting in reduced or total loss of durian harvests. Fungicide-resistant *Phytophthora* strains have been reported in many countries including Thailand. This becomes increasingly a more important issue to manage root rot disease in durian efficiently and safely. Additionally, the side effects of fungicides may come with risks, such as serious hazards to farmers, consumers, and the environment. (Kongtragoul et al., 2021)

Plant diseases, which are brought on by a variety of microorganisms, including fungi, bacteria, viruses, nematodes, and protozoa, have a significant negative impact on agricultural productivity and yield. Plant disease incidence has been decreased by the use of pesticides, less susceptible cultivars, crop rotation, and other management strategies, but their efficacy is often insufficient since soil-borne infections frequently persist and evolve resistance. In addition, employing synthetic pesticides excessively affects the environment and living organisms, disrupts ecosystems, and lowers agriculture's sustainability. (Miljakovic et al., 2020) Misusing chemical pesticides lead to soil and groundwater pollution and a rise in diseases and pesticide resistance. In addition, pesticides have negative effects on creatures that are not intended to be affected by them, such as beneficial insects like pollinators, soil microbiomes, and the health of terrestrial and aquatic ecosystems in general. (Tyskewickz et al., 2020)

Integrated Pest Management (IPM), organic farming, and other sustainable food production methods are used to protect the environment from the harmful effects of chemical fungicides. Biological Control Agents (BCAs), which are based on active microorganisms or their metabolites and natural products that reduce the population of plant pathogens, are one of these strategies. (Tyskewickz et al., 2020) BCAs have been developed in the past decades for the management of fungal and bacterial diseases. Some of the most intensively studied are bacterial and fungal belonging to the genus *Pseudomonas spp., Bacillus spp., Streptomyces spp., Trichoderma spp., Glomus mosseae, Gliocladium virens, Pythium oligandrum*, and *Beauveria bassiana*. They have been utilized as BCAs to successfully control the soil-borne diseases of valued crops caused by fungi, oomycetes, bacteria, and nematodes. To protect plants against pathogen infections, bacterial biocontrol agents employ a wide range of strategies. They may interact directly or indirectly with the pathogen to prevent or minimize plant disease via one or a combination of methods. (Tyskewickz et al., 2020: Bonaterra et al., 2022)

Citrus Greening Disease

Citrus is a genus of flowering trees and shrubs in the rue family, Rutaceae includes essential crops like oranges, lemons, grapefruit, and limes. There are hundreds of species that are expanding all over the world. Most of them have a pungent odor and contain essential oils in their foliage, blossoms, and fruits. Citrus trees in Thailand are divided into four categories, as shown below (Citrus, Wikipedia).

The orange group is divided into Sweet Oranges (*Citrus sinensis*) and Sour or Bitter Orange (*Citrus aurantium*).

Mandarin group including Satsuma Mandarin (*Citrus unshiu*), King Mandarin (*Citrus nobilis*), Mediterranean Mandarin (*Citrus delicoia*), and Common Mandarin (*Citrus reticulata*).

Pummelo and Grapefruits group including Pummelo (*Citrus maxima*) and Grapefruits (*Citrus paradise*).

Common acid member groups include Citron *(Citrus medica)* and Citrus lemon.

There are common citrus species grown in Thailand include Sweet Orange (*C. sinensis*), Tangerine (*C. reticulata*), Neck Orange (*C. nobilis*), Acidless Orange (*C. sinensis*), and Pummelo (*C. grandis* or *C. maxima*).

Citrus is a helpful fruit with a high nutrient content that is widely consumed. There are both local sales and exports to other countries. The most popular in the market is Tangerine. They rank among Thailand's major export crops roughly 128,046 rai are under cultivation, mostly in the north 95.3 percent were accountable. Citrus worth 1,138.26 million Baht were exported in 2021, totaling 47,318.94 tons. (Office of Agriculture Economics, 2021)

Citrus farmers frequently face the issue of greening disease, which is a disease that causes significant problems in citrus, resulting in lower output, no flavor, distorted fruit shape, and market unacceptability. Greening is a significant disease in citrus crops, especially mandarin and sweet orange, as well as pomelo (*Citrus maxima*) and small acid lime (*Citrus aurantifolia*). It causes citrus plant harm from the past to the present. (Jaikuankaew et al., 2016)

Citrus Greening Disease (CGD), also known as Huanglongbing (HLB), is important in citrus farming because of its severe damage, decreased yield, poor quality, and frequent disappearance before harvest. *Candidatus Liberibacter* species of bacteria are primarily accountable for the disease and cannot be grown on a culture medium (Poonyapitak et al., 2016; Jantasorn et al., 2007). These bacteria are limited to the phloem of the host plant and are found in low densities when living in the sieve tube of the host plant. Proteobacteria are alpha subgroups of bacteria that include two proteobacteria species, *Candidatus* Liberibacter asiaticus, and *Candidatus* Liberibacter africanus, that have peptidoglycan-like cell walls of gram-negative bacteria. (Jatasorn et al., 2007)

The symptoms discovered on the leaves will have two distinct characteristics: the first variety will have yellow symptoms with obvious green veins. On elder leaves, veins, and pulp may be more translucent than normal (vein clearing). Yellowing and blotchy mottling are frequently observed. The branches quickly dry out and perish. The end of the branch has a sign of mortality. Characteristic 2: The leaves are smaller, slenderer, longer, and thicker than normal. The vein is green, while the leaf area is yellow, this symptom is like signs of zinc deficiency. The blades tend to point upward. The characteristic signs of this disease used in observation are blotchy mottling patterns on leaves (Figure 5). (Tipu et al., 2021; Jantasorn et al., 2007)

Figure 5 Blotchy mottling patterns on leaves (A. Sornsaket)

One of the primary vectors for transmitting the disease by feeding on citrus leaves is the Asian Citrus Psyllid (ACP) or *Diaphorina citri* Kuawayama the insect in the family Psyllidae (Figure 6). ACP larvae and adults harm citrus trees by living on and sucking from the buds and new shoots. The larva secretes a white thread-like material like nectar, which causes black mold to grow. Furthermore, it will emit toxins, causing the tips to wither and the foliage to curl. If a serious injury causes the foliage to fall, the fruit is smaller or does not fruit at all. In the situation of greening disease, it functions as a vector for the infection to spread to other citrus trees. It can carry a bacterium for many days and can transmit to an uninfected plant when feeding on it. The phloem tissues of the roots and leaves are severely obstructed because of bacterial migration inside these two parts of the plant. As a result, obstruction to the circulation of nutrients and sugars in the interior tissues leads to leaf loss, uneven fruit size, which can impair the flavor and texture of fruits, premature fruit drop, and eventually the death of the tree. (Poonyapitak et al., 2016; Bendix and Lewis, 2018; Food Agriculture Organization of the United Nation [FAO]; Jantasorn et al., 2007; Tipu et al., 2021)

The excessive accumulation of starch in the remaining parenchyma cells of the aerial parts and photosynthetic cells is one of the most obvious symptoms of CGD in citrus trees. These can be detected by an iodine test. By observing how the leaves change color, one can tell whether a citrus tree is infected with the CGD by determining if the leaves turn blue or purple (positive test). If not, the leaves are healthy. (Whitaker et al., 2014)

Currently, there are no efficient methods or recommendations for controlling or managing Citrus Greening Disease. Academic articles frequently advise farmers to use pathogen-free seedlings or cultivar stems and to manage the insect vector Asian Citrus Psyllid. However, in farmers' growth circumstances, it has been discovered that pathogen-free citrus trees typically begin to exhibit signs of greening disease 1-2 years after planting, and because there are no concrete measures to manage the disease, bug vectors cannot be effectively controlled. Because the causative pathogen of the disease only lives in the plant's phloem cells, the injection of medicines such as ampicillin into the trunk is a common method for preventing or curing the disease. (Jaikuankaew et al., 2016; Stephano-Honedo et al., 2020).

Figure 6 Asian Citrus Psyllid (ACP) or Diaphorina citri Kuawayama

Source: https://www.kasetsanjorn.com/2172/

The use of antibiotics in field crops is, undoubtedly, constrained by the emergence of microbial resistance, which also poses a significant risk to human health due to its indirect effects (Stephano-Honedo et al., 2020.) The research from the Faculty of Pharmacy, Chiang Mai University, Thailand, found the accumulation of antibiotics in citrus fruit and remains for up to 90 days. They are seriously concerned about the citrus orchard's ecology, where antibiotic residues have begun to accumulate in the water, soil, and citrus plants. These might impact the food chain and result in bacterial resistance that can be transmitted to humans. In actuality, the disease cannot currently be treated with chemicals or any other types of controls. Therefore, it is obvious that a long-term cure for CGD infection in plants is required immediately (Jaikuankaew et al., 2016; Stephano-Honedo et al., 2020)

Agricultural innovations

In these three studies, agricultural innovations were used to promote development and manage plant diseases. Chitooligomers, silver nanoparticles (AgNPs), a composite of AgNPs and COAMs, innovative fertilizers, and integrated microorganisms are among the innovations used in these studies. The details are as follows.

Chitooligomers and Monomers (COAMs)

Shrimp shells are waste from the seafood industry that is dumped by millions of tons each year. Their structure consists of chitin polymer, a polysaccharide chain of N-acetyl-D-glucosamine (GlcNAc) bound together with β-1,4 glycosidic linkage. Chitosan is an acetylated derivative of chitin defined by the degree of acetylation (DA). The properties also depend on the degree of polymerization (DP). DA and DP determined general properties such as solubility and mode of chemical interaction, but another important property is the pattern of acetylation (PA), which can be defined as the sequence of glucosamine and N-acetyl glucosamine units along the molecular backbone. In the agricultural industry, chitosan and its oligomer are used to protect plants from bacteria, fungi, and viruses, as a plant growth regulator, and as a fertilizer additive. (Schmitz et al., 2019).

Chitosan oligomer is defined as chitosan with a degree of polymerization of less than 20 (for example DP6 = hexamer) and an average MW of less than 3.9 kDa (generally 0.2-3.0 kDa). It also has a greater solubility in addition to having outstanding qualities including biodegradability, biocompatibility, adsorptive capacities, and non-toxicity like chitin and chitosan. The structure of chitin, chitosan and chitosan oligomer show in Figure 7.

Currently, chitosan oligomer preparation techniques primarily include a chemical method (hydrogen peroxide oxidation, acid hydrolysis), a physical method (microwave treatment, UV radiation, and ultrasonic treatment), enzymolysis, an electrochemical method, and composite degradation methods developed from these methods. They have different advantages and disadvantages as shown in Table 1. (Liang et al., 2018)

Chitin and chitosan have been used in agriculture to control plant diseases. Both of them have demonstrated antiviral, antibacterial, and antifungal properties. Thus, they have been researched for many agricultural uses. They are also used to control disease or reduce the spread of plant diseases, to chelate nutrients and minerals, prevent pathogens from accessing them, or to enhance plant innate defenses. (El Hadrami et al., 2010)

Antimicrobial Activity of Chitosan and Chitosan oligomers

The antimicrobial activity of chitosan against bacteria and fungi has been reported in many articles. High-MW chitosan's possible antimicrobial effects included serving as a chelator of critical metals, preventing nutrients from being taken up from cells extracellularly, and modifying cell permeability since it is often unable to permeate the cell wall and cell membrane. However, low-MW chitosan has both intracellular and extracellular antibacterial action, which has an effect on mitochondrial function, RNA synthesis, and protein synthesis. Furthermore, the type of targeted microorganism has a significant impact on how chitosan acts as an antibiotic. (Ke et al., 2021) There are three proposed modes of action as antimicrobial mechanisms of chitosan and its derivatives as shown in Figure 8. (Ing et al., 2012; Ke et al., 2021)

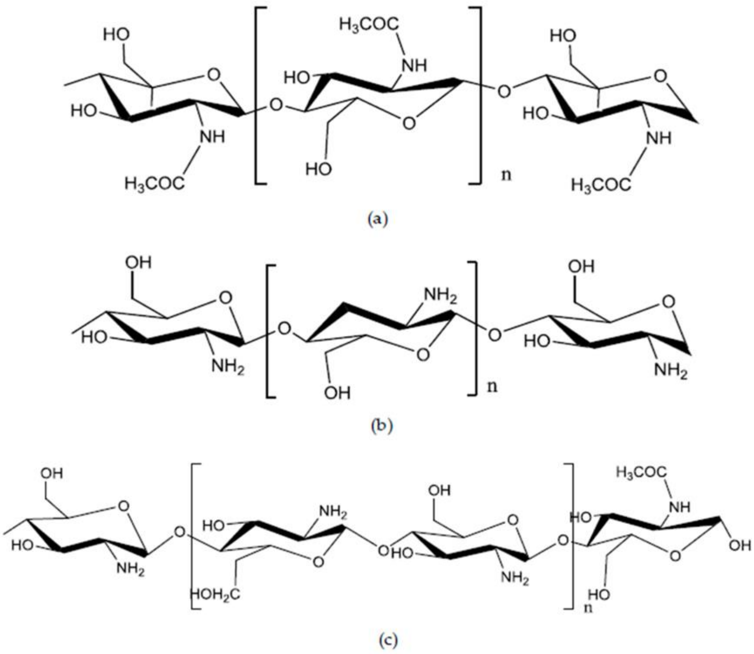


Figure 7 Structure of Chitin (a), Chitosan (b), and Chitosan Oligomer (c)

Source Liang et al. (2018)

Because of their positive charge, chitosan and its derivatives can interact with the phospholipids that make up the microbial membrane, which is negatively charged. As a result, the membrane's permeability will increase and cellular contents will leak out, eventually leading to cell death. (Ing et al., 2012)

By attaching to trace elements and blocking their availability for normal microbial development, chitosan and its derivatives can function as chelating agents.

Low molecular weight (low-MW) and oligo-chitosan can penetrate pathogen cell walls, bind to their DNA, and subsequently influence the synthesis of vital proteins and enzymes. Furthermore, ATP synthesis and mitochondrial activity are inhibited by low-MW chitosan and oligo-chitosan.

Table 1 The advantages and disadvantages of various preparation methods of COS

Preparation Methods of COS	Advantages	Disadvantages
Chemical degradation	Simple to handle	Difficult to separate and purify the products
Physical degradation	Easy purification and little contamination	Low productivity
Enzymatic degradation	Easy accessibility, no additional product modifications	High cost but low availability
Electrochemical degradation	Easy to operate and contamination-free	Short electrode life and easy to fail

Source: Liang et al. (2018)

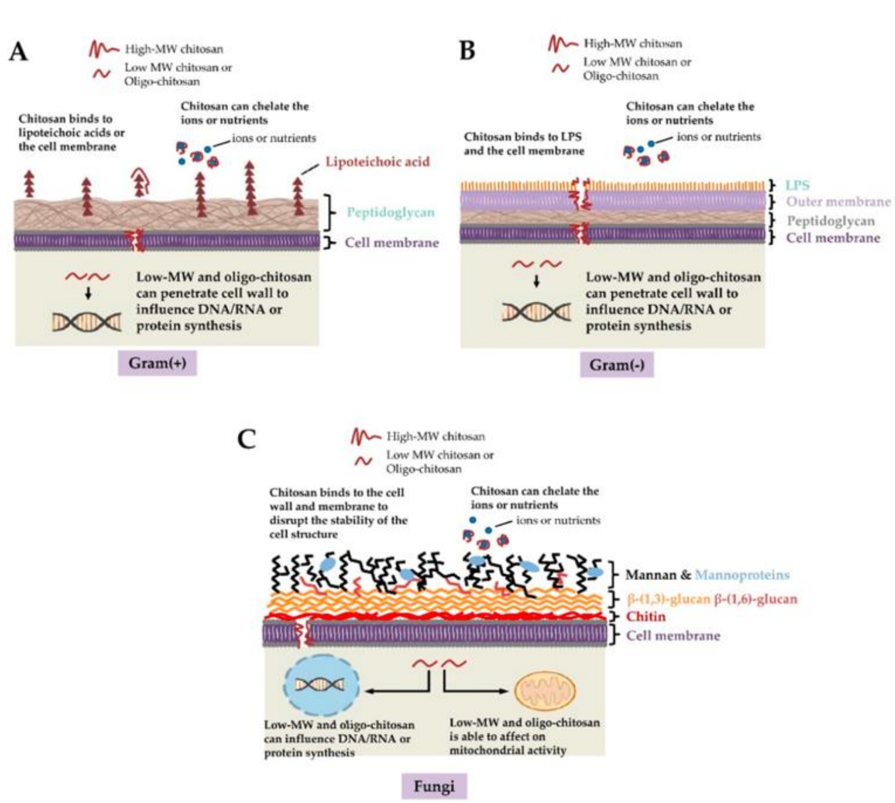


Figure 8 Antimicrobial Mechanism of bacteria gram-negative (A), bacteria gram-positive (B), and fungi (C).

Source Ke et al. (2021)

Chitosan has been demonstrated in several articles to have antibacterial and antifungal properties. (Ke et al., 2021) According to the research of Y.N. Fawzya (2019), the oligomers identified by TLC were monomer (1 unit) to hexamer (6 units) and showed their antifungal activity against the four fungi tested including Aspergillus *flavus*, *A. niger, Eurotium amstelodami,* and *Emericella nidulans*. The best inhibition was shown by 200 ppm of oligomer against *A. flavus.* (Fawzya et al., 2019) P. Li et al. (2016) investigate two kinds of oligochitosans with molecular weights less than 1500 Da and 3000 Da for the treatment of dry rot of *Zanthoxylum bungeanum* caused by *Fusarium sambucinum*. The results demonstrated that both oligochitosans can reduce *F. sambucinum* infection and strongly inhibit *F. sambucinum* radial colony and submerged biomass growth. These data suggest that the protective effects of oligochitosans on *Z. bungeanum* stems against dry rot may be due to a direct fungi toxic activity against pathogens as well as the elicitation of biochemical defensive responses in *Z. bungeanum* stems. (Li et al., 2016)

Bioactivity of Chitosan Oligomer and Monomer

Chitosan emerged as a promising agent used as a plant growth promoter and as an antimicrobial agent. It induces plant growth by influencing plant physiological processes like nutrient uptake, cell division, cell elongation, enzymatic activation, and synthesis of protein that can eventually lead to increased yield. It also acts as a catalyst to inhibit the growth of plant pathogens and alter plant defense responses by triggering multiple useful metabolic pathways. Several studies have shown how chitooligomer, a product of chitosan degradation, can promote plant growth. (Chakraborty et al., 2020)

A study by P.D. Dzung et al. (2017) on the effect of foliar application of oligochitosan with different molecular weights including 7.8, 5.0, and 2.5 kDa on growth promotion and fruit yield enhancement of chili plant. The results show oligochitosan with a molecular weight of 2.5 kDa proved to be the best, which increased the shoot fresh weight by 71.5%, shoot dry weight by 184%, total chlorophyll content by 12%, and fruit fresh weight by 49.8% for the control. Oligochitosan has the potential promising to apply as a biostimulant to enhance chili fruit yield significantly. (Dzung et al., 2017)

Chitosan oligomers induced genes in Arabidopsis that are principally related to vegetative growth, development, and carbon and nitrogen metabolism. Plants can respond well to a low-molecular-weight chitin mixture enriched to 92% with dimers (2mer), trimers (3mer), and tetramers (4mer). Compared with untreated plants, chitosan oligomers-treated plants had increased in vitro fresh weight (10%), radicle length (25%), and total carbon and nitrogen content (6% and 8%, respectively). The study shows that chitosan oligomers might play a role in nature as bio-stimulators of plant growth, and they are also a known direct source of carbon and nitrogen for soil biomass. (Winkler et al., 2017)

Chitosan oligomer can use as a plant growth promoter and as an antimicrobial agent as well. It induces plant growth by influencing physiological processes like nutrient uptake, cell division, cell elongation, enzymatic activation, and protein synthesis, which can lead to increased yield. It can also act as a catalyst to inhibit the growth of plant pathogens and alter plant defense mechanisms by triggering multiple useful metabolic pathways. (Chakraborty et al., 2020)

Chitosan oligomers' biological characteristics in fungal and plant systems have been studied. The heptamer is the oligomer that optimally stimulates plant defense mechanisms. Additionally, this size is optimal for inhibiting bacterial growth. The first-period chitosan was observed to be able to directly inhibit the growth of some plant pathogenic fungi when Hadwiger et al. discovered that it could stimulate the activity of a secondary pathway in plants that results in the accumulation of the phytoalexin pisantin, an antifungal isoflavonoid. According to a study by LA Hadwiger et al. (2017), the poly-glucosamine heptamer was able to induce a fully functional defensive response in pea tissue against a real pathogen of a pea. Their actions were related to the activation of defense genes known as pathogenesis-related (PR) genes, which caused the development of fungus to be slowed or stopped. The researcher added that chitosan oligomers' direct interaction with plant DNA chromatin resulted in the PR reaction. (Hadwiger, 2017)

Chito oligomers preparation methods

Chito oligomers or chitooligosaccharide (COS) are now produced using a variety of techniques, mainly chemical (hydrogen peroxide oxidation and acid hydrolysis), physical (microwave, UV, and ultrasonic treatment), enzymatic, electrochemical, and composite degradation methods derived from these techniques. Table 1 summarizes the advantages and disadvantages of various preparation techniques. (Liang et al., 2018)

Silver nanoparticles

Due to crop diseases that harm agricultural products all over the world. Plants are protected from disease using both synthetic and natural substances. The adverse impact on the environment of the chemicals used to treat plant diseases is very concerning. Researchers in the agriculture sector are looking for chemical alternatives. The application of nanotechnology in agricultural disease prevention holds a lot of potential for controlling insects and pathogens. The most researched and applied nanoparticles for enhancing the productivity, yield, and sustainability of agricultural crops are silver nanoparticles (AgNPs). Their strong pesticide, antifungal, antiviral, and bactericidal activities have long been acknowledged. The action of silver ions and nanoparticles on spore formation and disease development in plant pathogenic fungi may be the mechanism at play. AgNPs thus have a strong potential for application as nano pesticides for regulating phytopathogens. (Gupta et al., 2018)

Agriculture has already benefited from research on and the use of AgNPs. It is effective bactericidal, fungicidal, viral, and pesticidal properties have long been established (Gupta et al., 2018). There are several possible applications for silver, including the management of plant diseases. Due to its several modes of inhibitory effect against pathogens, silver can be used to treat a range of plant diseases as compared to synthetic fungicides. (Jo et al., 2019)

There are several studies of AgNPs demonstrate potential anti-phytopathogenic effects and plant disease management including fungi, bacteria, and viruses (Tariq et al., 2022). AgNPs were tested with three different isolated fungi from the sample of citrus, *Alternaria alternata* is the most abundant fungi pathogens isolated from citrus leaf and fruit spot followed by *Alternaria citri* and *Penicillium digitatum*. The result revealed that AgNPs have potent antifungal activity against isolated pathogens. (Abdelmalek and Salaheldin, 2016) The study of E.A. Salem et al. (2019), demonstrates the effect of silver nanoparticles on disease incidence and severity percentages of *Botrytis cinerea*-caused gray mold on tomato fruits during cold storage. It is also observed that any disease occurrence is not found nor did severity percentages of gray mold in tomato fruits treated with Nano silver particles throughout forty days of storage. (Salem et al., 2019) There was a study antifungal effects of AgNPs against various phytopathogenic fungi such as *Alternaria species*, *Botrytis cinerea*, *Cladosporium cucumerinum*, *Fusarium* species, *Pythium* species, etc. The outcomes showed that AgNPs have varying degrees of antifungal properties against these plant pathogens. (Kim et al., 2012)

The composite of silver nanoparticles and Chitooligomers

The application of AgNPs in the production of various hybrid products is being reconsidered due to their outstanding characteristics (Daniel et al., 2017). There are some studies for nanocomposite production of silver nanoparticles with chitosan and its derivative to produce nanocomposites with improved characteristics that might potentially be used as an antibacterial agent, silver was nano-mediated with chitosan as a reducing and stabilizing agent. (Sonseca et al., 2020)

L. Mei et al. (2020) have found the synergistic antibacterial effect of nanocomposite of chitosan oligosaccharide and silver nanoparticles. It is possible to enhance the surface charge of AgNPs by conjugating COS to them. This intensifies their electrostatic contacts and improves their capacity to cling to negatively charged bacterial cytoplasmic membranes. By functioning as an antibacterial agent in multivalent binding, COS can also make it easier for bacteria to absorb AgNPs. (Mei et al., 2020)

AgNPs and chitosan biopolymers can be combined to produce a more effective and diverse antibacterial effect. Incredibly strong antibacterial activity that acts against both gram-positive and gram-negative bacteria is observed in AgNPs-loaded chitosan composite. (Yang et al., 2016)

AgNPs were developed in a study by L.O. Cinterza et al. (2018) to balance toxicity and antibacterial activity. In order to stabilize the capping and increase biocompatibility, chitosan was used as the capping agent. The findings demonstrated that, in comparison to bare nanoparticles, chitosan stabilized AgNPs were nontoxic to normal fibroblasts, even at high concentrations, while considerable antibacterial activity was observed. (Cinteza et al., 2018)

There are some studies to show the efficacy of silver nano/oligomer for the treatment of plant diseases. L.T. Hien et al. (2022) have synthesized chitosan-stabilized silver nanoparticles (AgCSs) and evaluated antibacterial activity against *X. oryzae pv. Oryzae* bacteria caused the blight disease of rice. The study revealed that the use of AgCSs was effective in inhibiting those pathogens in rice. (Hien et al., 2022)

R. Dangtungee and P. Vatcharakajon, International Industry and Agriculture Innovation Research Center (IIAR), Maejo University International College, Chiang Mai, Thailand have successfully produced hybrid solutions composed of AgNPs and COAMs. It has previously been studied, and results indicate that it significantly inhibits the mycelial growth of an isolated strain of *Lasiodiplodia brasiliensis* from durian trees. This hybrid has been employed in field trials and has been successful in curing several plant diseases, including the Cassava mosaic virus, Phytophthora fungal infection, and Citrus greening disease.

Innovative fertilizer

Plant Nutrients

Natural chemical elements are required for plant development. All 17 elements are present in plant nutrition. Only carbon (C), oxygen (O), and hydrogen (H) can be trapped by plants from water and air, while the other 14 are Nitrogen (N), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg), Sulfur (S), Iron (Fe), Manganese (Mn), Boron (B), Molybdenum (Mo), Copper (Cu), Zinc (Zn), Chlorine (Cl), and Nickel (Ni) are nutrients that most plants absorb from the soil which is the main source of accumulation in nature. Plant nutrients are divided into two categories based on the quantity required by the plant. (DOA, 2000; Spring Green Evolution, n.d.; Plant nutrients, Wikipedia)

Macronutrients are the nine nutrients that plants require in significant amounts to develop and can be divided into three groups as follows.

1. The macronutrients found in water and air include Carbon (C), Oxygen (O), and Hydrogen (H).

2. The soil contains macronutrients such as Nitrogen (N), Phosphorus (P), and

Potassium (K) is what plants require in large amounts. These three elements are frequently inadequate to meet the demands of plants in natural soils. As a result, in the production of various kinds of agricultural fertilizers, these three elements are the primary ones to increase production and encourage plant development.

3. Secondary nutrients in the soil are Calcium (Ca), Magnesium (Mg), and Sulfur (S), which plants require after macronutrients. These nutrients are generally available to meet the requirement of plants.

Micronutrients are minerals that plants require in minute quantities. The soil contains the following nutrients: Iron (Fe), Manganese (Mn), Boron (B), Molybdenum (Mo), Copper (Cu), Zinc (Zn), Chlorine (Cl), and Nickel (Ni).

All plant nutrients, whether primary or secondary macronutrients, are equally essential to plant growth. Only the amount of demand drives the division of these nutrients into subcategories for easier study and comprehension. As a result, all of these nutrients are required for plants to live, blossom, fruit, and develop healthily. The following are the roles of plant nutrients (Spring Green Evolution, n.d.; Plant nutrients, Wikipedia; Office of The Permanent Secretary of Ministry of Agiculture and Cooperative-OPSMOAC, 2020):

<u>Primary macronutrients group</u>

Nitrogen (N): Plant development elements, specifically in the production of amino acids, nucleic acids, proteins, and different hormones, as well as being directly involved in the process of photosynthesis, where nitrogen is one of the primary components of chlorophyll, which turns plants green.

Phosphorus (P): Promotes and accelerates the development of plant roots. It is a component that influences blooming, fruiting, and seed production. It is also necessary for many activities, including photosynthesis, energy storage, and transmission as well as the plant respiration mechanism.

Potassium (K): Aids in the synthesis of sugars, starches, and proteins promoting sugar, starch, and oil transportation mechanisms, as well as plant water efficiency and yielding. It also increases plant resistance to certain diseases and insects.

Secondary macronutrients group

Calcium (Ca): One of the primary components of plant development that contributes to cell division, pollination, seed germination, and the formation of foliage and roots.

Magnesium (Mg): Important chlorophyll components aid in the synthesis of amino acids, vitamins, lipids, and sugars, allowing the acid-base conditions in the cells to be appropriate and promoting seed germination. Furthermore, magnesium aids in the absorption process as well as bringing phosphorous to good use.

Sulphur (S): A necessary component of amino acids, proteins, and vitamins in plants. Contributes to chlorophyll synthesis and seed development. Furthermore, sulfur is a component of volatile compounds that give some plants a unique smell.

Micronutrients group

Boron (B): Helping plants absorb calcium and nitrogen more effectively, as well as contributing to plant blooming and pollination. It is also essential in the transmission of sugar to the fruit, hormonal translocation, and plant cell division.

Copper (Cu): One of the components that contribute to the production of chlorophyll. It is one of the catalysts or stimuli in various plant processes such as respiration, enzyme function, food production, and reproductive processes, all of which influence plant blooming and fruiting.

Iron (Fe): One of the elements found in protein that contributes to photosynthesis and plant food production. It plays a role in the completion of the respiration process and growth.

Manganese (Mn): Nutrients that contribute to photosynthesis and enzyme activity influence the development of foliage, blooms, and fruiting. Furthermore, manganese regulates different activities involved in the effective use of iron and nitrogen.

Molybdenum (Mo): Nutrients that enable bacteria and soil microbes to trap nitrogen from the air, which influences protein synthesis and nitrogen function in plants, and also contribute to chlorophyll formation and phosphorus compound deformation.

When growing plants on the ground, the quantity of various nutrients in the soil inevitably varies with the rate of absorption and usage of plants. Some of these nutrients are used for development, while others are kept in different parts of the plant, such as leaves, stems, fruits, or flowers. As a result, when the harvest season ended and the product was removed from the area, the nutrients that used to accumulate in these soils were also permanently removed from the area. (Spring Green Evolution, n.d.)

Fertilizers

Substances or elements are added to the soil for the purpose of releasing plant nutrients, particularly nitrogen, phosphorus, and potassium, which plants still lack in order to grow well and produce higher yields. Fertilizers are classified into two types: organic fertilizers and chemical or scientific fertilizers. (Pimdee, 2017)

Organic fertilizers include manure, compost, green manure, and some organic industrial waste products. Important manures including swine, duck, and poultry manure, etc. are widely used. Manure is comparatively low in nitrogen, phosphorus, and potassium, approximately 0.5% nitrogen, 0.25% phosphorus, and 0.5% potassium. Furthermore, compost is the fertilizer obtained by composting plant leftovers prior to their incorporation into the soil to make fertilizer. (Pimdee, 2017)

Chemical or scientific fertilizer, these fertilizers are manufactured or synthesized industrially from native minerals or as a byproduct of some industrial factory. Chemical fertilizers are divided into two categories. A single fertilizer is one that contains only one nutrient in the formula, whereas complex fertilizers contain two or more nutrients in the formula. (Pimdee, 2017; Nippan, 2018)

Bio-fertilizers are fertilizers containing live microorganisms with special characteristics that can synthesize nutrient compounds for plants or transform plant nutrients that are not beneficial to plants into absorbable and usable forms. There are two types of microbes used in biofertilizers. (1) Microorganisms that can produce plant nutrient compounds such as Nitrogen by themselves, such as rhizobium, Frankia, and blue-green algae. (2) A group of microorganisms that aid in the dissolution of plant nutrients in the soil to make them more useful to plants, such as mycorrhiza, which enables phosphorus to be dissolved in the soil in a form that plants can absorb and use. (Nippan, 2018)

Dross fertilizer

Aluminium dross is an unavoidable by-product obtained from the aluminium smelting process which consists of metallic aluminium (60-75), oxides (20-30%), and salts (5-10%). The smelting process of 1000 kg of molten aluminium produced ca.20kg of dross (Sultana et al., 2013). Normally this dross is treated in rotary kilns to recuperate the remaining aluminium, and the resultant waste materials (salts) may still contain a small fraction of dross that is disposed of by landfills. When disposed of landfills the aluminium dross may come in contact with water and emits harmful gases such as NH_3, H_2, CH_4, and H_2S and contaminate the environment (Das et al., 2007). New environmental regulations have forced the aluminium industries across the world to recycle dross rather than landfill for the prevention of waste and environmental conservation (Capuzzi et al., 2018). The recycling of aluminium dross is becoming increasingly important because of environmental issues and high landfill costs. The process that could convert dross to value-added aluminium products is necessary for the profitability of dross recycling (Hong et al., 2010; Meshram and Singh, 2018).

Mostly, the dross treatment comprises crushing, grinding, and sieving, followed by water leaching to remove the salt. Later the salt is recovered by filtering and evaporation and is useful for applications such as mortar components, inert filling, etc. The produced alumina and alloying element can be used in various industries after washing (Yoshimura et al., 2008; Liu et al., 2013). The main element composition in dross raw material was aluminium oxide reacting with moisture and releasing ammonia gas about 47 % wt of dross as presented in Equation 1 and Equation 2. (Narayanan and Sahai, 1997; Mailar et al., 2016)

$$2AlN + 3H_2O\ (l) = Al_2O_3(s) + 2NH_3\ (g) \qquad (K = 4.88x10) \qquad (1)$$

$$2AlN + 6H_2O\ (l) = 2Al(OH)_3 + 2NH_3\ (g) \qquad (K = 8.11x10^{57}) \qquad (2)$$

Numerous studies have been reported on the conversion of aluminium dross into a useful resource. Gireesh et al (Mailar et al., 2016) reported that the mortar with 20% of aluminium dross yields optimum strength and durability for the concrete. In an interesting work, Yoshimura et al. (Yoshimura et al., 2008) successfully used aluminium dross as raw materials in refractories. In an important study, Dash et al. (Dash et al., 2008) leached out 85% of alumina from aluminium dross by acid dissolution in sulphuric acid. Pratumma et.,al. (Pratumma et al., 2016) applied the aluminium dross as a plant fertilizer by the acid treatment (HCl and H_3PO_4) process and found that acid treatment of aluminium dross provided a strong effect on the germination index (GI) of the seed. The efficiency of nitrogen trapping forming as ammonium ion ($NH4^+$) depended on the types of acid. HCl treatment caused an irreversible reaction equation; however, H_3PO_4 treatment provided a reversible reaction equation as shown in Equation 3 to Equation 8.

$$HCl\ (aq) \longrightarrow H^+ + Cl^- \qquad (3)$$

$$NH_3\ (g) + H^+ \longrightarrow NH_4^+ \qquad (4)$$

$$NH_4^+ + Cl^- \longrightarrow NH_4Cl\ (s) \qquad (5)$$

$$H_3PO_4 \longleftrightarrow H^+ + H_2PO_4^- \qquad (6)$$

$$NH_3\ (g) + H^+ \longrightarrow NH_4^+ \qquad (7)$$

$$NH_4^+ + H_2PO_4^- \longrightarrow NH_4H_2PO_4\ (s) \qquad (8)$$

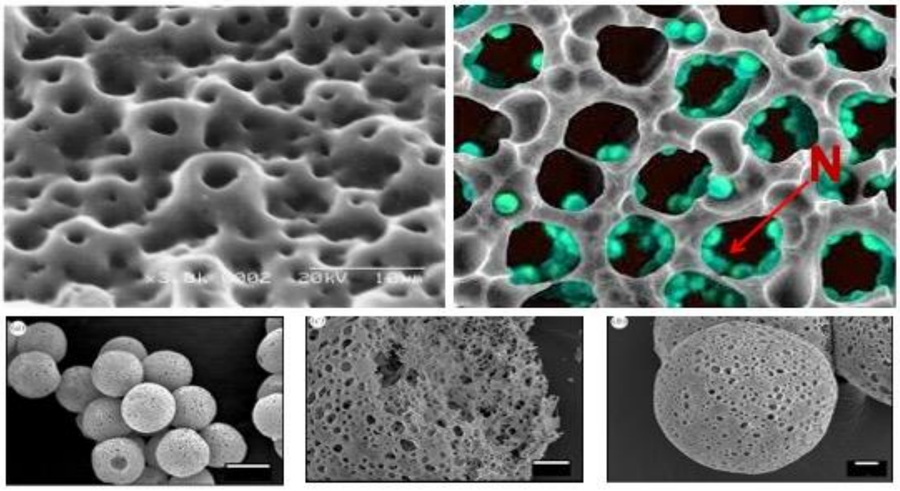


Figure 9 After acid treatment, the porous structure of aluminium dross allows it to trap nitrogen.

Microorganisms for agriculture

Plant diseases, which are brought on by a variety of microorganisms, including fungi, bacteria, viruses, nematodes, and protozoa, have a significant negative impact on agricultural productivity and yield. Approximately 20–40% of agricultural output losses are attributable to pathogenic diseases. Plant disease incidence has been decreased by the use of pesticides, less susceptible cultivars, crop rotation, and other management strategies, but their efficacy is often insufficient since soil-borne infections frequently persist and evolve resistance. In addition, employing synthetic pesticides excessively affects the environment and living organisms, disrupts ecosystems, and lowers agriculture's sustainability. (Miljakovic et al., 2020)

Chemical pesticides, the most common means of defending plants against fungus diseases, have significantly strained the agricultural environment in recent decades. Chemical plant protection solutions have a high level of performance, but their safe administration, effects on the environment, and harm to human and animal health raise concerns. The misuse of chemical pesticides leads to soil and groundwater pollution as well as a rise in diseases and pesticide resistance. In addition, pesticides have negative effects on creatures that are not intended to be affected by them, such as beneficial insects like pollinators, soil microbiomes, and the health of terrestrial and aquatic ecosystems in general. (Tyskewickz et al., 2022)

Currently, research is focused on more sustainable methods of managing plant diseases and enhancing crop yield, which is advised within an integrated crop management system (ICMS). Biological control, a crucial element of an ICMS, is the employment of advantageous organisms to lessen the impact of plant diseases and encourage favorable responses from the plant. The most common approach to biological control is the selection of antagonistic microorganisms, studies on their mechanisms of action, and the development of a biocontrol preparation. (D. Miljakovic et al., 2020)

Integrated Pest Management (IPM), organic farming, and other sustainable food production methods are used to protect the environment from the harmful effects of chemical fungicides. Biological Control Agents (BCAs), which are based on active microorganisms or their metabolites and natural products that reduce the population of plant pathogens, are one of these strategies. (Tyskewickz et al., 2022)

Antagonist: Organisms or antagonistic microbes used in biological plant disease control have four methods for regulating or inhibiting plant pathogens, which are as follows (Saranukrom Thai).

1. Competition

Antagonist microbes can compete with plant pathogens in a variety of activities, including nutrient and air utilization, and area occupancy, leading plant pathogens to not develop or live in areas where antagonists exist. The plant will become stronger and more productive. The most prevalent type of rivalry is the use of nutrients or substances for development found in the soil or in the surroundings, causing pathogens to lack nutrients, be unable to grow, and damage plants. For example, the antagonist bacteria *Pseudomonas fluorescens* produces siderophore substances that enable natural iron bonds better than the fungi *Gaeumannomyces graminis* var. tritici that cause wheat all-encompassing disease (Figure 10). This fungus is unable to damage wheat roots, allowing wheat to develop properly and generate a higher yield.

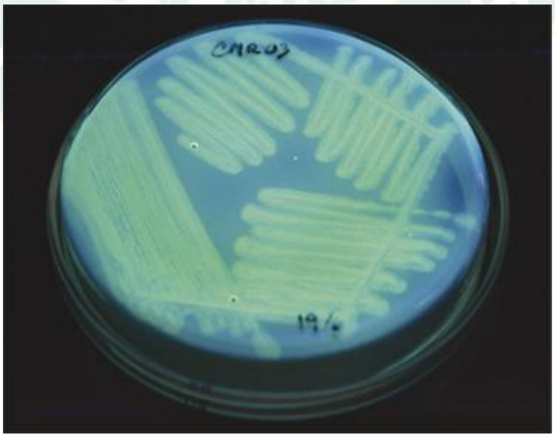


Figure 10 Pseudomonas fluorescence, an antagonist bacterium, produces a siderophore in order to thrive and compete with plant fungi pathogens.

Source Saranukrom Thai

2. Antibiosis

The antagonistic bacteria that have been chosen and are interested in the biological control of plant disease primarily concentrate on detrimental properties against pathogens. These antagonists can produce chemicals that inhibit or destroy pathogens, such as toxins or antibiotics. (Figure 11) The mechanism was discovered to be the first effective biological control of plant disease by antagonistic bacteria *Agrobacterium radiobacter* strain K84, which produces a bacteriocin called Agrocin 84 that inhibits or eliminates the *Agrobacterium tumefaciens* biotypes 1 and 2 that cause crown gall diseases in plants.

Figure 11 Bacillus bacteria produce antibiotics to destroy plant pathogens.

Source Saranukrom Thai

3. Parasitism

Bacteria with parasitic properties that invade and eradicate other organisms are rare. The use to control plant diseases is not as effective as a detrimental reaction. *Erwinia urediniolytica*, for example, eliminates the pedicel of rust disease fungal spores, as does *Pasteuria penetrans*, a parasite of the nematode *Meloidogyne incognita*, the cause of root-knot disease. Furthermore, *Trichoderma* spp. (Figure 12) was used to eliminate root rot caused by *Phytophthora* spp., and *Bacillus penetrans* was used to eradicate the root-knot nematode *Meloidogyne* spp.

4. Disease resistance induction

The mechanism of disease resistance induction is an interesting one to investigate. This is because microorganisms such as fungi or bacteria, particularly those that have lost their ability to cause disease, can initiate or encourage plants to develop resistance to pathogen destruction. Mutations in a single gene of the fungi *Colletotrichum magna*, the causative agent of anthracnose in cucurbits, for example, will not produce disease but will develop in the plant to help it resist the initial pathogen's infestation. In the instance of a living nonvirulent strain of *P. solanacearum*, the plant can be induced to produce tomatine at the roots, causing the tomato more resistant to the native *P. solanacearum* strain. Figure 13 shows plants that have been induced to acquire disease resistance by antagonistic microorganisms.

Figure 12 Trichoderma fungi are parasites that attack plant pathogens.

Source Saranukrom Thai

Figure 13 Plants that have been induced to acquire disease resistance by antagonistic microorganisms

Source Saranukrom Thai

BCAs have been developed in the past decades for the management of fungal and bacterial diseases. Some of the most intensively studied are bacterial and fungal belonging to the genus *Pseudomonas spp., Bacillus spp., Streptomyces spp., Trichoderma spp., Glomus mosseae, Gliocladium virens, Pythium oligandrum*, and *Beauveria bassiana*. They have been utilized as BCAs to successfully control the soil-borne diseases of valued crops caused by fungi, oomycetes, bacteria, and nematodes. To protect plants against pathogen infections, bacterial biocontrol agents employ a wide range of strategies. They may interact directly or indirectly with the pathogen to prevent or minimize plant disease via one or a combination of methods. (Tyskewickz et al., 2022; Bonaterra et al., 2022)

Most BCAs are filamentous fungi, particularly species of the genus *Trichoderma*, which belong to the phylum Ascomycota. It has been demonstrated that avirulent *Trichoderma* strains have beneficial characteristics that make them effective for biofertilization, biostimulator, and plant protection. *Trichoderma* species are effective biocontrol agents in soil ecosystems due to their capacity for rapid growth, adaptability to a wide range of substrates, and resistance to numerous toxic chemicals, such as fungicides (including azoxystrobin, 3,4-dichloroaniline, and trifloxystrobin, herbicides, and other organic pollutants. Additionally, it was shown that *Trichoderma* degrades several hazardous pollutants using cellulose/lignin degradation enzymes that have been proven to have the ability to metabolize xenobiotics. *Trichoderma* also produces copious amounts of conidia and chlamydospores and can adapt to changes in its environment. (Tyskewickz et al., 2022)

Trichoderma strains started out whitish and cottony, then developed into yellowish-green to deep green compact clumps, especially in the center of a growth area or in concentric ring-like zones on the agar surface, as shown in Figure 14 (Zin and Badaluddin, 2020). *Trichoderma atroviride*, *Trichoderma hamatum*, and *Trichoderma harzianum* are used to control fungal pathogens. Kasetsart University's Kamphaeng Saen campus research demonstrates that *Trichoderma harzianum* (Figure 15) was found in natural soils in Thailand, and strain CB-Pin-01 was highly effective in controlling the disease of various economic crops.

There are many studies of *Trichoderma* spp. against plant pathogens. F. Kerroum et al. (2015) conducted a study to determine the efficacy of *Trichoderma harziannum* in the treatment of *Fusarium* crown and root rot disease of tomato. The result shows the antagonistic effect of *T. harzianum* against *F. oxysporum* f. sp. radicis-lycopersici causing crown and root rot disease in tomatoes. (Kerroum et al., 2015) In the field, W. Nuangmek et al. (2015) studied the impact of *Trichoderma* sp. on the development and management of cantaloupe disease. Mildew and wilt diseases were not detected in plots inoculated with *Trichoderma*, whereas plots without *Trichoderma* had incidences of 26.70% and 80.00%, respectively. (Nuangmek et al., 2015)

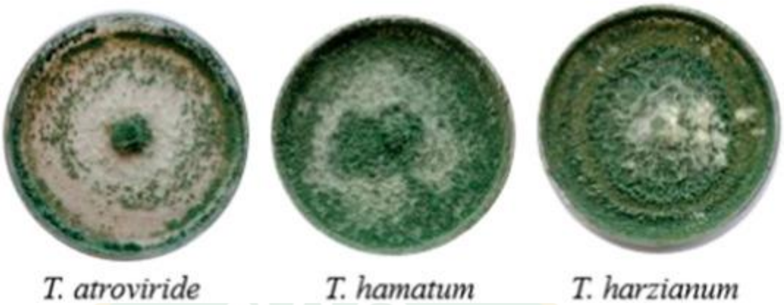


Figure 14 Three different isolated strains of Trichoderma spp.

Source Zin and Badaluddin (2020)

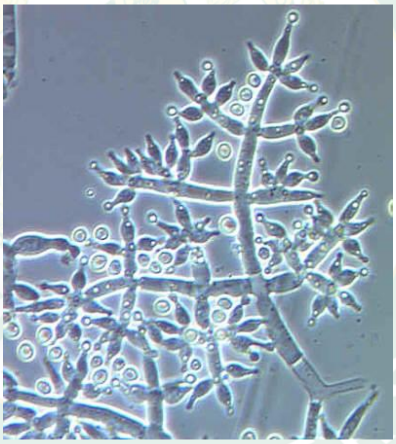


Figure 15 Trichoderma harzianum

Source https://en.wikipedia.org/wiki/Trichoderma#/media/ File:Trichodermaharzianum.jpg)

Non-pathogenic soil *Bacillus* species offer several advantages over other kinds of organisms, including the ability to produce endospores and the ability to endure harsh pH, temperature, and osmotic conditions. Some *Bacillus* species have been shown to colonize the root's surface, encourage plant growth, and lyse fungal mycelia. (Ashwini and Srividya, 2014) The focus of the current review is on the interaction between *B. subtilis* (Figure 16) and host plants in the rhizosphere via root colonization, their biocontrol capability and mechanism, and the use of *B. subtilis* to maintain and/or improve agricultural yield in the field under biotic and abiotic stress. (Hashem et al., 2019)

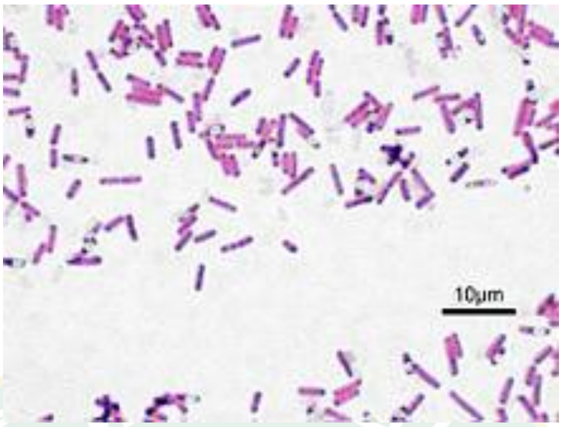


Figure 16 Bacillus subtilis

Source https://en.wikipedia.org/wiki/Bacillus_subtilis#/media/File:Bacillus_subtilis_Gram.jpg)

N Siwakorn et al. investigated the effectiveness of *B. subtilis* microorganisms and discovered that this antagonistic microbe inhibited development against *Phytophthora palmivora*, the causative agent of durian root and stem rot. (Siwakorn et al., 2016)

Chaetomium

Chaetomium is a saprophytic fungus that belongs to the Chaetomiaceae genus in the Ascomycetes group, which is one of the biggest saprophytic ascomycetes groups. A perithecium is produced, with a sterile hypha or hair encircling the outer cell wall. Ascospores are produced as a club-shaped ascus containing 8 spores. Chaetomium is a soil-borne fungus that thrives in humus, decomposing animal carcasses, and organic debris. It is sex propagated and tolerates a wide range of climatic circumstances. Because this fungus produces cellulase and xylanase enzymes, it is also found in organic fertilizers that can degrade cellulose and other organic matter. Furthermore, Chaetomium fungi can generate a variety of secondary compounds that can inhibit plant pathogenic fungi, including benzoquinone, chaetomanone, ergosterol, ergosteryl palmitate, chrysophanol, chaetoglobosin C, alternariol monomethyl ether, echinuline, and isochaetoglobosin D. (Maumoon et al., 2020; Kedves et al., 2021)

S. Chomchid et al. (2020) isolated *Phytophthora* spp. and *Pythium* spp. pathogens from the soil of a citrus tree afflicted with root rot disease. The results show that an ethyl acetate preparation of *Chaetomium* can effectively suppress the growth and development of pathogen fungus chlamydospores. (Chomchid et al., 2020)

CHAPTER III

RESEARCH METHODOLOGY

Chitosan oligomers and monomers (COAMs) benefit and its application in innovative organic methods for root rot disease treatment in durian crops.

Materials

Chitooligomers and Monomers (COAMs)

COAMs were produced by degrading chitosan, which was supplied by a local company. Non-thermal or cold plasma and ultrasonic treatment are used in the degradation technique. The oligomers and monomers were mixed in the optimum proportions to create the commercial brand name G-plus by Navatec (Thailand) Co., Ltd. Chiang Mai, Thailand.

The instruction for use: COAMs were diluted in clean water at a ratio of 1:500 for foliar application.

Integrated Microorganisms (IMOs)

IMOs are antagonist microorganisms including *Trichoderma harzianum* and *Bacillus subtilis* and some microorganisms used for soil amendment in crop areas. One kilogram of sugar is added to 500 grams of IMO, which has been dissolved in 200 liters of pure water with a pH range of 5.5 to 7.0. Keep the mixing under a shaded area for 24 hours and stir well before applying. IMO was provided by Kasetatchariya Co., Ltd., Samutprakarn, Thailand.

Methods

Relative inhibition

The effectiveness of COAMs against the fungus *Colletotrichum gloeosporioides* and *Fusarium pseudensiforme*, which cause the diseases anthracnose and dieback, respectively, were tested in a laboratory. The inhibition test was performed using the poisoned food technique. Briefly, 90 ml of sterilized potatoes dextrose agar was mixed with 10 ml of concentrate COAMs and poured onto sterile glass plates. The plates were left to solidify overnight. After that, the center of each plate was inoculated with a 12 mm size plug of the 7-day-old mycelial tip of each fungus, followed by incubating at 30 C in an incubator. The plates containing only PDA or 0.005% carbendazim served as negative and positive controls, respectively. The radial growth of mycelium was determined on day 7. The anti-fungal activity of COAMs was calculated from the formula: (DN-DT) *100/DN, where DN and DT were colony diameters of negative control and the test plates, respectively. The experiments were performed in 5 replicates. Moreover, COAMs treated with cold atmospheric Argon (Ar) or Helium (He) plasma jet (Ar-COAMs and He-COAMs respectively) were also used to test for anti-fungal activity. COAMs have been prepared by enzymatic method labeled to COAMs. The other was prepared by non-thermal plasma method, using Ar and He labeled to Ar-COAMs and He-COAMs respectively.

Determination of Inhibitory Concentration 50 value

The IC50 values of COAMs against *C. gloeosporioides* and *F. pseudensiforme* were determined by using the poisoned food technique. The different concentrations of COAMs ranging from 1/640 (v/v) to 1/5 (v/v) were used. The percent relative inhibition of each concentration was calculated according to the formula mentioned earlier. Seven to eight values of percent relative inhibition were fitted with the non-linear regression dose-response curve, which was generated by using the IC50 function in GraphPad Prism program (GraphPad Software Inc., San Diego, CA, USA).

Crop field test

Durian crops in the eastern area of Thailand including Chanthaburi, and Trat provinces are selected. The crops which have got the problems of root rot disease are recruited in this field test. Each crop selects 20 durian trees which 10 are the testing group compared to 10 as the control group. The severity scale and improvement score collection is considered as results.

The control group received metalaxyl 50 grams mixed with 20 liters of water, a common and traditional therapy used by farmers. Metalaxyl was applied by brushing on the infected area and spraying on leaves every 7 days for 4 times.

The testing group was treated with the organic innovative method including COAMs, and IMO. The mixing of IMO 2 to 5 liters per tree was sprayed on the soil around the durian tree for the first day of treatment in the testing group. Then, using a COAM mixture of around 1-2 liters per tree, thoroughly spray the underside of the leaves, branches, and trunk every 7 days 4 times. The application and examination for each group shows in Table 2.

Table 2 The application and examination schedule of the control and the testing group

Treatments	Day of treatment	Phloem tissues examination	Control group	Testing group
1^{st}	Day 0	Before treatment	metalaxyl	COAMs + IMO
2^{nd}	Day 7	-	metalaxyl	COAMs
3^{rd}	Day 14	Before treatment	metalaxyl	COAMs
4^{th}	Day 21	-	metalaxyl	COAMs
No treatment	Day 28	Examine	-	-

A digital microscope is used to examine the phloem tissues of the durian tree to identify the severity of the root rot disease. The steps before examining the phloem tissues are (1) using a sharp knife, (2) cracking durian tree bark until seeing the phloem, and then (3) using a digital microscope to examine the appearance of phloem tissues as shown in Figure 17. The phloem tissues are evaluated as a severity scale before the first treatment in all group.

An infection's severity is measured on a scale from 0 to 5. Scale 0 is no infection. Scale 1-2.99 is mild infection with slightly dry and light brown tissues. Scale 3 to 4.99 are moderate infections with dry and brown tissues. Scale 5 is a severe infection with very dry and dark brown tissues as shown in Figure 18 Durian trees with a severity rating of five cannot be treated; instead, they must be burnt to prevent the spread. The selected durian tree must have a severity scale of 4 or above.

Figure 17 Step of determining phloem tissue, (1) Use a sharp knife, (2) Crack durian tree bark until seeing phloem, (3) Use a digital microscope to examine the appearance of phloem tissues.

Durian trees commonly renew phloem tissues to replace the infected area as they get cured or recover from the root rot disease. When comparing the reconstructed phloem tissues to the tissues before treatment under a digital microscope as shown in Figure 18, the improvement criteria are applied. The improvement scores criteria as shown in Table 3. Using the criteria of improvement scores. After 14 days and 28 days of the initial treatment, the phloem tissues in all groups are scored for improvement.

Determine the difference between control and testing using the Excel program to analyze data from a t-test Two-Sample Assuming Unequal Variance at 99% confidence ($p<0.01$).

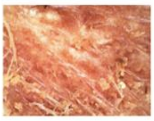
Phloem tissues by digital microscope	Severity scale	Definition	Appearance and Color of phloem tissues
	0	No infection	Wet tissues, Amber-Yellow
	1 - 2.99	Mild infection	Slightly dry tissues, Light brown
	3 - 4.99	Moderate infection	Dry tissues, Brown
	5	Severe infection	Very dry tissues, Dark brown

Figure 18 The severity of infection scale and description

Figure 19 Improvement scores 0 to 5

Table 3 Improvement score criteria

Improvement Score	Changing compared to before treatment	Definition
4 – 5	Obviously changing	Cured and improved
2 – 3.99	Moderate changing	Better improvement
>0 – 1.99	Slightly changing	Slightly improvement
0	No change	No improvement

Aluminium dross neutralization and its application as plant fertilizer

Materials

ADC12 and 6063 aluminium dross were procured from the smelting process factory, SRI Metal Products Co, Ltd, Suphanburi, Thailand. Hydrochloric acid and phosphoric acid were procured from Sigma-Aldrich, USA. Plant seeds including Chinese cabbage, sweet corn, basil, and spring onion seeds were procured from Chia Tai Co., Ltd., Bangkok, Thailand. Coconut husk for planting was procured from the local market.

Methods

1 Primary nutrient in aluminium dross the aluminium dross was sieved (100 µm) and was dried in an oven at 80°C for 3 hours and later neutralized with phosphoric acid and hydrochloric acid. The concentration of the acid used is 14 volume/volume percent (v/v%). The acid neutralization was determined by a pH meter. Dross ADC12 and 6063 grades were analyzed the chemical composition of primary nutrients and secondary nutrients.

2. Plant growth determination – Height and weight measurement the effect of 14 v/v% of HCl and H_3PO_4 treated and untreated aluminium dross fertilizer was analyzed by determining the height and weight of the plant along with the control plant. Crushed coconut husk and aluminium dross fertilizer were mixed at various proportions as shown in Table 4. The content of fertilizer was varied at 3, 5, 10, 20, 30, and 50wt%, respectively. 0.13g of Chinese cabbage seed (20 seeds) were used for pot experiment studies under controlled temperature, light intensity, and humidity for 13 days. 2 mL of water was applied to each pot and the physiological parameters were recorded. After treatment, the plant was uprooted from the pot and then eliminated the crushed coconut husk from the root followed by washing with water.

3. Effect of treated and untreated aluminium dross fertilizer on the plant growth 0.13g (around 20 seeds) of Chinese cabbage, sweet corn, basil, and spring

onion seeds were planted in soil under controlled temperature, light intensity, and humidity. Then 2 mL of water was applied and physiological parameters of treated and untreated aluminium dross fertilizer with 14v/v% of HCl and H_3PO_4 were recorded along with the control plants for 20 days.

4. Effect of dross on soil pollution ADC12 treated with HCl and H_3PO_4 (10wt%) of soil was compared with the original soil. Chinese cabbage was planted in soil for 20 days under controlled temperature, light intensity, and humidity. Then, the soil was dried in an oven at 60-70°C for 12h. The chemical element composition of the soil was measured by using the XRF technique (Horiba XGT-5200).

Table 4 The weight of crushed coconut husk and aluminium dross at the various ratios

The content of aluminium dross (wt%)	Crushed coconut husk (g)	Aluminium dross (g)
control	20.00	0.00
3	19.40	0.60
5	19.00	1.00
10	18.00	2.00
20	16.00	4.00
30	14.00	6.00
50	10.00	10.00

Chitooligomer-Silver nano hybrid solution for the treatment of Huanglongbing/Citrus Greening Disease

Material – Hybrid solution preparation

Silver nanoparticles (AgNPs) were prepared by the extraction of natural silver metal 99.99%. Chitooligomers and monomers (COAMs) were prepared by chitosan degradation with cold plasma and ultrasonic methods. Mix AgNPs with COAMs by heating and continue stirring for a period of 1-3 hours. Then add the adhesion enhancer at room temperature and stir continuously for 12 hours.

Methods

Citrus Greening Disease (CGD) testing

Diagnosis criteria for showing symptoms of CGD consider the splotchy mottling of the entire leaf as shown in Figure 20.

Figure 20 Citrus leaves with CGD show the splotchy mottling of entire leaves.

Use an iodine test kit to determine whether citrus trees were infected by the greening disease.

1. Crush the citrus leaves (3 g) with distilled water (3 ml) in the mortar. Then filter the crushed solution and put it in a transparent sachet.
2. Dip the iodine litmus paper into the sachet, left it for 1 minute, and then observe the color change.
3. Use a dropper to drop iodine solution into the sachet, left it for 1 minute, and then observe the color change.
4. The observed color of the solution in a sachet will turn blue or purple color meaning a positive result or infected tree detection while no color change means no infection or negative result as shown in Figure 21.

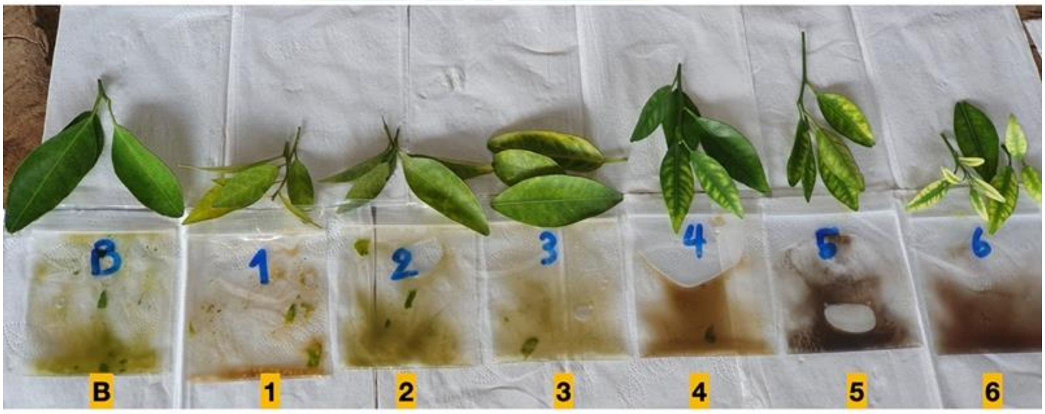


Figure 21 The color of the testing solution of CGD (1-6) compared to baseline or no symptom (B)

Field crops test

A citrus plantation in Fang district, Chiang Mai province, was selected for 50 citrus trees that had CGD which was confirmed by iodine test kits. The 10-year-old citrus trees were between 2.00 and 2.50 meters tall, with planting and row spacing of 4.5 meters. The canopy of the citrus tree is 2 meters wide.

An experiment with a completely randomized design (CRD) was designed. Three different substances-ampicillin, AgNPs, and AgNPs-COAMs or hybrid solution — were used in five different ways, three times a repetition every month for each plant. Gibberellic acid is a plant hormone which added to groups 3 and 5. The methods are shown in Table 5.

Table 5 Group of treatment

Group	Treatment
1	Ampicillin
2	AgNPs
3	AgNPs + Gibberellic acid
4	The hybrid solution
5	The hybrid solution + Gibberellic acid

In accordance with the experimental plans, testing substances at the specified concentration were injected into the trunks of the chosen citrus trees (trunk injection), and every group receives an equal 800 ml.

Criteria of treatment and statistic

1. The CGD is confirmed by liquid and litmus iodine test kits.

2. Physical changes in citrus leaves are observed as the criteria for disease improvement. (Table 6)

3. The statistical F-test will be used to verify the impact of physical changes at 99% confidence, $p < 0.01$.

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Table 6 Physical citrus leaves criteria and determination

Physical leaves criteria	Determination
Shoot length	Centimeter (cm)
Number of leaves	Number of leaves
Leaf area	Centimeter square (cm^2)
Greenness of leaves	Greenness SPAD index

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CHAPTER IV
RESULTS

Chitosan oligomers and monomers (COAMs) benefit and its application in innovative organic methods for root rot disease treatment in durian crops.

COAMs preparation and properties

1. Chitooligomers and monomers (COAMs) have been prepared by the degradation of chitosan. The degradation method used non-thermal or cold plasma and ultrasonic treatment.

2. The physical properties and molecular weight of COAMs solutions were analyzed. Their appearances are a yellow liquid, with less viscosity, and a pH of 5.0. The molecular mass of COAMs was measured by Gel Permeation Chromatography (GPC) and viscometry with the treatment for 0 to 10 minutes. The results show in Table 7.

Table 7 The molecular weight of COAMs measured by viscometry and GPC.

Sample after treatment	Viscosity [η], dl/g	Molecular weigh $M\eta \times 10^{-3}$ (viscometry)	Molecular weight $Mw \times 10^{-3}$ (GPC)
1 (10 minutes)	0.078	1.98	1.94
2 (7 minutes)	0.156	3.84	3.80
3 (3 minutes)	0.183	4.54	4.50
4 (0 minute)	0.206	5.06	5.09

3. The preliminary results of COAMs treated with cold plasma and ultrasonic showed that the molecular weight measured by the viscosity and GPC technique was significantly reduced ($p<0.05$).

4. Particle sizes were measured by Nano Particle Analyzer Brand: HORIBA model: SZ-100V2 of NSTDA Characterization and Testing Service Center (NCTC), Thailand. The average particle size of the solution is 15.12 ± 0.69 nm as shown in Table 8.

Table 8 The particle size of the hybrid solution

Measurement	Z average	PI
1	14.8	0.314
2	14.4	0.312
3	15.2	0.383
4	16.3	0.285
5	15.4	0.339
6	14.6	0.316

Laboratory and crop field test

Relative inhibition

Relative inhibition has been tested. The inhibition of mycelial growth was performed to examine the anti-fungal activity of COAMs. The result showed that COAMs, Ar-COAMs, and He-COAMs could inhibit the mycelial growth of *C. gloeosporioides*, with the average percent inhibition of 96.62+5.19%, 99.66+3.42%, and 100%, respectively. These values were comparable to that of 0.005% carbendazim, which showed a percent relative inhibition of 95.52+2.52% (Figure 22) As COAMs, Ar-COAMs, and He-COAMs showed no different percent relative inhibition against *C. gloeosporioides*, only COAMs was chosen for further inhibition study against *F. pseudensiforme*. The result showed that COAMs could also inhibit the mycelial growth of *F. pseudensiforme*, with a percent relative inhibition value of 99.77+0.51%. Interestingly, it was found that all samples of COAMs treatment exhibited slightly good inhibition and better than carbendazim. Plasma treatment on Argon and Helium transfer was not different as much but good significant than a non-treatment sample.

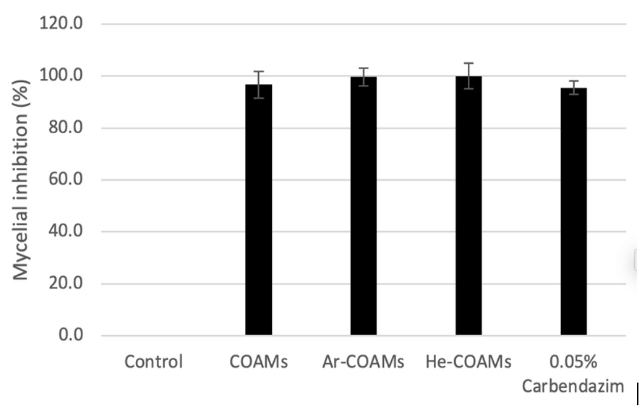

Figure 22 Comparison of mycelial growth inhibition (%) of COAMs, Ar-COAMs, He-COAMs, and carbendazim

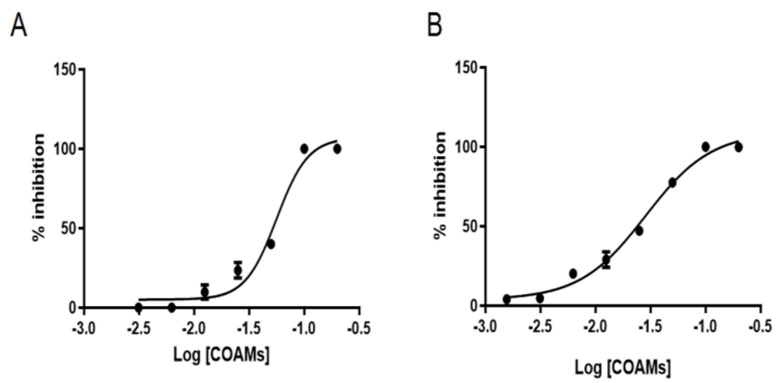

Figure 23 Non-linear regression dose-response plot determining the IC50 values of COAMs against (A) C. gloeosporioides and (B) F. pseudensiforme.

Determination of Inhibition Concentration 50 (IC50)

The determination of the IC50 value was investigated. The nonlinear regression dose-response curves of COAMs against *C. gloeosporioides* and *F. pseudensiforme* were generated to calculate the IC50 values (Figure 23A and 23B), and the IC50 values are summarized in Table 9.

Table 9 The IC50 values of COAMs against C. gloeosporioides and F. Pseudensiforme

Fungus	IC_{50} (v/v)
C. gloeosporioides	0.0562 ± 0.0014
F. pseudensiforme	0.0285 ± 0.0028

Crop field test

Crop field test result on the target area. Durian crops in Chanthaburi and Trat province, Thailand have been found root rot disease and selected. A total of 12 crops (around 1,200 rai of area) were selected in this study. There are 9 crops from Chanthaburi and 3 crops from Trat as shown in Table 10. The number of durian trees is 180 and 60 from Chanthaburi and Trat respectively.

The severity scales of Chanthaburi in the control and testing groups are 4.75 and 4.65, respectively. The scales in Trat are 4.80 for the testing group and 4.78 for the control group. Table 11 shows the severity scale of all crops in Chanthaburi and Trat. The findings in the table show that in both provinces, there was no significant difference ($p>0.01$) in the severity scale between the control and testing groups.

Table 10 Data of selected crops and number of durian trees by provinces

Province	Number of tested crops
Chanthaburi	9
Trad	3
Total	12

Table 11 Severity scale by durian crop in Chanthaburi and Trat

Number of crops	Chanthaburi		Trat	
	Control group	Testing group	Control group	Testing group
1	4.70	4.50	4.75	4.79
2	4.75	4.80	4.85	4.80
3	4.70	4.60	4.80	4.75
4	4.65	4.45		
5	4.70	4.50		
6	4.80	4.80		
7	4.85	4.75		
8	4.80	4.70		
9	4.80	4.75		
average	4.75	4.65	4.80	4.78
t-Test	ns		ns	

Note: ns = not significantly different, ** = significant difference at $p \leq 0.01$

Phloem tissues were examined by a digital microscope before the first treatment of each group (day 0), before the 3rd treatment (day 14), and 7 days after the last treatment (day 28). The results of phloem tissues of durian crop at Chanthaburi and Trat province as shown in Figure 24. Durian tissues were rarely or slightly improved in the Chanthaburi and Trat control groups, but changes were noticeable in the testing group beginning on day 14 and were highly obvious by day 28. It was discovered that improvement was the same for all the testing group's crops.

The durian crops have also been evaluated and the improvement score has been recorded on the 14 and 28 days after the first treatment as shown in Table 4.6. Consider the statistics in Table 12 for Chanthaburi Province, the average improvement scores are 0.34 for the control group and 4.66 for the testing group. The results revealed that durian trees in the testing group had a statistically significant improvement score higher than durian trees in the control group (p<0.01). Durian trees in Trat province showed an average improvement score of 0.67 and 4.83 for the control and testing groups respectively. The findings revealed that the testing group outperformed the control group by a statistically significant difference.

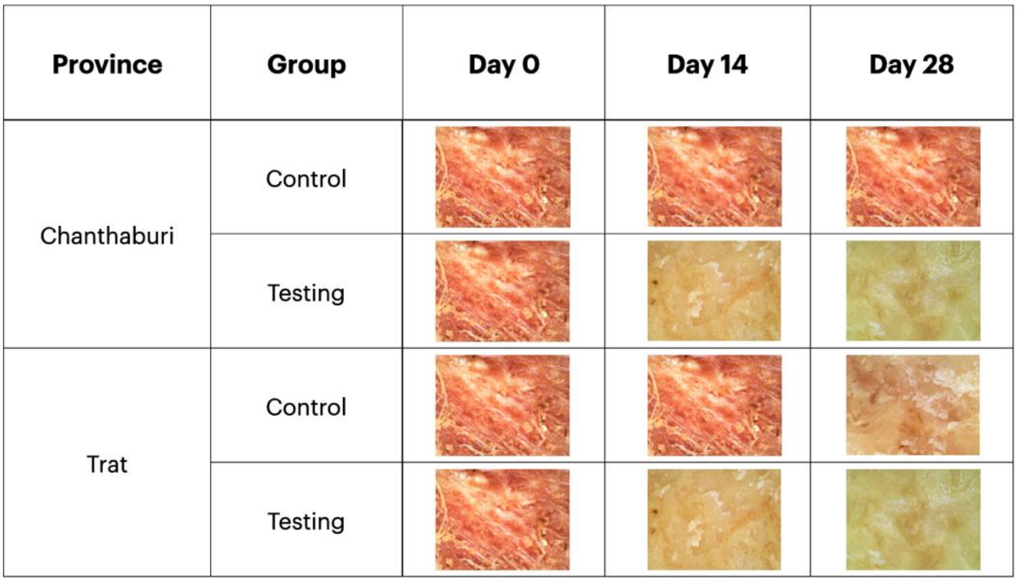

Figure 24 Phloem tissues of root rot disease durian tree viewed through a digital microscope.

Table 12 The improvement scores on 28 days after the first treatment

Number of crops	Chanthaburi		Trat	
	Control group	Testing group	Control group	Testing group
1	0.30	4.60	0.70	4.85
2	0.36	4.55	0.64	4.90
3	0.40	4.75	0.67	4.74
4	0.35	4.80		
5	0.40	4.70		
6	0.20	4.55		
7	0.35	4.60		
8	0.40	4.75		
9	0.30	4.64		
average	0.34	4.66	0.67	4.83
t-Test	**		**	

Note: ns = not significantly different, ** = significant difference at p $\leq$ 0.01

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After treatment with COAMs and IMO, use a sharp knife to crack the durians bark until seeing phloem as shown in Figure 25 and 26. The outside look is white or light yellow. This demonstrates that the infected durian trees started to recover.

The phloem tissues were also viewed through a digital microscope shown in Figure 27. The tissues were found to be properly wet and amber yellow in color, indicating that durian tissue has been cured and improved from root rot disease.

Figure 25 The external appearance of rebuilt phloem tissues after COAMs+IMO treatment

Before treatment After treatment

Figure 26 Phloem tissue appearance of durian tree before (left) and after (right) COAMs + IMO treatment.

Researchers also found durian leaves changing in color to dark green, thick, and shiny, and there are new shooting leaves as shown in Figure 28. The result implies that the anti-pathogen was successful and improved the growth-up branch of the durian tree.

Figure 27 Cured and improved phloem tissues viewed through a digital microscope.

Figure 28 The shooting of new leaves

Aluminium dross neutralization and its application as plant fertilizer

Primary nutrients in aluminium dross

The chemical composition of primary nutrients and secondary nutrients was examined as shown in Table 13.

Table 13 Chemical analysis data of dross ADC12 and 6063 grade.

Composition	ADC12 (wt%)	6063 (wt%)
Aluminium oxide (Al_2O_3)	55.17	45.39
Sodium oxide (Na_2O)	18.07	47.65
Magnesium oxide (MgO)	9.40	0.02
Silicon dioxide (SiO_2)	1.44	0.53
Potassium oxide (K_2O)	2.12	0.10
Calcium oxide (CaO)	4.97	0.79
Titanium dioxide (TiO_2)	2.48	4.19
Iron oxide (Fe_2O_3)	2.95	1.23
Zinc oxide (ZnO)	3.37	0.06

Plant growth determination

Aluminium dross treated with acid was non-toxic to plants which can be clearly seen that GI of plants with dross fertilizer showed better than GI of the control (DI water) as shown in Figure 29 (Pratumma et al., 2016).

The average height of Chinese cabbage planted in treated ADC12 and 6063 aluminium dross fertilizer with H_3PO_4 are shown in Figures 29 and 30, respectively. According to Figures 29 and 30, the best results are obtained for 10wt% aluminium dross fertilizer, irrespective of ADC12 and 6063, which presents the highest average height of ca. 9 and 8.5cm, respectively. Interestingly, above 10wt% of treated aluminium dross fertilizer, the height of the plant dropped which may be due to the high NaCl content in dross fertilizers as shown in Table 14 (Shrivastava et al., 2015). NaCl provided a bad effect on plants because Na was absorbed in its root decreasing osmotic pressure at that area obstructing water-absorbing ions for nourishment. Another reason may be that an increase in the content of NaCl caused a reduction in the absorption of nutrients (Razaq et al., 2017).

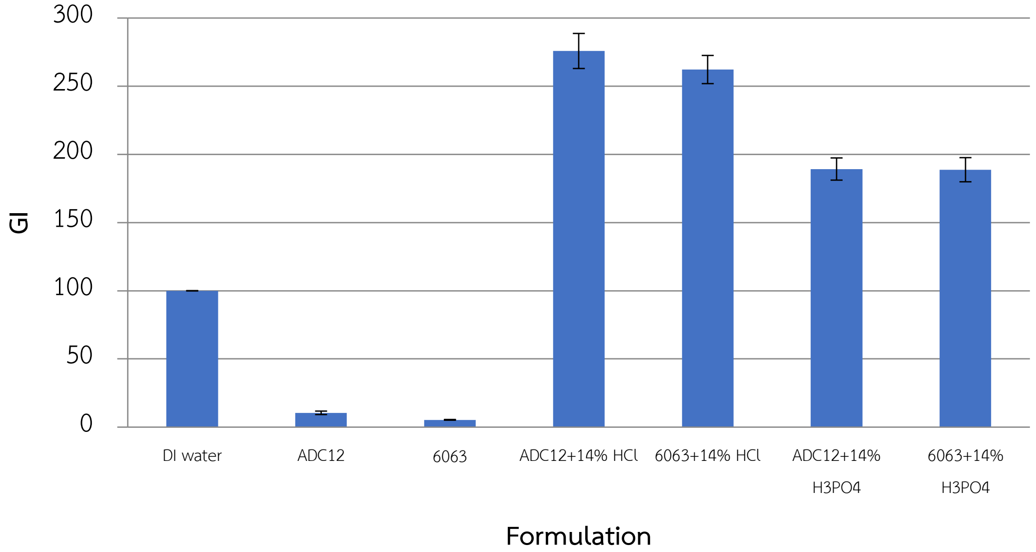

Figure 29 Variation of germination index (GI) as a function of concentration of hydrochloric acid and phosphoric acid

Plant height

The average height of the Chinese cabbage planted in treated ADC12 and 6063 aluminium dross fertilizer with HCl are shown in Fig 31 and 32, respectively. The best results were observed at 3, 10, and 20wt% treated aluminium dross fertilizer irrespective of ADC12 and 6063. The maximum height of Chinese cabbage is ca.10 cm for HCl-treated ADC12 and 9 cm for HCl treated 6063.

The 10wt% of the dross fertilizer showed enhancement in the height of the plant when compared with control and other treated samples. A comparison of the average height of Chinese cabbage planted in 10wt% of aluminium dross fertilizer is shown in Fig 33 Treated ADC12 aluminium dross fertilizer with HCl contributed to the highest average height of Chinese cabbage.

Table 14 Level of salty in planting material

Aluminium dross (wt%)	Salty ($mS.cm^{-1}$)			
	Aluminium dross ADC12		Aluminium dross 6063	
	Before treating	After treating	Before treating	After treating
0	0.02	0.02	0.02	0.02
3	0.47	0.46	0.49	0.49
5	0.83	0.83	0.83	0.81
10	1.65	1.68	1.63	1.62
20	3.28	3.23	3.29	3.30
30	4.97	5.10	4.91	5.00
50	8.15	8.16	8.25	8.21

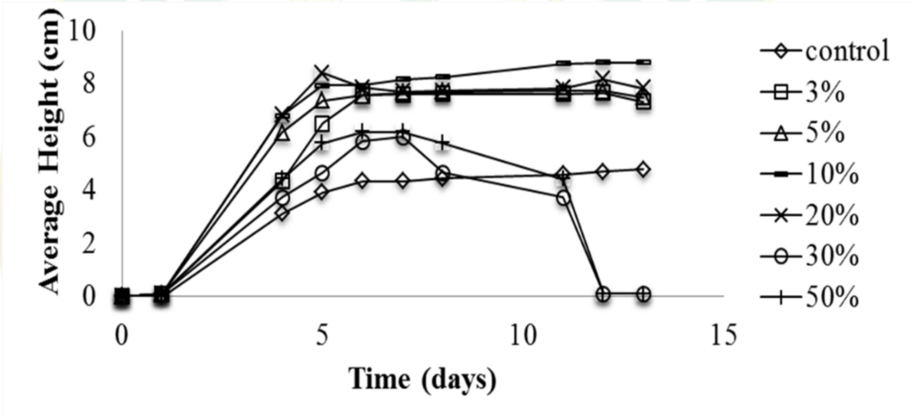

Figure 30 Average height of Chinese cabbage planted in treated dross ADC12 grade with H_3PO_4

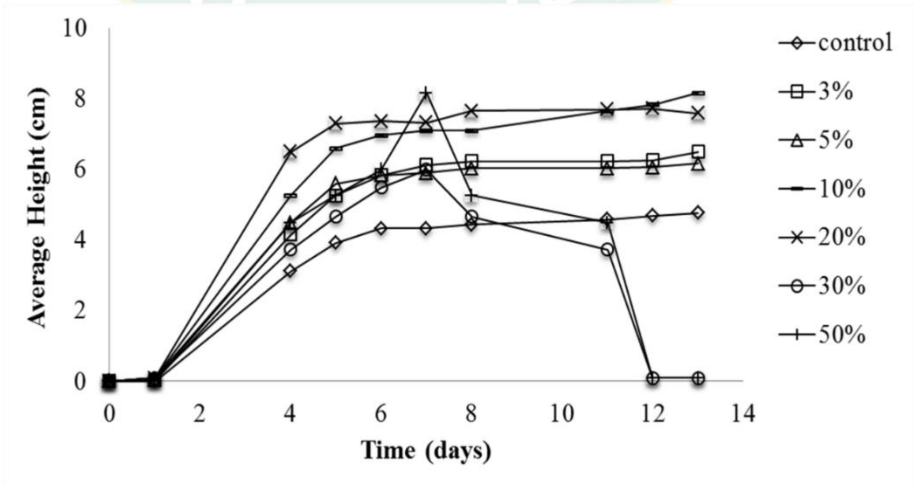

Figure 31 The average height of Chinese cabbage planted in treated dross 6063 grade with H_3PO_4

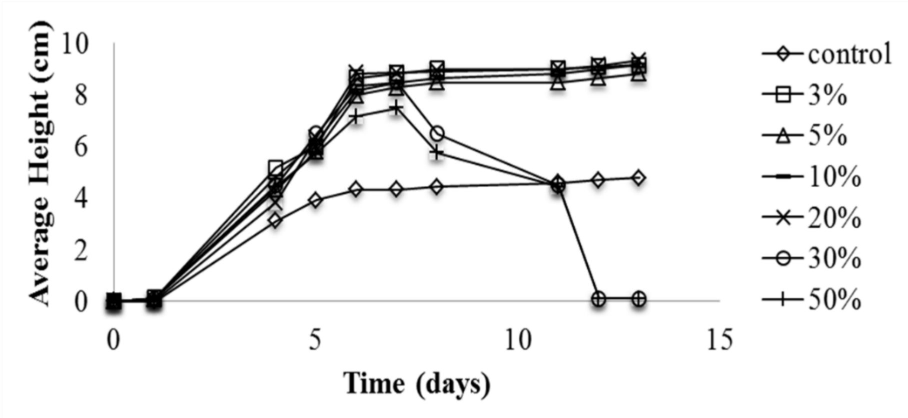

Figure 32 Average height of Chinese cabbage planted in treated dross ADC12 grade with HCl

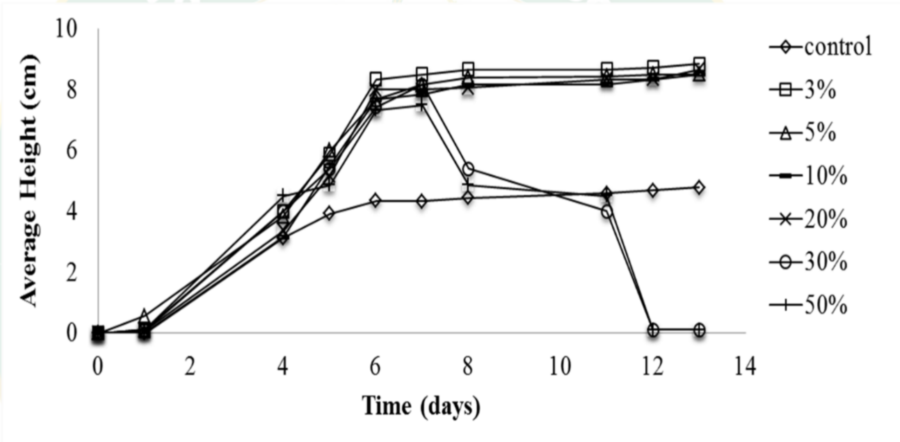

Figure 33 Average height of Chinese cabbage planted in treated dross 6063 grade with HCl

From Figure 29-32 irrespective of the type and content of the fertilizers, the growth of the plant from the first day to the fifth day sharply increased. After the fifth day of planting, the plant growth is constant. On the other hand, after 5 days the plant is withered and desiccated at 30wt% and 50wt% of fertilizer. Hence, the results showed that 10wt% of the aluminium dross fertilizer is the optimum content for planting. And, it can be concluded that treated ADC12 aluminium dross fertilizer with HCl was the best fertilizer in this experiment.

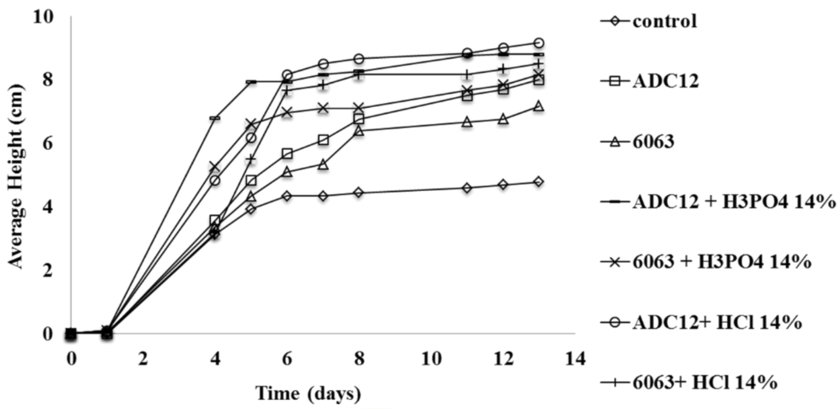

Figure 34 Comparison of the average height of Chinese cabbage planted in different aluminium dross at 10% and control.

Plant Weight

After 13 days, the Chinese cabbage was uprooted for weighing the plant including its root as shown in Fig 34 and 35. The maximum weight was found when treated with ADC12 aluminium dross fertilizer with HCl (Fig. 36). From this, it can be concluded that the weight of the plant including root and stem was strongly affected by HCl dross treatment when compared to that of control plants. At 20wt% of HCl-treated aluminium dross fertilizer, the weight of Chinese cabbage was higher than 10wt% fertilizer since its root is bigger. However, it can be concluded that 10wt% of aluminium dross fertilizer showed the optimum content as an economic reason. The weight of Chinese cabbage planted in treated dross with HCl had significant plant growth enhancement than those with H_3PO_4 treated dross. This is because the treated dross with HCl had higher nitrogen content which is required for the growth and development of plants as evidenced in the work of A. Pratumma (Pratumma et al., 2016)) since HCl treatment caused an irreversible reaction equation; however, H_3PO_4 treatment provided a reversible reaction (Demmig et al., 1986). In a comparison of the average height and average weight of Chinese cabbage, the results showed that the average height and average weight of Chinese cabbage planted in aluminium dross were better than the control around 80% and 50% respectively. It can be concluded that the aluminium dross fertilizer treated with acid provided good effect on height more than weight of plant.

Figure 35 Untreated and treated dross of Chinese cabbage with HCl in 13 days

Figure 36 10 wt% of treated aluminium dross with HCl with Chinese cabbage

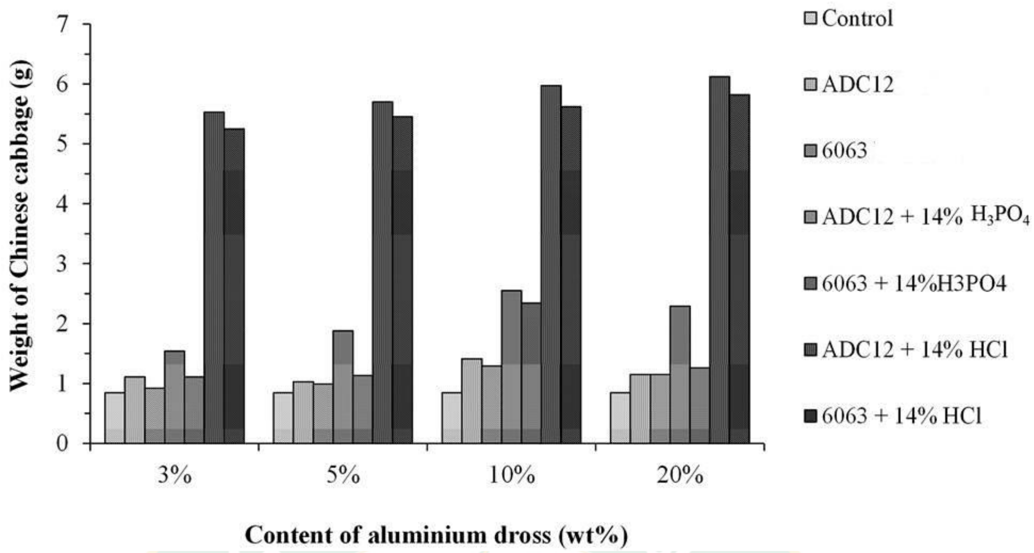

Figure 37 Plant growth-promoting effect of aluminium dross fertilizer

Effect of treated and untreated composition on plant growth

From the above results, it can be clearly seen that 10wt% of aluminium dross fertilizer was the optimum content since it resulted in the maximum height and weight of the plant. The growth of the plant with 10wt% treated, untreated aluminium dross fertilizer and control were compared. The apparent growth of Chinese cabbage is shown in Figure 37. Here HCl treated ADC12 and 6063 aluminium dross fertilizer in the soil led to the maximum growth of the plants. However, the growth was lower in the pot without aluminium dross (control sample). Figure 38 shows the growth of sweet corn with enhanced physiological parameters by adding HCl-treated aluminium dross fertilizer. In the case of Basil also, HCl-treated aluminium dross fertilizer showed enhanced growth parameters as indicated by the increase in physiological parameters (Figure 39). The same was also observed in the case of spring onion (Figure 40). Moreover, it can be summarized that planting in soil with treated aluminium dross fertilizer with HCl also led to enhanced growth as indicated by the increase in height and weight of the plants. The efficiency of HCl-treated aluminium dross in promoting plant growth is due to the generation of ammonium ions during the neutralization process which acts as fertilizer (Pratumma et al., 2016).

Figure 38 Chinese cabbage growth at 0, 10, and 20 days at 10wt% fertilizer content.

Remark (A) control
(B, C) untreated ADC12 and 6063 aluminium dross fertilizer
(D, E) treated aluminium dross fertilizer with H_3PO_4
(F, G) treated aluminium dross fertilizer with HCL,

Figure 39 Sweet corn growth at 0, 10, and 20 days at 10wt% fertilizer content.

Remark (A) the control sample
(B, C) untreated ADC12 and 6063 aluminium dross fertilizer
(D, E) treated aluminium dross fertilizer with H_3PO_4
(F, G) treated aluminium dross fertilizer with HCl

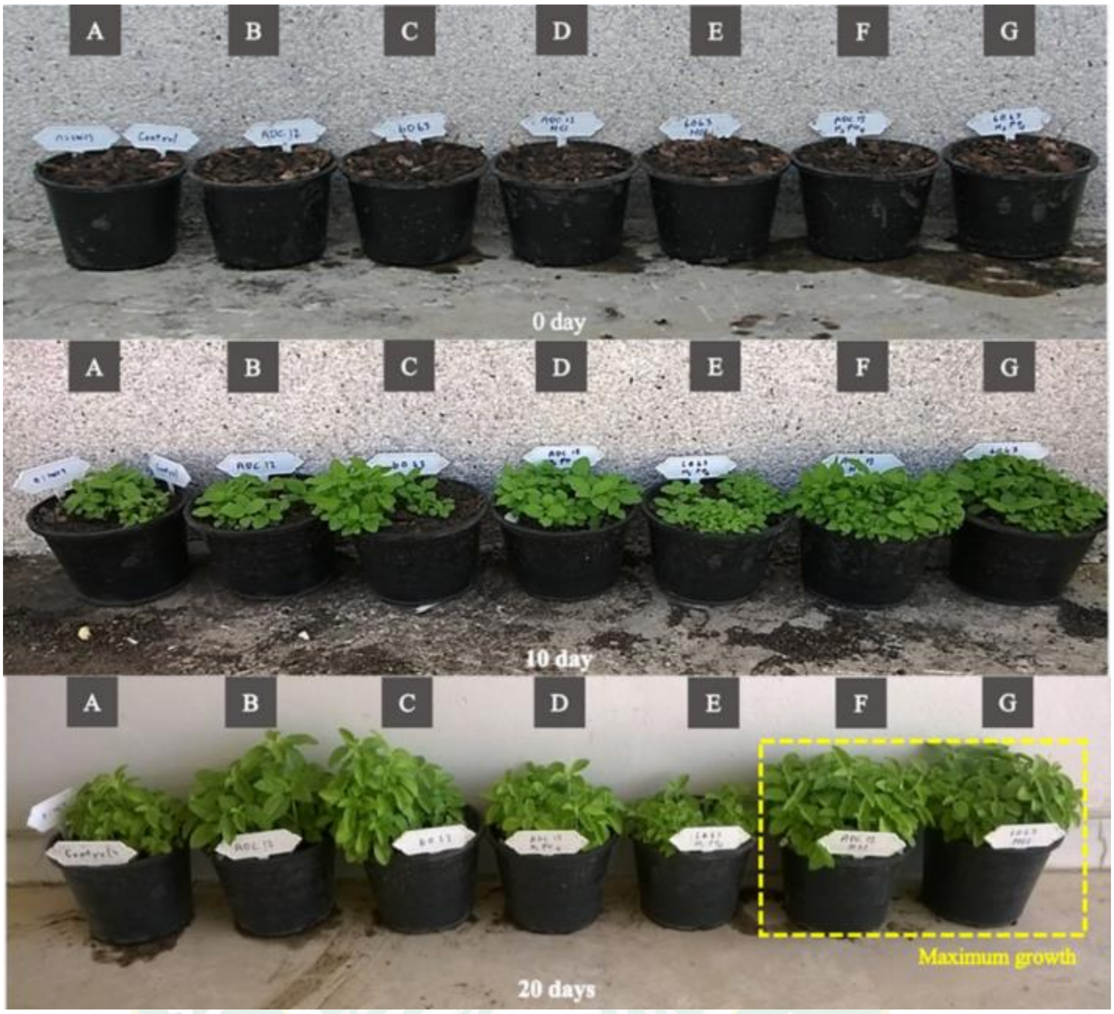

Figure 40 Basil growth at 0, 10, and 20 days
at 10wt% fertilizer content.

Remark: (A) the control sample
(B, C) untreated ADC12 and 6063 aluminium dross fertilizer
(D, E) treated aluminium dross fertilizer with H_3PO_4
(F, G) treated aluminium dross fertilizer with HCl.

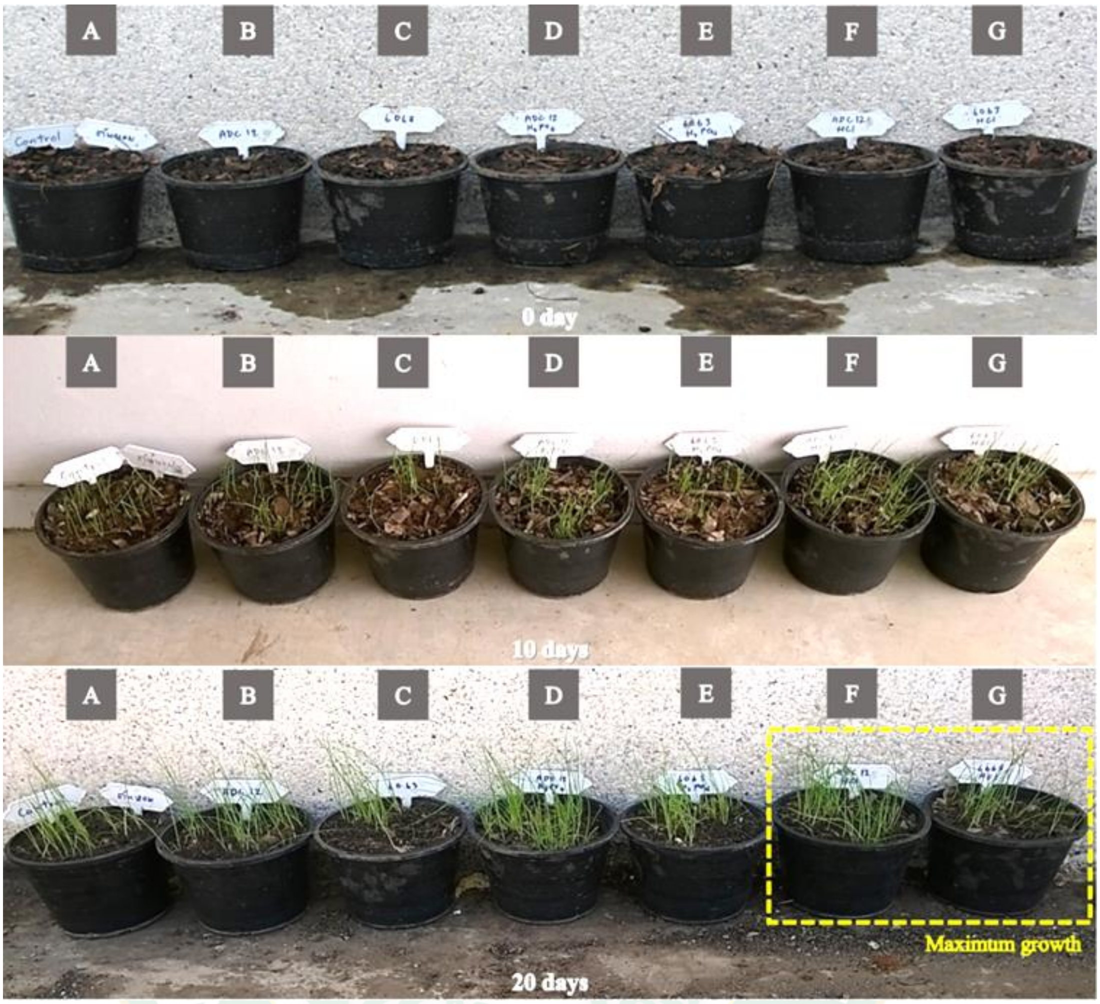

Figure 41 Spring onion growth at 0, 10, and 20 days at 10wt% fertilizer content.

Remark: (A) the control sample
(B, C) untreated ADC12 and 6063 aluminium dross fertilizer
(D, E) H_3PO_4 treated aluminium dross fertilizer
(F, G) HCl treated aluminium dross fertilizer.

Effect of dross on soil pollution

The residual composition of the dross fertilizer in the soil should be examined to determine whether aluminium dross fertilizer released any toxic substance or heavy metal elements. From the previous results, 10wt% ADC12 aluminium dross fertilizer showed the highest efficiency for planting, therefore, it is used for examining the chemical composition of the samples. According to Table 15, soil from planting Chinese cabbage without aluminium dross fertilizer consisted of silicon dioxide (SiO_2) and aluminium oxide (Al_2O_3) as the major composition accounting for 80.50 and 9.28wt% respectively. The main minor component is Iron (III) oxide (Fe_2O_3). Other components include calcium, potassium, magnesium, and phosphorus oxides. The residual components of soil after planting with the addition of ADC12 aluminuim fertilizer treated with H_3PO_4 and HCl showed an increase of 7.22 and 5.12%of Al_2O_3 respectively with respect to control samples. For treated ADC12 aluminium dross fertilizer with H_3PO_4, there is no existence of phosphorus pentoxide (P_2O_5) and sulfur trioxide (SO_3). On the other hand, for treated aluminium dross fertilizer with HCl, Fe_2O_3 and P_2O_5 are absent in the soil. Thus, it can be clearly observed that there is no existence of residual chemical composition in soil from using aluminium dross fertilizer. It can be concluded aluminium dross fertilizer is not harmful to the environment.

Table 15 Residue chemical composition analysis of planting soil of ADC12 aluminium dross

Composition	Control sample (%)	Treated with H_3PO_4 (%)	Treated with HCl (%)
SiO_2	80.5	75.20	76.80
Al_2O_3	9.28	14.40	16.50
Fe_2O_3	3.39	3.31	-
CaO	2.60	2.93	2.02
K_2O	2.42	2.41	2.54
MgO	1.30	1.54	1.30
P_2O_5	0.24	-	-
SO_3	-	-	0.55

Chitooligomer-Silver nano hybrid solution for the treatment of Huanglongbing/Citrus Greening Disease

Hybrid solution physical properties and particle size.

The hybrid solutions' appearance is brown-gold liquid, with normal viscosity, and pH of 5-7. It was measured particle size by NSTDA Characterization and Testing Service Center (NCTC), Thailand. The average particle size of the hybrid solution is 282.48 + 3.56 nm as shown in Table 16.

Table 16 The particle size of the hybrid solution

Measurement	Z average	PI
1	278.8	0.314
2	284.3	0.203
3	284.8	0.186
4	283.6	0.234
5	277.3	0.339
6	286.1	0.200

Citrus Greening Disease testing results

Citrus leaf test results before and after hybrid solution treatment are shown in Figure 41. The iodine test kit both liquid and litmus were positive in the pre-treatment group (A-C); after the hybrid solution treatment (D-F), they were negative.

Field test results

The data was collected during the period of January-March 2022 and March-May 2022. Iodine test kits were used to evaluate all treatments, and the results of all groups were negative. Citrus trees treated with AgNPs + COAMs hybrid solution have shoot lengths that are significantly ($p<0.01$) longer than those treated with Ampicillin over the course of two periods, but there are no differences in the number of leaves, leaf area, or greenness index across treatments. Tables 17 and 18 show the results. The characteristic of leaves of all treatments is also shown in Figure 42.

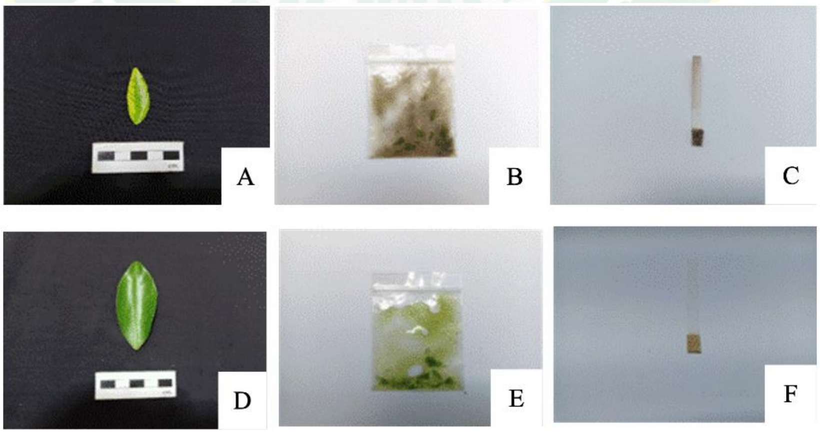

Figure 42 The characteristic of leaves of all treatments

Remark: (A) Citrus leaf showing symptoms of greening disease
(B) the blue/purple color of iodine test kit
(C) the blue/purple color of iodine litmus paper test kit

(D) no symptoms of Greening disease
(E) no color changing of liquid iodine test kit
(F) no color changing of iodine litmus paper test kit

Table 17 Result of treatments for shoot length and the number of leaves

Treatment	January–March 2022		March-May 2022	
	Shoot length (cm)	The number of leaves (no.)	Shoot length (cm)	The number of leaves (no.)
1. Ampicillin	9.62	7.09	9.78	7.42
2. AgNPs	10.20	7.23	10.22	7.57
3. AgNPs + Gibberellic acid	16.85	8.65	17.37	8.99
4. The hybrid solution	11.58	7.53	12.43	8.29
5. The hybrid solution + Gibberellic acid	17.10	8.45	17.10	8.78
F-test	**	ns	**	ns
c.v. %	5.19	1.11	17.56	21.82

ns = not significantly different, ** = significant difference at $p \leq 0.01$

Table 18 Result of treatments for leaf area, greenness index, and iodine test kit

Treatment	January–March 2022			March-May 2022		
	Leaf area (cm^2)	Greenness Index (SPAD)	Iodine test kit (-/+)	Leaf area (cm^2)	Greenness Index (SPAD)	Iodine test kit (-/+)
1. Ampicillin	10.30	59.92	negative	10.42	60.42	negative
2. AgNPs	9.87	54.59	negative	9.94	54.99	negative
3. AgNPs + Gibberellic acid	8.87	54.97	negative	9.27	55.54	negative
4. The hybrid solution	10.51	54.26	negative	10.75	54.86	negative
5. The hybrid solution + Gibberellic acid	6.87	59.28	negative	6.93	59.91	negative
F-test	ns	ns	-	ns	ns	-
c.v. %	6.41	20.55	-	25.07	8.06	-

ns = not significantly different

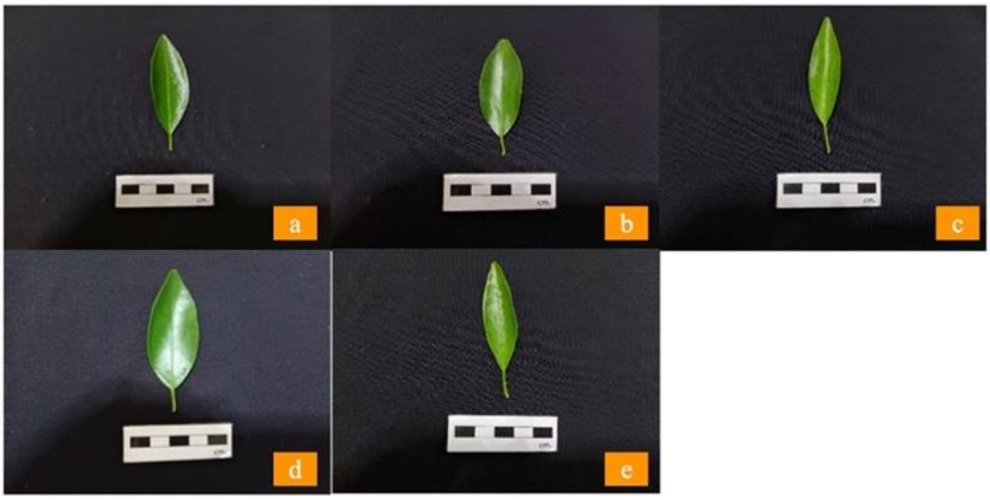

Figure 43 Characteristics of citrus leave after receiving the treatment of 1st to 5th, a to e respectively.

Citrus fruits have been collected and sent to the Institute of Product Quality and Standardization, Maejo University to analyze for residues of silver in the fruits. The report shows no silver found in the fruits as shown in Table 19.

In this experiment, the mixing that contained the hybrid solution and water at a ratio of 1:500 was also employed to treat the disease by foliar application. According to the preliminary report, the foliar application of a hybrid solution results in negative iodine test kits when compared to the baseline (before treatment). The plants began to exhibit more shooting shoot tips (terminal buds), young leaves, and flowers after 10 days of treatment, and by 25 days, the plants had more shooting leaves and flowers. The results are shown in Figure 43

The results also show physical features of citrus trees have changed. Figure 44 demonstrates how the same tree displays improvement in shooting leaves and flowering as compared to prior treatment, and Figure 45 demonstrates that they are also exceedingly fruitful after the treatment.

Table 19 Test report of silver residues in citrus fruits

Test item	Results	Unit	Method
Silver	Not found[1]	mg/kg	AOAC (2019) , 999.10 detected by ICP-OES

Note: 1. [1]Limit of Detection (LOD) 0.01 mg/L

Figure 44 The physical appearance of the citrus trees treated with the hybrid solution:

Remark: before treatment (A),
10 days after treatment (B),
25 days after treatment (C)

Figure 45 Shooting leaves and flowering after treatment (1-3), compared to before treatment (4)

Figure 46 Fruitful of the citrus tree after hybrid solution treatment

The cost of three treatments, including trunk injections of hybrid solution and ampicillin as well as foliar applications of hybrid solution, was determined as shown in Table 20.

Table 20 Comparison of the total cost of three treatments.

Criteria of Comparison	Ampicillin	The hybrid solution	The hybrid solution
Application method	Trunk injection	Trunk injection	Foliar application
Mixing ratio	1 kg per 20 liters of water	1 lit per 40 liters of water	1 lit per 500 liters of water
Apply mixing per citrus tree	800 ml	800 ml	2 liters
Number of workers	4 workers	4 workers	4 workers
*Capacity per day	40 citrus trees	40 citrus trees	1,750 citrus trees per 4,000 liters of mixing
**Total Cost per citrus tree (THB)	112.80 THB	64.00 THB	7.43 THB

*Number of citrus trees that can apply treatment per day.
**The calculation is based on a labor cost of 450 THB per day.

According to cost calculations that considered the number of workers and treatment capacity that could be applied each day, ampicillin cost 112.80 THB, while the application of hybrid solution by trunk injection and foliar spraying cost 64.00 THB and 7.43 THB, respectively.

Based on the percentage of incomplete citrus fruits and the total yield in kilograms, it was determined from the citrus harvest data in this experiment that the ampicillin treatment group's percentage of incomplete fruits was 25% and the hybrid solution's percentage was 5% in both the trunk injection and foliar application groups. The results are shown in Table 21.

Table 21 The yield of citrus trees

Criteria of Comparison	Ampicillin	AgNPs-COAMs	AgNPs-COAMs
Application method	Trunk injection	Trunk injection	Foliar application
Yield weight per citrus tree (kg)	95	110	115
% Of incomplete fruits	25%	5%	5%
Net of Yield (kg)	71.25	104.50	109.25

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CHAPTER V
CONCLUSION AND DISCUSSION

Chitosan oligomers and monomers (COAMs) benefit and its application in innovative organic methods for root rot disease treatment in durian crops

Chitooligomers also known as chitooligosaccharides (COS) or chitosan oligomers produced by the degradation of chitosan. Their degrees of polymerization (DPs) are less than 20, and their average molecular weight is less than 3900 Da. It also has a greater solubility in addition to having outstanding qualities including biodegradability, biocompatibility, adsorptive capacities, and non-toxicity like chitin and chitosan. According to several studies, applying chitosan and its derivatives on plants can inhibit pathogens, particularly fungi, boost the immune system, and promote plant growth. (Hadwiger, 2017; Winkler et al., 2017; Schmitz et al., 2019)

The study of LA Hadwiger (2017) revealed that the chitooligomer that optimal induces plant defense mechanism is a heptamer (7-mer). This oligomer size is also optimal to inhibit pathogens' growth. The short-chain oligomer can pass through the cell wall to activate the PR-response gene in plant chromatin, thereby causing a plant defense mechanism. (Hadwiger, 2017 There are several investigations have verified the antimicrobial effect of oligomers and monomers. Y.N. Fawzya et al. (2019) examine the COAMs containing monomer (1 unit) to hexamers (6 units) for antifungal activity. They found that COAMs inhibited *A. flavus, A. niger, Eurotium amstelodami*, and *Emericella nidulans* (Fawzya et al., 2019). P. Li et al, (2016) evaluate low-MW oligochitosan (< 3 kDa) and found significant protective effects on *Zanthoxylum bungeanum* stems against dry rot caused by *Fusarium sambucinum*. (Li et al., 2016) As a result, the researcher was interested in chitooligomers, which could be developed and used to manage plant diseases.

Chitosan, a biopolymer, can be depolymerized to produce a short-chain oligomer with high efficacy in stimulating plant defense and inhibiting pathogens. There are several methods for producing chitooligomers and monomers, each with its own set of advantages and disadvantages. (Liang et al., 2018) The researcher discovered that in Thailand, no one has yet been capable of developing oligomers with DP less than 20 and a molecular weight less than 3.9 kDa that has been studied to prove their efficacy and cost-effectiveness in the treatment of plant diseases. For these reasons, the researchers sought an effective depolymerizing method to produce COAMs.

In this study, the researchers have successfully produced COAMs with novel methods which can certainly produce oligomers and monomers. Chitosan was broken down using a cold plasma treatment with Helium (He) or Argon (Ar) and ultrasonic treatment to produce COAMs. By using the viscosity and GPC techniques to determine the MW of COAMs after the treatment, it was discovered that the molecular weight was significantly decreased ($p<0.01$).

The MW examined in this study is 1.94-1.98 x 10^{-3} Daltons. S. Liang et al., (2018) indicate that the molecular oligomers and monomers typically range from 0.2 to 3.0 kDa. This study demonstrates that oligomers and monomers are the main components of the COAMs produced. Further study is required to distinguish the molecular weight range to examine the number of oligomers and monomers.

The particle size of COAMs was measured by Nano Particle Analyzer in this study. Its molecule is nanoparticles with an average size of 15.12 nm or 0.015 microns which allow it to readily pass through the plant leaf's stomata size of 19.1-71.5 microns (JG Jordan et al., 2015) and enables it to use as a foliar application.

The test of *C. gloeosporioides* and *F. pseudensiforme*'s mycelial growth inhibition in this study provided additional evidence of their antipathogen characteristics. It was discovered that all COAM samples showed somewhat better inhibition than carbendazim.

The novel approach of introducing IMOs containing *Trichoderma harzianum*, *Bacillus subtilis*, and certain microorganisms for antipathogens and soil amendment in agricultural areas and COAMs was applied for the treatment of the root rot disease in durians. The outcome demonstrates that in field testing, COAMs and IMOs had a considerable impact on durian root rot pathogens, and significant improvement was reported when compared to chemical fungicides. After 30 days of the last treatment, the phloem tissues examined under a digital microscope had been rebuilt to replace infected tissues, indicating a cure and improvement. Additionally, the appearances of durian trees show thick, shiny, dark green leaves that exhibit indicators of healthy development. These may be the antifungal and growth-promoting properties of COAMs, which are based on the improvement of infected tissue. (Hadwiger, 2017; Winkler et al., 2017; Schmitz et al., 2019).

According to the findings of this research, after using IMO and COAMs in the treatment of fungi in durian. It was discovered using digital microscopy that durian tissues had improved and recovered from fungal infection, supporting the efficacy of COAMs on fungal elimination. The findings of this study are congruent with YN Fawzya et al. (2019) study revealing that the oligomers identified by TLC were monomer (1 unit) to hexamer (6 units) and showed their antifungal activity against the fungi tested (Fawzya et al., 2019). Many research investigations have described the mode of action of chitosan and its derivatives against pathogens including bacteria and fungi. The effects may be the positive charge to interact with the negatively charged pathogens' membranes, chelating and binding to trace elements of pathogens, and low molecular weight of oligo-chitosan can penetrate to the cell wall and bind to DNA of pathogens to inhibit mitochondrial function and ATP production. (Ing et al., 2012; Ke et al., 2021)

It was also discovered that tissue recovered from fungus infection regenerated and could be examined under a digital microscope. This depicts COAMs' bioactivities as plant growth stimulators. COAMs induce plant growth by influencing plant physiological processes like nutrient uptake, cell division, cell elongation, enzymatic activation, and synthesis of protein that can eventually lead to increased yield. This study's results correspond to those of P.D. Dzung et al. (2017), who discovered that foliar spray of low-MW oligochitosan (2.5 kDa) can stimulate plant growth. Oligochitosan has the potential promising to apply as a biostimulant to enhance chili fruit yield significantly (Dzung et al., 2017). The impact of durian tissue development after the use of COAMs provided further confirmation as a plant growth stimulator in the author's research.

According to this study's findings, COAMs and IMOs are effective treatments for durian root rot disease. Interesting biological characteristics of COAMs include biocompatibility, non-toxicity, and biodegradability. They are used in agriculture because of their antimicrobial properties, particularly their ability to fight off fungi and regulate plant growth. The use of IMO is one of the Biological Control Agents (BCAs), which are based on active microbes or their metabolites and natural products that decrease the population of plant pathogens (Tyskewickz et al., 2022). COAMs and IMOs are novel substances that can be used to defend durian trees against root rot disease.

P. Kongtragoul et al., (2021) discover that *P. palmivora* isolated from infected durian trees are resistant to metalaxyl suggesting that this fungicide may be ineffective in suppressing *Phytophthora* infections in durians. Fungicide-resistant *Phytophthora* strains have been reported in many countries including Thailand. This becomes increasingly a more important issue to manage root rot disease in durian efficiently and safely. Additionally, the side effects of fungicides may come with risks, such as serious hazards to farmers, consumers, and the environment. (Kongtragoul et al., 2021)

There is a promise to employ COAMs and IMOs as natural fungicides to partially replace the usage of synthetic fungicides due to COAMs' notable effectiveness in the management of root rot disease in durian trees drastically reducing chemical use and improving the quality of life for farmers and consumers.

Aluminium dross neutralization and its application as plant fertilizer

Aluminum dross is a byproduct of the foundry process produced by the oxidative interaction between molten aluminum and oxygen from the air, which results in slag separation. The quantity of aluminum slag produced by the aluminum smelting process is substantial and grows annually in response to demand. In Thailand, aluminum dross production is anticipated to reach 30,000 tons in 2015. Because of the high amount of aluminum dross, there is an issue with the unlawful disposal of industrial refuse in public areas. This has an impact on the environment and is one of Thailand's present issues. The increasing demand for the development of an eco-friendly approach to the treatment of industrial waste is of great significance in day-to-day life. Furthermore, sustainable development has paved the way for the development of an environment-friendly approach.

When aluminum dross ADC12 and 6063 grades were analyzed for chemical composition using X-ray Fluorescence Spectrometry, it was discovered that most of the aluminum dross in both grades, more than 60-80%, was Al_2O_3, with the rest being other metal oxides. The primary result of the reaction of aluminum dross with moisture is ammonia vapor. The properties of ammonia gas vary based on the pH value. At pH 7, ammonia nitrogen is in the form of ammonium ions ($NH4^+$), and as the pH rises, more ammonium ions will change to free ammonia (NH_3).

The effectiveness of this dross fertilizer lies in its ability to modify the aluminum dross by neutralizing the pH of the slag, which results in the conversion of ammonia gas to ammonium ions, which is a significant nutrient that plants can utilize and use technology to fix ammonium ions trapped in the pores of aluminum dross by selecting to use phosphoric acid or other acids that are already used in the soil amendment industry as a treatment. Furthermore, elements such as SiO_2, Fe_2O_3, MgO, CaO, Na_2O, and others can be found in aluminum dross. These compounds can be used by plants as secondary macronutrients and micronutrients.

In the study, Aluminium dross (ADC12 and 6063), the by-products of aluminium production were taken as the raw materials for the production of plant fertilizer by neutralization. Plant growth results showed that 10wt% of aluminium dross fertilizer was the optimum content for planting. Among them, ADC12 aluminium dross treated with HCl showed improved weight and height of Chinese cabbage, basil, sweet corn, and spring onion.

The soil residue was evaluated by gathering soil before and after planting Chinese cabbage with treated aluminum dross grad ADC12 10%wt. The Al_2O_3 content of the soil was discovered to have risen by 7.22%. This is because Al_2O_3, the major component of aluminum dross, is not categorized as a primary macronutrient, secondary macronutrient, or micronutrient, and thus is not absorbed and utilized by plants. Even though aluminum oxide residues remain in the soil, it still has characteristics as a soil amendment because it makes the soil crumbly and creates more gaps in the soil, allowing the soil to better retain hydration. As a result, plants can absorb water more effectively during the growth process. Aluminum oxide, by the way, decreases nitrogen and potassium losses. In other terms, aluminum oxide can fix nitrogen and potassium in the soil. As a result, the soil contains a high level of nitrogen and potassium residue. Because aluminum oxide can bond nitrogen in the form of ammonium in tiny porous, Nitrosomonas and Nitrobacter are unable to transform ammonium to nitrate via the nitrification process. Consequently, nitrogen in the form of ammonium is absorbed in its native form and is not readily leached. This stored ammonium will then liberate nitrogen for further use by plants. Therefore, even though aluminum oxide remains in the soil after planting, it is still helpful for enhancing soil and increasing crop yields. It can be summarized that aluminium dross fertilizer was safe for planting due to its environmentally friendly nature.

Chitooligomer-Silver nano hybrid solution for the treatment of Huanglongbing/Citrus Greening Disease

Thailand's agricultural industry has a constant development rate. There has been expanded cultivation of oranges resulting in increased demand for inputs in cultivation but the epidemic of Citrus Greening Disease (CGD) or Citrus Huanglongbing (HLB) which is important in citrus farming because of its severe damage, decreased yield, poor quality, and frequent disappearance before harvest, affecting citrus exports in Thailand with a value of up to 1.1 trillion baht per year. (Office of Agricultural Economics, 2021)

CGD is caused by *Candidatus* Liberibacter species, which is spread by the Asian citrus psyllid (Poonyapitak et al., 2016). Since the beginning of the disease, a variety of techniques have been applied to treat CGD including eradicating the insect vector, administering insecticides, and using broad-spectrum antibiotics to treat the symptoms in infected trees. The use of antibiotics in field crops is, undoubtedly, constrained by the emergence of microbial resistance, which also poses a significant risk to human health due to its indirect effects. In actuality, the disease cannot currently be treated with chemicals or any other types of controls. Therefore, it is obvious that a long-term cure for CGD infection in plants is required immediately. (Stephano-Honedo et al., 2020).

As a result, the study team has investigated the creation of a substance that can be used as a replacement for antibiotics, while also being effective in eradicating plant pathogens and safe for both humans and animals, with no residue that harms the environment.

The research team found that AgNPs demonstrate potential anti-phytopathogenic effects and plant disease management including fungi, bacteria, and viruses. (Tariq et al., 2022) The application of AgNPs in the production of various hybrid products is being reconsidered to their outstanding characteristics (Daniel et al., 2017). There are some studies for nanocomposite production of silver nanoparticles with chitosan and its derivative in order to produce nanocomposites with improved characteristics that might potentially be used as an antibacterial agent, silver was nano-mediated with chitosan as a reducing and stabilizing agent. (Sonseca et al., 2020)

The study team was successful in creating an antipathogenic hybrid of AgNPs and COAMs to replace antibiotics. Preliminary testing revealed that the hybrid significantly inhibits the mycelial growth of an isolated strain of *Lasiodiplodia brasiliensis* from durian trees. The research team also examined the hybrid solution's properties and discovered that its molecule is nanoparticles with a size of 282.48 nm or 0.28 microns (Jordan et al., 2015), allowing it to easily pass through the plant leaf's stomata size of 19.1-71.5 microns and allowing it to be used as a foliar application.

Citrus plants exhibiting indications of CGD were recruited in citrus crop field trials by observing evident symptoms such as yellowing and blotchy mottling. *Candidatus* Liberibacter species of bacteria are primarily accountable for CGD and cannot be grown on a culture medium (Poonyapitak et al., 2016), so CGD must be confirmed using another efficient technique.

The excessive accumulation of starch in the remaining parenchyma cells of the aerial parts and photosynthetic cells is one of the most obvious symptoms of CGD in citrus trees. These can be detected by an iodine test. By observing how the leaves change color, one can tell whether a citrus tree is infected with the CGD by determining if the leaves turn blue or purple (positive test). If not, the leaves are healthy. (Whitaker et al., 2014)

In this study, a liquid iodine test kit was used to confirm that citrus trees were infected with CGD. Furthermore, the study team collaborated on the development of an iodine litmus paper test kit to ease testing in citrus-growing areas. The findings showed that citrus trees with CGD signs on their foliage tested positive, and the intensity of the color changes varied according to the severity of the disease. This could contribute to the creation of assays that are practical and simple to use in citrus-growing areas, as well as those that can determine the severity of the disease. This will improve the CGD therapy strategy.

The iodine test was negative after using the hybrid solution to cure the CGD of citrus trees and examining the treated citrus leaves, proving that the hybrid was successful in eliminating CGD. This may be because AgNPs in the hybrid solution demonstrate potential anti-phytopathogenic effects and plant disease management including fungi, bacteria, and viruses (Tariq et al., 2022) by numerous mechanisms including the positive charge of silver ions attached to the negative charge cell wall of pathogens which causes cell death (Gupta et al., 2018). There are synergistic antibacterial effects of AgNPs-COAMs. By conjugating COAMs to AgNPs, one can increase the surface charge of those particles. This increases their ability to adhere to negatively charged bacterial cytoplasmic membranes and amplifies their electrostatic interactions. COAMs can enhance bacterial absorption of AgNPs by acting as an antibacterial agent in multivalent binding. (Mei et al., 2020) The outcome also reveals a notable increase in shoot length. This could be the growth stimulation of a hybrid solution due to the properties of COAMs.

Three therapies, ampicillin and hybrid solution for trunk injection and hybrid solution for foliar application were assessed in this research to compare the efficiency of CGD treatment in terms of cure and improvement effects verified by the iodine test kit, along with cost-effectiveness. The findings showed that all three treatments can control CGD in citrus trees, which was confirmed by the negative iodine test results.

The expense of treatment for each therapy was calculated using labor capability per day, which is the number of citrus trees that can apply therapy per day. According to the cost study of each treatment, Ampicillin by trunk injection was the most expensive, followed by hybrid solution by trunk injection and foliar application, respectively. This is because the trunk injection method is less efficient than the foliar spray method since it's more difficult to operate, requires more complicated tools, and takes longer to operate per citrus tree than the foliar application.

In comparison to ampicillin, the hybrid therapy for CGD, particularly foliar application, has been shown to be highly cost-effective (Table 4.14). This hybrid approach might replace antibiotics for the treatment of CGD since antibiotics in field crops are obviously limited by the emergence of microbial resistance, which also poses a serious risk to human health related to its indirect effects (Stephano-Honedo et al., 2020). These can be applied to other plant diseases that require further field research.

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APPENDIX

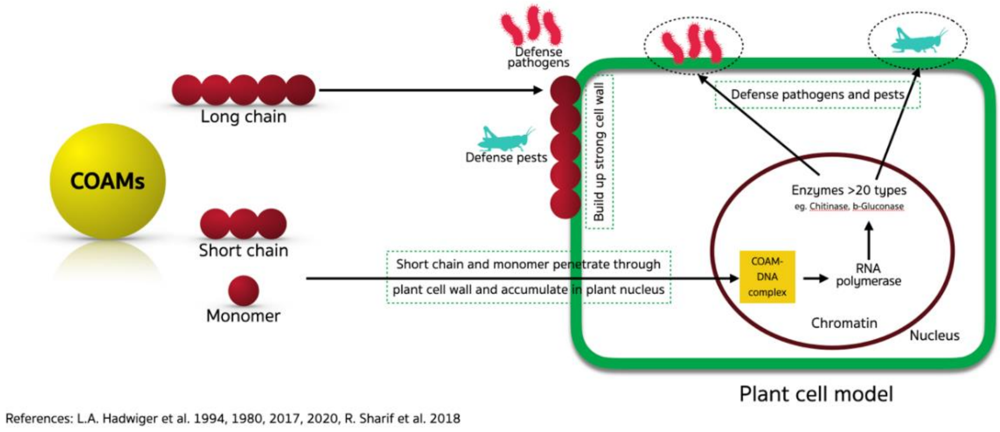

Figure 47 Chito oligomers and Monomers (COAMs) Mechanism of Action

Group	Day 0	Day 7	Day 14	Day 21	Day 28
Control Group	Metalaxyl	Metalaxyl	Metalaxyl	Metalaxyl	-
Testing Group	IMO COAM	COAM	COAM	COAM	-
Phloem Tissues Examination	Before 1st treatment		Before 3rd treatment		7 days after 4th treatment
Severity Scales Examination	Before 1st treatment				
Improvement Scores Examination			Before 3rd treatment		7 days after 4th treatment

Figure 48 Study schedule of treatment and examination

Province: .... **Crop No.** ....

☐ Control	☐ Severity Scales ☐ Improvement Scores				
☐ **Testing**	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
1					
2					
3					
4					
5					
6					
7					
8					
9					
10					

Figure 49 Results Recording Form

MAEJO UNIVERSITY

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	2023	Shareholder and President, Innovay (Thailand) Co., Ltd.