Journal of Sustainability Science and Management
Volume 18 Number 3, March 2023: 110-124

eISSN: 2672-7226

SOIL BURIAL DEGRADATION OF STARCH-BASED FILMS ON MICROBIAL LOAD AND PLANT GROWTH

KATAKRIT CHAISUWAN[1], DAHLAL ANURAKUMPHAN[1], SUKALLAYA HEMMANEE[2], JAREERAT RUAMCHAROEN[2] AND MONTIRA LEELAKRIANGSAK[2]*

[1]Demonstration School of Prince of Songkla University, Prince of Songkla University Pattani campus, 94000, Pattani, Thailand. [2]Department of Science, Faculty of Science and Technology, Prince of Songkla University Pattani campus 94000, Pattani, Thailand.

**Corresponding author: montira.l@psu.ac.th*

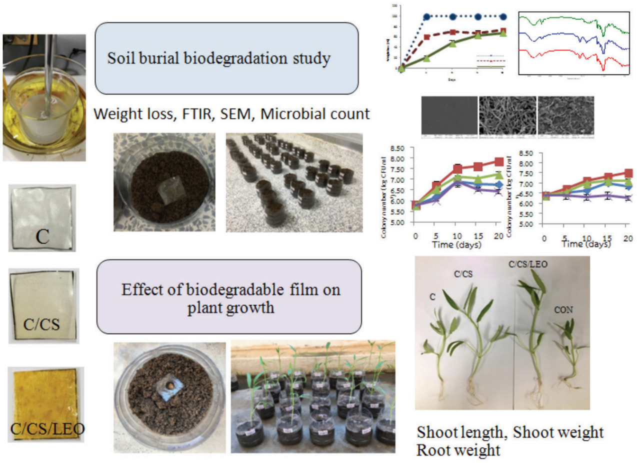
Submitted final draft: 15 December 2022 *Accepted: 20 January 2023* http://doi.org/10.46754/jssm.2023.03.008

Abstract: The biodegradability of cassava starch (C) film, cassava starch/chitosan (C/CS) film and cassava starch/chitosan/lemongrass essential oil (C/CS/LEO) film by soil burial for 20 days was studied using weight loss, Fourier-transform infrared spectroscopy (FTIR) and a scanning electron microscope (SEM). The FTIR analysis revealed that the removal of the functional group corresponded to starch film weight loss. Observations made from the SEM showed that the film's appearance changed during degradation. The number of soil microorganisms after 20 days of burial was determined using the plate count method. On day 20, the control sample showed significantly less microbial count ($P < 0.05$) than all treatments. The effect of starch films on water convolvulus (*Ipomoea aquatica*) growth for 21 days was studied by measuring the shoot length, root fresh weight, and shoot fresh weight. It was found that water convolvulus planted in soil for 21 days with C/CS and C/CS/LEO films showed similar shoot length, shoot fresh weight, and root fresh weight. However, it was significantly higher ($P < 0.05$) compared with water convolvulus grown in soil with C film and control. The study concludes that the released chitosan affects water convolvulus growth.

Keywords: Biodegradable film, cassava, chitosan, lemongrass essential oil.

Abbreviations:
Cassava starch: C
Cassava starch/chitosan: C/CS
Cassava starch/chitosan/lemongrass essential oil: C/CS/LEO
Essential oils: EOs
Lemongrass essential oil: LEO

Graphical abstract



Introduction

The slow decay of plastic has become one of the most pressing environmental issues and results in environmental pollution. A biodegradable film, which is susceptible to biodegradation and eco-friendly product, is an alternative replacement for plastic (Torres *et al*., 2011; Omotoso *et al*., 2015; Luchese *et al*., 2018). Starch is one of the most widely available natural polysaccharides used as raw material for biodegradable polymeric films that do not contribute to environmental pollution (Torres *et al*., 2011; Jiménez *et al*., 2012; Omotoso *et al*., 2015). Research on starch-based film has increased due to its potential as an environmentally friendly biodegradable biopolymer, especially in food packaging and agricultural applications (Jiménez *et al*., 2012). However, it has some drawbacks. It is water-sensitive, has a brittle nature, and poor mechanical properties (Jiménez *et al*., 2012; Luchese *et al*., 2018; Thakur *et al*., 2019). Incorporating kaolinite clay and chitosan could improve the water vapour barrier and mechanical properties (Ren *et al*., 2017; Luchese *et al*., 2018; Ruamcharoen *et al*., 2019). The mixture of chitosan and starch reduced film solubility (Ren *et al*., 2017; Brandelero *et al*., 2019). Besides its excellent film-forming ability, chitosan exhibits antimicrobial activity and enhances plant growth (Dutta *et al*., 2009; Goy *et al*., 2009; Rahman *et al*., 2018). The incorporation of lipophilic materials, such as essential oils (EOs), has been demonstrated for its effectiveness in improving starch films' barrier properties to water vapour and exhibiting antimicrobial activity (Jiménez *et al*., 2012; Moore-Neibel *et al*., 2012; Mith *et al*., 2014). Lemongrass essential oil (LEO) exhibited antimicrobial activity against *Salmonella enterica*, resulting in the shelf life extension of vegetables (Moore-Neibel *et al*., 2012). Recently study of ZnO and pineapple leaf fibre incorporated with cassava starch/chitosan showed that the physical, mechanical and antibacterial properties of bioplastic have improved and it could be completely decomposed in 21 days in ordinary soil and 18 days in seawater (Armynah *et al*., 2022). The inclusion of some additives or reinforcement agents improves starch films' properties. Therefore, they can be used as bioactive packaging.

Biodegradable films that can be degraded in the soil, activated sludge or compost after the end of service life have been proposed as a solution to environmental problems (Ishigaki *et al*., 1999; Avella *et al*., 2005; Torres *et al*., 2011; Omotoso *et al*., 2015;). The application of potato starch film modified with urea as a potential plasticiser and fertiliser had been reported to improve plant growth (Rychter *et al*., 2016). Therefore, biodegradable and compostable films could benefit from sustainable development since they can be used as agricultural fertiliser (Li *et al*., 2019). Biodegradation utilises microorganisms to convert polymers to small-molecule fragments. These small-molecule fragments can be further degraded to carbon dioxide and water. The conversion takes place in a three-step process. First, macromolecular chains are depolymerised into monomers and oligomers, then simple units are taken in the microbial cell as biomass, and finally respiration of biomass takes place to complete the degradation process (Pathak & Navneet, 2017; La Mantia *et al*., 2020).

Chitosan, a deacetylated derivative of chitin, is a linear hydrophilic polysaccharide that displays biodegradability, biocompatibility, and non-toxicity. These properties make it a promising and versatile biopolymer (Dutta *et al*., 2009; Goy *et al*., 2009; Raafat & Sahl, 2009). Chitosan has been investigated due to its antimicrobial activity against a wide range of microorganisms, like bacteria, yeasts, and fungi (Savard *et al*., 2002; Goy *et al*., 2009; Goy *et al*., 2016; Jiang *et al*., 2016; Yuan *et al*., 2016). Chitosan exhibited antimicrobial activity against both Gram-positive bacteria *Staphylococcus aureus*, and Gram-negative bacteria *Escherichia coli* (Goy *et al*., 2016). Spore germination and mycelia growth of *Botryotinia fuckeliana*, the postharvest pathogenic fungi, were significantly inhibited by chitosan (Jiang *et al*., 2016). Studies have reported the rate of chitosan degradation and its effect on bacterial community changes in soils (Sato *et al*., 2010; Sawaguchi *et al*., 2015). Chitosan added in silty soil decreased to less than half after 10 days (Sawaguchi *et al*., 2015). The increase in the bacterial community of a chitosan-added soil was also observed by denaturant gradient gel electrophoresis (DGGE) between 28 and 195 days (Sato *et al*., 2010). The weight loss of chitosan film buried in paddy soils and red clay was 79.2% and 83.2%, respectively, after burying for one month (Nakashima *et al*., 2005). This proves that the chitosan degradation rate differed depending on the soil type (Nakashima *et al*., 2005; Sawaguchi *et al*., 2015). In agriculture, chitosan has been widely used to stimulate plant defence and enhance plant growth (Raafat & Sahl, 2009; Akter Mukta *et al*., 2017). It has also affected seed germination and weed seedling growth by up-regulating Indole-3-acetic Acid (IAA) synthetic genes (Li *et al*., 2019). Growth enhancement in strawberry plant roots and fruit yield were observed following treatment with chitosan solution (Akter Mukta *et al*., 2017; Rahman *et al*., 2018). In addition, the total antioxidant activities and contents in strawberry fruits increased with the spray application of chitosan (Rahman *et al*., 2018). Chitosan coatings have prevented moisture losses during postharvest storage and retarded microbial growth. Hence, the overall quality of fruits and vegetables is maintained (Yuan *et al*., 2016; Perdana *et al*., 2021). It is considered environmentally friendly, easily degradable, and safe to use (Raafat & Sahl, 2009).

Recently, cassava starch and cassava starch/chitosan films incorporated with lemongrass essential oil (LEO) have been successfully made through the casting method (Perdana *et al*., 2021). Their mechanical properties and antimicrobial activities against some pathogenic microorganisms have been tested. Besides, the application was successfully conducted using a coating system. The results showed that they prolonged chilies' storage by lowering the total microbial growth and reduced weight loss. However, the biodegradability of the films has not been studied. To the best of our knowledge, this is the first study to present the results of the application of starch/chitosan film as a potential plant growth enhancer. Therefore, this research aims to investigate the biodegradation of three formulations of starch-based films using soil burial tests and study the effects of biodegradation on soil microorganisms and growth of water convolvulus (*Ipomoea aquatica*).

Materials and Methods

Films Preparation

The starch solution was prepared using a solution-casting method described by Perdana *et al*. (2021). The gelatinised starch solution was made by heating cassava starch (Fish brand, Thailand) solution (3% w/v) at 80°C under stirring at 300 rpm for 20 min. It was mixed with glycerol (Univar, Australia) (20 wt% of starch) and kaolinite clay (Amarin Ceramics Co., Ltd, Nonthaburi, Thailand) 4 wt% of starch and stirred for 15 min to obtain the cassava starch (C) film. Chitosan (low molecular weight 75-85% deacetylated, Sigma Aldrich Co., Ltd, USA) 40 wt% of starch dissolved with glacial acetic acid was added to C to prepare the cassava starch/chitosan (C/CS) film. The LEO (AP Operation Co., Ltd, Chonburi, Thailand) was prepared with Tween-80 (Univar, Australia) (0.8% v/v) and added separately to obtain 2% of concentration to yield cassava starch/chitosan/lemongrass essential oil (C/CS/LEO) film. The solutions were cooled until the temperature was approximately 30°C, then it was casted onto the polystyrene disk and dried at 50°C in an oven for two days.

Biodegradation by Soil Burial Testing

Biodegradation studies of starch films were carried out on a laboratory scale using a soil burial test. Garden soil was sieved and mixed. Water bottles (7 cm) were filled with 100 g of soil. Three formulations of 2x2 cm^2 starch films (C, C/CS, and C/CS/LEO) were weighed and placed on the soil, and then covered with 50 g of soil. Bottles without film were used as control. All treatments were watered (3 ml) every five days. The experiment was carried out for 20 days. Starch films were taken from the bottles at 5, 10, 15, and 20 days. The soil was gently removed from the films. The biodegradability of the starch films was evaluated through weight loss using the following equation:

$$\%\, weight\ loss = \frac{Wi - Wt}{Wi} \times 100 \qquad (1)$$

Where W_i represents the initial weight and W_t is the weight after the established time.

Fourier-transform Infrared Analysis

Starch film samples were collected before and after burial for 0, 5, 10, 15, and 20 days, respectively. FTIR spectra of three formulations of film were determined using the Bruker Tensor 27 FT-IR spectrometer (Bruker, Germany) with the attenuated total reflectance (ATR) mode. All spectra were scanned five times at a wave number ranging from 400 to 4000 cm^{-1}.

Scanning Electron Microscopy (SEM)

Samples from days 0, 10, and 20 were taken to observe SEM morphology changes (LEO 1455VP, UK). The samples were mounted on stub and sputter-coated with gold. The morphology of the film surface was observed using 10 kV as the operating voltage with thermionic emission. All SEM images were performed at 25°C.

Determination of the Number of Soil Microorganisms

The number of culturable microorganisms in the soil was estimated as described by Sawaguchi *et al*. (2015) with modification. The soil sample was taken before and after the burial test for 0, 5, 10, 15, and 20 days. Soil sample weighing 1 g around the tested films was suspended in 9 ml of 0.1% peptone water (Becton, Dickson and company, USA) and shaken. The suspension supernatant was diluted with 0.1% peptone water. It was then mixed into melted nutrient agar (NA, Himedia, Mumbai, India) and incubated at 37°C for 24 h to obtain the total bacteria count. The spread plate method was used to isolate fungi in potato dextrose agar (PDA, Himedia, Mumbai, India) with the addition of 100 $\mu g\ ml^{-1}$ streptomycin and incubated at 33°C for 72 h. After the incubation, the surviving bacteria or fungi in the single-colony forming unit per ml were calculated. The experiments were repeated three times.

Assessment of Biodegradable Films' Effect on Plant Growth

As mentioned above, the biodegradable films' effect on the plant growth of water convolvulus, or *Ipomoea aquatica* (Chia Tai Co., Ltd, Thailand), was determined in the soil burial test with modification. Seeds were soaked in warm water overnight to determine the percentage of germinated seeds. Only germinated seeds were selected for the plant growth experiment. A square 2x2 cm^2 of each film (C, C/CS, and C/CS/LEO) was used to punch a 5 mm diameter hole. Then, a seed was placed in the middle of the hole. Twenty seeds were planted for each treatment and seeds without film were used as control. Plants were grown for 21 days and the shoot length was recorded weekly. Plants were watered twice daily. On day 21, the root and shoot fresh weight were determined. Tests for each treatment were performed in triplicate.

Statistical Analysis

All experiments were carried out in triplicate and the results were analysed using a one-way analysis of variance (ANOVA). Multiple comparisons were performed using Duncan's multiple range test (DMRT) to determine the significant variation ($P < 0.05$).

Results and Discussion

Soil Burial Biodegradation Studies

Film Weight Loss

Measuring weight loss is one of several methods used to follow polymer films' biodegradation rate (Ishigaki *et al*., 1999; Torres *et al*., 2011; Rychter *et al*., 2016; La Mantia *et al*., 2020). As shown in Figure 1, the films were buried and observed every five days until day 20 to evaluate the biodegradability rate of the three film formulations (C, C/CS, and C/CS/LEO). The addition of LEO to films affected the film' surface colour, moving it towards yellowness. After 20 days, C/CS and C/CS/LEO films were still intact, whereas the C film decomposition occurred entirely on day 5. Weight loss of 100% was recorded on day 5 for C film, while the weight loss of C/CS and C/CS/LEO films was 61.44±3.67% and 19.37±4.69%, respectively (Figure 2). The weight loss percentage of C/CS film slightly increased and reached 75.22±0.92% on day 20. More than a 37% increase in C/CS/LEO film weight loss from day 5 to day 10 was observed and it reached 63.47±0.96% on day 20. A weight loss of 99.35% was observed during the biodegradation of cassava starch-based film after 31 days (Torres *et al*., 2011). The beginning of degradation of cassava starch-based film with yerba mate extract occurred on day 6 and almost the entire film was decomposed on day 12 (Medina Jaramillo *et al*., 2016). The addition of chitosan in starch film affected the biodegradability reduction rate in a soil burial test (Brandelero *et al*., 2019).

Biodegradability is accomplished by living organisms (Torres *et al*., 2011). Soil microorganisms play a vital role in decomposing organic matter and releasing nutrients into plant-available forms (Saxena *et al*., 2020). Outdoor soil degradation is a complex process involving hydrolysis and enzymes from microorganisms. Soil microorganism types and numbers vary between soil types and they are influenced by soil organic matter content, texture, moisture, oxygen, and other factors (Chen *et al*., 2003). Bacteria are the most abundant microorganisms

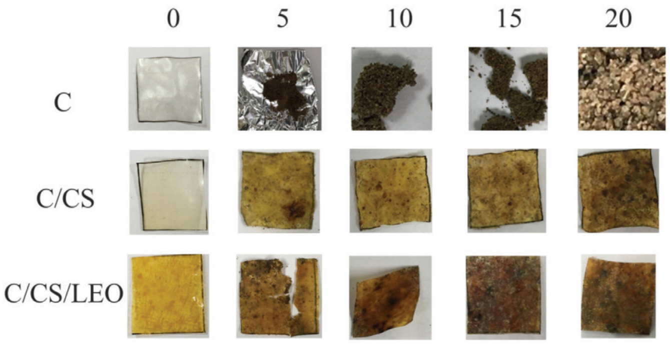


Figure 1: Digital photograph of cassava starch (C), cassava starch/chitosan (C/CS) and cassava starch/chitosan/lemongrass essential oil (C/CS/LEO) films before (0) and after the soil burial test (5, 10, 15, and 20 days)

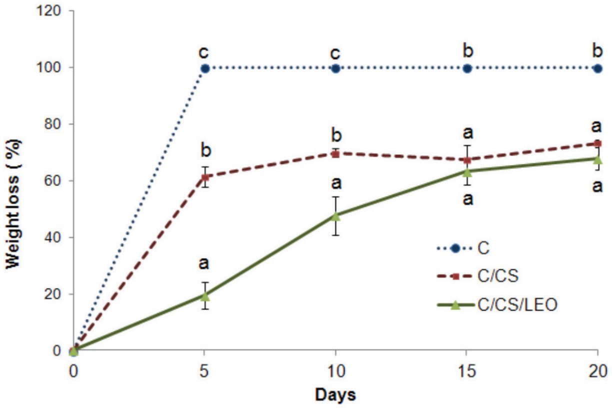


Figure 2: Weight loss (%) of the three formulations of starch films during the soil burial test. Different small vertical letters indicate significantly difference ($P < 0.05$) when analysed with Duncan's Multiple Range Test. The value is mean ± SE

in soil and they exist in the range of 10^8-10^9 per gramme of soil. This is followed by actinomycetes in the range 10^7-10^8 per gramme of soil, fungi in the range 10^5-10^6 per gramme of soil, and algae in the range 10^4-10^5 per gramme soil, respectively (Chen *et al.*, 2003). Amylase-producing bacteria, fungi and other microorganisms are known to have the ability to secrete amylases to digest glycosidic linkages found in starch. *Bacillus* spp. are the predominant bacteria found in soil and have been reported to produce a large variety of extracellular enzymes, in particular amylases (Saxena *et al.*, 2020).

The weight loss of chitosan films buried in paddy soil was higher than those buried in red soil, and 100% weight loss was observed after burial for two months (Nakashima *et al.*, 2005). The added chitosan was degraded in silty soil, but it was not observed in sandy soil (Sawaguchi *et al.*, 2015). This implies that the chitosan degradation rate would depend on the type of soil. Moreover, chitosan films prepared with acetic acid showed a higher rate of weight loss compared with those prepared with other acids, such as propionic acid, formic acid, and butyric acid (Nakashima *et al.*, 2005). The addition of urea as a plasticiser of starch film improved its mechanical properties and increased the weight loss and disintegration of samples (Rychter *et al.*, 2016).

Starch consists of two types of polysaccharides, the repeating of α-1,4 glycosides links in linear amylose and α-1,4 linkages and α-1,6 branch linkages in amylopactin (Moran *et al.*, 2011). Biodegradability and mechanical properties of starch films depend on the type of starch used to prepare these films (Torres *et al.*, 2011). Films prepared from cassava and sweet potato starch had high values of elongation at break, resulting in strong and tough properties (Torres *et al.*, 2011). Torres *et al.* (2011) studied the biodegradability of starch films by weight loss using a compost test. The highest percentage of weight loss was observed in cassava starch-based films, whereas gold potato starch-based films showed the lowest percentage of weight loss. The difference observed may be explained in terms of the amylose content of the different starches (Moran *et al.*, 2011). This weight loss study demonstrated a degradation behaviour that can be illustrated in two stages. In the first 5 days, the high weight loss was mainly associated with the leaching of glycerol.

This is in agreement with the observation of substantial intensity decrease in the spectrum peak around 3300 cm^{-1}, which is related to hydroxyl groups present in the starch/glycerol film (Torres *et al.*, 2011). This observation is attributed to the fact that α-glycosidic linkages in starch are easily broken down by hydrolytic enzymes (Moran *et al.*, 2011; Torres *et al.*, 2011). During the second stage of 5-20 days, the weight of C/CS films declined steadily. The degradation mechanism at this stage is associated with biological activity as the numbers of fungi and bacteria were higher (Figure 5). However, it depends on EO concentrations. Therefore, the lower weight loss of C/CS/LEO films when compared to C/CS films (Figure 2) corresponded with the FTIR results (Figure 3) and bacteria and fungi count (Figure 5).

Fourier-transform Infrared (FTIR) Analysis

The ATR/FTIR spectra of different films before and after the soil burial test are presented in Figure 3. Similar trends in FTIR spectra of cassava starch-based film was observed, with the absorption peak being around 3300 cm^{-1} and 1100-1000 cm^{-1} corresponding to the stretching of -OH group and C-OH belonging to starch, glycerol, and water (Torres *et al.*, 2011; Medina Jaramillo *et al.*, 2016) [Figure 3 (a)]. The peak at 2925 cm^{-1} is associated with the C-H stretch vibration (Medina Jaramillo *et al.*, 2016). The FTIR spectrum analysis showed similar intensity peaks for C/CS and C/CS/LEO films. The peaks at 1700-1500 cm^{-1} and 1500-1400 cm^{-1} are associated with -NH and C-H/CH_3/CH_2 stretching vibrations, respectively, which are the characteristics of chitosan [Figure 3 (a)]. Both peak regions gradually disappeared, which corresponded to the degradation, as highlighted with the dashed lines in [Figure 3 (a) to 3 (c)]. The more pronounced decreasing intensity of

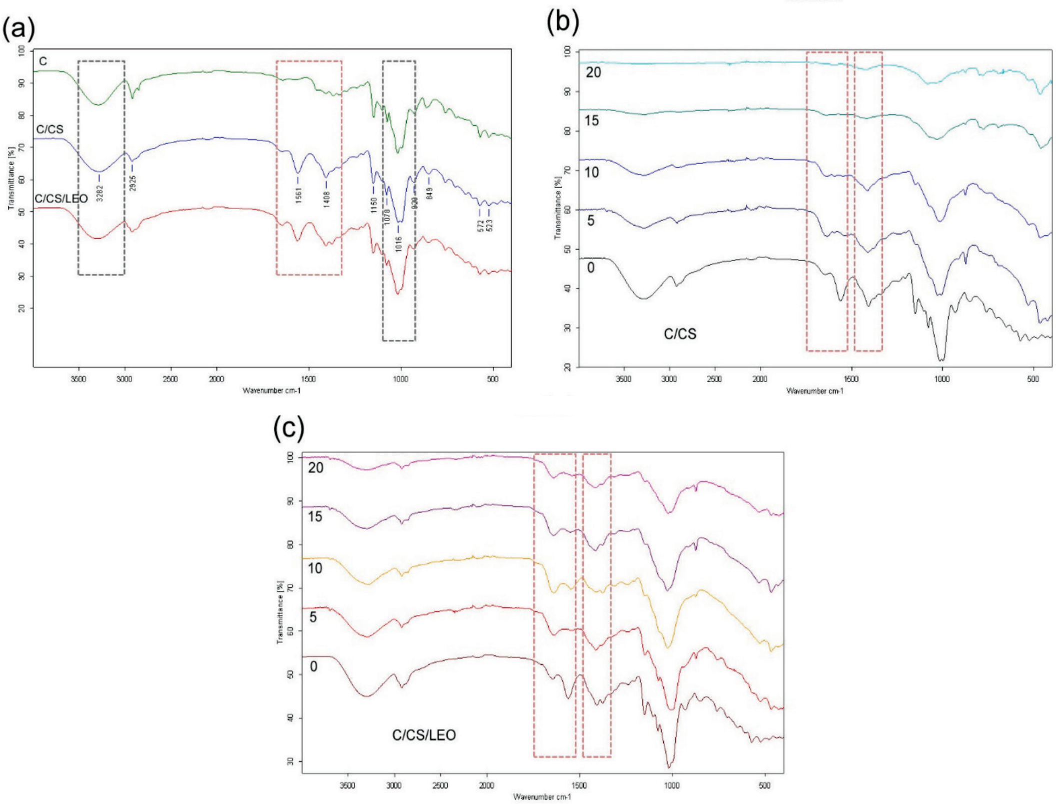


Figure 3: FTIR spectrum of cassava starch (C), cassava starch/chitosan (C/CS) and cassava starch/chitosan/lemongrass essential oil (C/CS/LEO) films before the soil burial test (a), C/CS (b) and C/CS/LEO (c) before (0 day) and after soil burial test for 5, 10, 15 and 20 days

peaks referring to OH- and C-OH was observed after degradation, with almost complete reduction within 20 days [Figure 3 (b)]. After 24 h of composting sweet potato starch film, FTIR spectra showed a decreased peak that corresponded to the hydroxyl groups (Torres *et al*., 2011). The peak at 1150 cm^{-1} associated with C-O-C in the glycosidic linkage was similar in all three formulations, and disappeared after five days of degradation [Figure 3 (b)]. The peak at 1561 cm^{-1}, which was attributed to the N-H function of chitosan, disappeared after 20 days, whereas C/CS/LEO films showed less reduction than C/CS films [Figure 3 (a) to 3 (c)].

The FTIR spectra confirmed the biological degradation by the action of α-amylase as the decreasing of the peaks is associated with starch glycosidic linkages at 1100-1000 cm^{-1} [Figure 3 (a) to 3 (c)]. The decreased peak intensity in the chitosan film was confirmed and showed that it was attributed to microorganisms (Altun *et al*., 2020). However, C/CS/LEO films' weight loss was significantly lower ($P < 0.05$) than C and C/CS films. The film weight loss increased until day 15 and remained steady (Figure 2). This behaviour may be caused by the addition of a lipophilic component in the formulation, which could reduce water vapour permeability (Ojagh *et al*., 2010; Jiménez *et al*., 2012) and enhance antimicrobial activity (Moore-Neibel *et al*., 2012; Souza *et al*., 2013; Hernández-García *et al*., 2021; Perdana *et al*., 2021). The moisture content value decreased as cinnamon essential oil (CEO) was added into chitosan-based film, resulting in the film network's compactness (Ojagh *et al*., 2010). The lower α-amylase activity was demonstrated by slowly decreasing peaks around 1100-1000 cm^{-1} [Figure 3 (c)], which corresponded with the weight loss results (Figure 2). A previous study showed that LEO had the least minimum inhibitory concentration towards Gram-positive and Gram-negative bacteria, yeast, and fungi (Perdana *et al*., 2021). CEO release profiles from C films were studied and it was found that CEO was released in 2 h on a petri dish (Souza *et al*., 2013). FTIR results suggested that the chitosan degradation rate in C/CS/LEO films was lower than in C/CS films. It should be taken into account that C films were degraded entirely within 5 days, therefore, no FTIR results were reported.

Electron Microscopy Observation of the Degraded Film Surfaces

SEM images of the film surfaces before and after degradation are shown in Figure 4. The films before burial exhibited a smooth and homogeneous surface (0 day). The addition of chitosan, when compared to C films without CS, exhibited a smoother surface. A rough surface with appearance of fibrils was observed in C/CS and C/CS/LEO films after 10 days of burial. The existence of holes was observed on the surface of both films. C/CS films seemingly showed a fewer number of holes compared with C/CS/LEO films. Further degradation of fibrils was detected after 20 days of burial.

Effects of Biodegradable Films on the Number of Microorganisms

The patterns observed are a consequence of the increase in microbial count. The numbers of fungi and bacteria were significantly lower ($P < 0.05$) in the control soil sample compared with the treatments (Figure 5). The microbial numbers were higher in the presence of starch film. The total number of bacteria trended to increase more than fungi during degradation. Therefore, bacteria possibly are a major player in the first stage of starch degradation. However, the specific bacteria that dominated during degradation are unknown. Soil with C/CS film exhibited the highest microbial numbers compared with other films. C, C/CS,

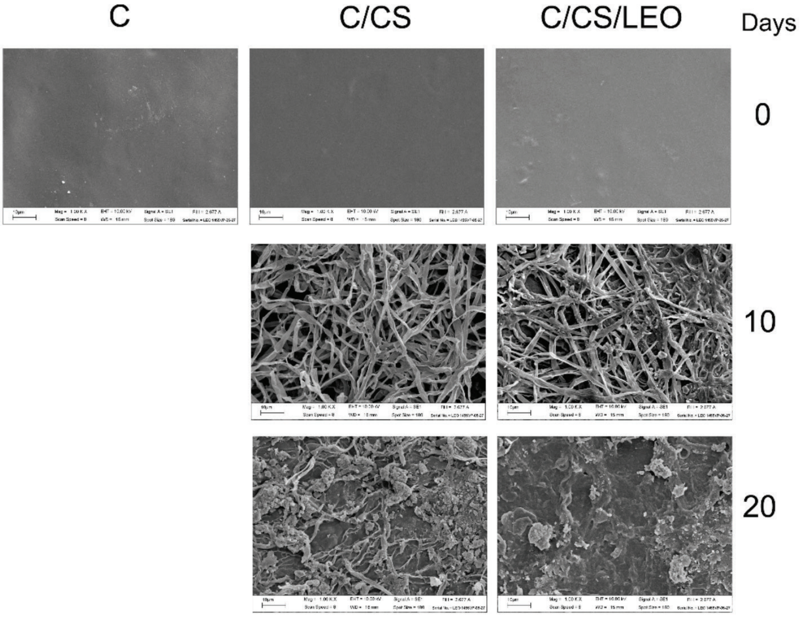


Figure 4: Scanning electron micrographs of cassava starch (C), cassava starch/chitosan (C/CS) and cassava starch/chitosan/lemongrass essential oil (C/CS/LEO) films before (0) and after the soil burial test (10 and 20 days). The bar is 10 µm

and C/CS/LEO films buried in soil for 20 days had significantly increased bacterial [Figure 5 (a)] and fungal [Figure 5 (b)] numbers ($P < 0.05$) compared with control (without film). However, the increase of microbial numbers was not significantly different ($P > 0.05$) among the three film formulations. The soil burial biodegradation study of corn-starch-sodium-alginate-based liquid mulch film showed that the film was completely degraded after 25 days and soil organic matter content was increased, involving the abundance of beneficial soil microorganisms (Gao *et al*., 2022). During the soil biodegradation, the abundance and diversity of bacterial community decreased, while the abundance of fungal communities increased significantly. The study suggested that fungi were the main microorganisms responsible for biodegradation (Gao *et al*., 2022).

Chitosan is considered non-toxic to humans and environmentally friendly for agriculture use (Raafat & Sahl, 2009). Chitosan-degrading bacteria, such as *Sphingobacterium multivorum*, were separated from the soil and responded to the high biodegradability (Nakashima *et al*., 2005). The study of biodegradation of chitin and chitosan films show that the weight losses of chitosan films buried in paddy soil were higher than those buried in red clay, and the decomposition rate of chitin films was higher than that of chitosan films (Nakashima *et al*., 2005). The decomposition of powdered chitosan in silty and sandy soils was observed and the numbers of fungi and bacteria were significantly higher by the end of day 11 of chitosan incubation (Sawaguchi *et al*., 2015). The numbers of bacteria and fungi were significantly higher, about 20 times by the end of day 11 in silty soil with chitosan (Sawaguchi *et al*., 2015). Some chitosan-utilising bacteria dominated the community structure, and the bacterial community structure became diverse after the complete degradation of chitosan (Sawaguchi *et al*., 2015). The addition of powdered chitosan in silty soil is associated with bacterial community changes as analysed by DGGE (Sawaguchi *et al*., 2015). Actinobacteria belonging to genera *Streptomyces* and *Kitasatospora* highly increased after chitosan addition and are possibly involved in the initial degradation process of chitosan (Sawaguchi *et al*., 2015). PCR-DGGE revealed that species belonging to the phylum Proteobacteria genus *Cellvibrio* had become predominant after the addition of chitin or chitosan (Sato *et al*., 2010). Some of other species that might not be involved in the degradation of chitin and chitosan appeared at the later stage of the experimental period (Sato *et al*., 2010). The dominant bacterial and fungal communities during the degradation of chitosan

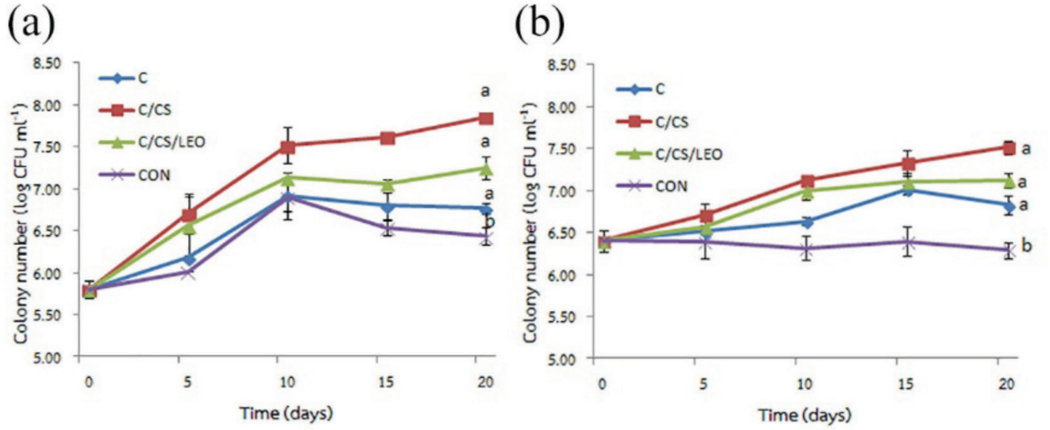


Figure 5: Total bacteria count (a) and fungi count (b) from pre-burial soil samples (control) and after soil burial test for 5, 10, 15, and 20 days. The same small vertical letters indicate no significantly difference ($P > 0.05$) when analysed with Duncan’s Multiple Range Test. The value is mean ± SE

under controlled thermophilic conditions were observed and it was found that Ascomycota and Proteobacteria played a significant role in biodegradation (Altun *et al*., 2020). On day 7 of degradation in the composting reactor, chitosan film had the highest fragmentation (Altun *et al*., 2020). Moreover, adding chitosanase-producing strains accelerated the chitosan degradation process (Altun *et al*., 2020). The studies mentioned above indicate that changes in bacterial community structures were affected by chitosan and some bacterial species, perhaps chitinolytic bacteria, dominated the community structure. Most of them are unidentified bacterial species, which might be unculturable (Nakashima *et al*., 2005; Sato *et al*., 2010; Sawaguchi *et al*., 2015). In addition, the antibacterial effect of chitosan might restrain the growth of other bacteria (Nakashima *et al*., 2005).

Effects of Biodegradable Films on Water Convolvulus Growth

Soil with all tested films had a significant effect on water convolvulus growth compared with control. Shoot length was significantly higher ($P < 0.05$) after 7 days and 21 days of planting (Table 1). However, it was not significantly different between C/CS and C/CS/LEO films. The highest shoot length of 19.5±0.50 cm, which increased 50% compared with control, was observed in the C/CS treatment after 21 days. Interestingly, shoot length similarly increased 11-12% with C treatment compared with control after 7, 14, and 21 days. However, the highest shoot length, 7.85±0.32 cm, was measured from the C/CS treatment after 7 days. After 21 days of planting with C/CS and C/CS/LEO films, shoot and root weights were significantly higher ($P < 0.05$) compared with C and control (Table 1).

Starch-based composite film's poor mechanical properties are commonly known due to the strong intermolecular and intramolecular hydrogen bonding in the amylose and amylopectin chains, making it too brittle (Jiménez *et al*., 2012; Omotoso *et al*., 2015; Thakur *et al*., 2019). Adding plasticisers, such as PEG and glycerol, promote interactions through hydrogen bonding, causing the shift of FTIR's main peaks, yet there is no effect on biodegradation (Kammoun *et al*., 2013). It has been reported that incorporating chitosan into cassava starch films could improve the mechanical properties and antimicrobial activity (Luchese *et al*., 2018; Perdana *et al*., 2021). There are several studies on the application of chitosan through soaking, spraying, and coating on plant productivity to avoid the use of chemical products and dangerous farming practices (Guan et al., 2009; Akter Mukta *et al*., 2017; Choudhary *et al*., 2017; Malerba & Cerana, 2018; Li *et al*., 2019). To the best of our knowledge, this is the first report on the effects of chitosan released from cassava starch film during biodegradation on plant growth. The effects of chitosan nanoparticles (CSNPs) and chitosan (CS) on seed germination and seedling growth of wheat (*Triticum aestivum* L.) were compared and reported (Li *et al*., 2019). The study indicated that the adsorption of CSNPs

Table 1: Effects of biodegradable films on shoot length, shoot fresh weight, and root fresh weight of water convolvulus (*Ipomoea aquatica*)

Treatment	**Shoot length (cm)**			**Shoot fresh weight (g)**	**Root fresh weight (g)**
	7 days	**14 days**	**21 days**	**21 days**	
Control	5.74 ± 0.17^{a}	9.22 ± 0.91^{a}	13.0 ± 0.80^{a}	0.68 ± 0.04^{a}	0.09 ± 0.01^{a}
C	6.75 ± 0.42^{b}	10.27 ± 0.97^{a}	14.5 ± 0.80^{b}	0.75 ± 0.03^{ab}	0.11 ± 0.03^{a}
C/CS	7.85 ± 0.32^{c}	11.55 ± 1.00^{a}	19.5 ± 0.50^{c}	0.85 ± 0.02^{b}	0.19 ± 0.01^{b}
C/CS/LEO	7.46 ± 0.18^{bc}	10.98 ± 1.02^{a}	18.9 ± 0.50^{c}	0.78 ± 0.02^{b}	0.17 ± 0.02^{b}

Values are mean±SE of three independent replications. Mean values with the same letter in the same column are not significantly different from each other (ANOVA, DMRT, $P > 0.05$)

on the surface of wheat seeds was higher than that of CS. CSNPs at a lower concentration than CS had a positive effect on chlorophyll (Chl) synthesis in wheat seedling and promote wheat growth by activating the indole-3-acetic acid (IAA) signalling pathway (Li *et al*., 2019).

The study on Cu-chitosan NPs as plant growth agents by treating maize seeds with Cu-chitosan NPs followed by foliar spray in pot experiment showed increased plant height, stem diameter, root length, root number, and Chl content (Choudhary *et al*., 2017). In addition, chitosan nanoparticles-supplemented rhizospheric *Pseudomonas aeruginosa* was found to have enhancement effect on the shoot length and fresh weight of *Vigna unguiculata* plants (Panichikkal & Krishnankutty, 2022). Besides, Cu-chitosan NPs also serve as an antifungal agent against curvularia leaf spot disease to protect the plant from oxidative stress. The elevation activities of antioxidant (superoxide dismutase and peroxidase) and defence enzymes (polyphenol oxidase and phenylalanine ammonia-lyase) were observed in NP-treated samples (Choudhary *et al*., 2017). Rahman *et al*. (2018) reported that both fresh and dry shoot and root weight increased significantly strawberries applied with chitosan compared with untreated control. Moreover, total carotenoids, anthocyanins, flavonoids, phenolics and antioxidant activity increased with the spray application of chitosan in a dose-dependent manner (Rahman *et al*., 2018). Transcriptome analysis of strawberries treated with chitosan showed in an increase in gene signatures associated with the plant immune system, hormone metabolism, biotic and abiotic stresses, system-acquired resistance, photosynthesis, heat-shock proteins, and reprogramming of protein metabolism (Landi *et al*., 2017).

In the present study, there was a significant increase in shoot length after 7 days of water convolvulus planting with C films (Table 1). Additionally, the total microbial numbers were higher in the presence of the starch film after burial for 5 days as shown in Figure 5. Microorganisms in the soil are known to have various direct and indirect mechanisms of plant growth promotion (Saxena *et al*., 2020). The findings of this study may explain that the increase of bacteria and fungi as a result of secondary effects produced from starch improved plant growth indirectly through the acquisition of nutrients. In addition, a significant increase in shoot length and root weight was recorded after 21 days of water convolvulus planting with C/CS and C/CS/LEO films (Table 1). Using the solution casting method, the biodegradation of films resulting in chitosan release improved plant growth. The decreased intensity of the chitosan film peak was more pronounced in C/CS than C/CS/LEO films analysed by FTIR [Figure 3 (b) to 3 (c)]. These results indicate that the released chitosan maximises water convolvulus shoot length, shoot and root weights in the C/CS treatment (Table 1). The utilisation of potato starch films plasticised with urea for plants' fertilisation was also reported, and urea release rates were dependent on time and concentration (Rychter *et al*., 2016). The completed biodegradation process generates a fertiliser for the ground (La Mantia *et al*., 2020). Moreover, the application of plant and probiotic bacteria indicates a significant increase in strawberry fruit yield. However, the yield provided was not as high as the chitosan treatment (Akter Mukta *et al*., 2017). Our results confirm that the degradation of biodegradable film releasing chitosan results in the improvement of plant growth. Further study on the bacterial community during the biodegradation process would be helpful in better understanding the complicated interaction between bacterial species, details of the biodegradation mechanisms and plant growth.

Conclusion

This study demonstrated that starch-based composite films exhibited a limited lifetime in compost conditions that makes them eco-friendly after disposal in landfills. The presence of chitosan and LEO might help the compact structure of C/CS/LEO films and cause the film to degrade slower. Starch-based films buried in soil significantly increased soil bacteria and fungi and significantly increased the shoot length of water convolvulus after planting for 21 days. The analysis of film stability, durability and shelf life could provide additional information to select the potential application of the films. It should be noted that the effect of released chitosan on plant growth was confirmed. Therefore, the utilisation of starch-based films modified with chitosan in agriculture or horticulture purposes, which quickly degrades, as a plant growth promoter could be a great potential application for sustainable production of high-quality plants.

Acknowledgements

The authors thank Muhammad Iqbal Perdana for technical assistance and the Language Centre, Prince of Songkla University, Pattani Campus, and Ana Crawford and Dr. Christina Spevak for proofreading this article. This study was part of the Science classroom in a university-affiliated school supported by the Ministry of Science and Technology, Thailand.

References

Akter Mukta, J., Rahman, M., As Sabir, A., Gupta, D. R., Surovy, M. Z., Rahman, M., & Islam, M. T. (2017). Chitosan and plant probiotics application enhance growth and yield of strawberry. *Biocatalysis and Agricultural Biotechnology*, *11*, 9-18.

Altun, E., Çelik, E., & Ersan, H. Y. (2020). Tailoring the microbial community for improving the biodegradation of chitosan films in composting environment. *Journal of Polymers and the Environment*, *28*(5), 1548-1559.

Armynah, B., Anugrahwidya, R., & Tahir, D. (2022). Composite cassava starch/chitosan/Pineapple Leaf Fiber (PALF)/Zinc Oxide (ZnO): Bioplastics with high mechanical properties and faster degradation in soil and seawater. *International Journal of Biological Macromolecules*, *213*, 814-823.

Avella, M., De Vlieger, J. J., Errico, M. E., Fischer, S., Vacca, P., & Volpe, M. G. (2005). Biodegradable starch/clay nanocomposite films for food packaging applications. *Food Chemistry*, *93*(3), 467-474.

Brandelero, R. P. H., Alfaro, A. T., Marques, P. T., & Brandelero, E. M. (2019). New approach of starch and chitosan films as biodegradable mulching. *Revista Virtual de Quimica*, *11*(3), 686-698.

Chen, G., Zhu, H., & Zhang, Y. (2003). Soil microbial activities and carbon and nitrogen fixation. *Research in Microbiology*, *154*(6), 393-398.

Choudhary, R. C., Kumaraswamy, R. V., Kumari, S., Sharma, S. S., Pal, A., Raliya, R., Biswas, P., & Saharan, V. (2017). Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays* L.). *Scientific Reports*, *7*(1), 9754-9754.

Dutta, P. K., Tripathi, S., Mehrotra, G. K., & Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications. *Food Chemistry*, *114*(4), 1173-1182.

Gao, X., Fu, C., Li, M., Qi, X., & Jia, X. (2022). Effects of biodegradation of corn-starch-sodium-alginate-based liquid mulch film on soil microbial functions. *International Journal of Environmental Research and Public Health*, *19*(14).

Goy, R. C., de Britto, D., & Assis, O. B. G. (2009). A review of the antimicrobial activity of chitosan. *Polimeros*, *19*(3), 241-247.

Goy, R. C., Morais, S. T. B., & Assis, O. B. G. (2016). Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on *E. coli* and *S. aureus* growth. *Revista Brasileira de Farmacognosia*, *26*(1), 122-127.

Guan, Y.-j., Hu, J., Wang, X.-j., & Shao, C.-x. (2009). Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *Journal of*

Zhejiang University Science B, *10*(6), 427-433.

Hernández-García, E., Vargas, M., González-Martínez, C., & Chiralt, A. (2021). Biodegradable antimicrobial films for food packaging: Effect of antimicrobials on degradation. *Foods*, *10*(6), 1256.

Ishigaki, T., Kawagoshi, T., Ike, M., & Fujita, M. (1999). Biodegradation of a polyvinyl alcohol-starch blend plastic film. *World Journal of Microbiology and Biotechnology*, *15*, 321-327.

Jiang, H., Sun, Z., Jia, R., Wang, X., & Huang, J. (2016). Effect of chitosan as an antifungal and preservative agent on postharvest blueberry. *Journal of Food Quality*, *39*(5), 516-523.

Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2012). Edible and biodegradable starch films: A review. *Food and Bioprocess Technology*, *5*(6), 2058-2076.

Kammoun, M., Haddar, M., Kossentini Kallel, T., Dammak, M., & Sayari, A. (2013). Biological properties and biodegradation studies of chitosan biofilms plasticized with PEG and Glycerol. *International Journal of Biological Macromolecules*, *62*, 433-438.

La Mantia, F. P., Ascione, L., Mistretta, M. C., Rapisarda, M., & Rizzarelli, P. (2020). Comparative investigation on the soil burial degradation behaviour of polymer films for agriculture before and after photo-oxidation. *Polymers*, *12*(4), 753.

Landi, L., De Miccolis Angelini, R. M., Pollastro, S., Feliziani, E., Faretra, F., & Romanazzi, G. (2017). Global transcriptome analysis and identification of differentially expressed genes in strawberry after preharvest application of benzothiadiazole and chitosan. *Frontiers in Plant Science*, *8*, 235-235.

Li, R., He, J., Xie, H., Wang, W., Bose, S. K., Sun, Y., Hu, J., & Yin, H. (2019). Effects of chitosan nanoparticles on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *International Journal of Biological Macromolecules*, *126*, 91-100.

Luchese, C. L., Pavoni, J. M. F., Dos Santos, N. Z., Quines, L. K., Pollo, L. D., Spada, J. C., & Tessaro, I. C. (2018). Effect of chitosan addition on the properties of films prepared with corn and cassava starches. *Journal of Food Science and Technology*, *55*(8), 2963-2973.

Malerba, M., & Cerana, R. (2018). Recent advances of chitosan applications in plants. *Polymers*, *10*(2), 118.

Medina Jaramillo, C., Gutiérrez, T. J., Goyanes, S., Bernal, C., & Famá, L. (2016). Biodegradability and plasticizing effect of yerba mate extract on cassava starch edible films. *Carbohydrate Polymers*, *151*, 150-159.

Mith, H., Duré, R., Delcenserie, V., Zhiri, A., Daube, G., & Clinquart, A. (2014). Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria. *Food Science & Nutrition*, *2*(4), 403-416.

Moore-Neibel, K., Gerber, C., Patel, J., Friedman, M., & Ravishankar, S. (2012). Antimicrobial activity of lemongrass oil against *Salmonella enterica* on organic leafy greens. *Journal of Applied Microbiology*, *112*(3), 485-492.

Moran, J. I., Cyras, V. P., Vazquez, A., & Foresti, M. L. (2011). Characterization of chemically modified potato starch films through enzymatic degradation. *Journal of Polymers and the Environment*, *19*(1), 217-224.

Nakashima, T., Nakano, Y., Bin, Y., & Matsuo, M. (2005). Biodegradation characteristics of chitin and chitosan films. *Journal of Home Economics of Japan*, *56*(12), 889-897.

Ojagh, S. M., Rezaei, M., Razavi, S. H., & Hosseini, S. M. H. (2010). Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. *Food Chemistry*, *122*(1), 161-166.

Omotoso, M. A., Adeyefa, O. S., Animashaun, E. A., & Osibanjo, O. O. (2015). Biogradable starch film from cassava, corn, potato and yam. *Chemistry and Materials Research*, *7*(12), 15-24.

Panichikkal, J., & Krishnankutty, R. E. (2022). Chitosan and gold nanoparticles supplementation for augmentation of indole-3-acetic acid production by Rhizospheric *Pseudomonas aeruginosa* and plant growth enhancement. *Current Microbiology*, *79*(6), 185.

Pathak, V. M., & Navneet. (2017). Review on the current status of polymer degradation: A microbial approach. *Bioresources and Bioprocessing*, *4*(1), 15.

Perdana, M. I., Ruamcharoen, J., Panphon, S., & Leelakriangsak, M. (2021). Antimicrobial activity and physical properties of starch/chitosan film incorporated with lemongrass essential oil and its application. *LWT - Food Science and Technology*, *141*, 110934.

Raafat, D., & Sahl, H. G. (2009). Chitosan and its antimicrobial potential-A critical literature survey. *Microb Biotechnol*, *2*(2), 186-201.

Rahman, M., Mukta, J. A., Sabir, A. A., Gupta, D. R., Mohi-Ud-Din, M., Hasanuzzaman, M., Miah, M. G., Rahman, M., & Islam, M. T. (2018). Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. *PloS One*, *13*(9), e0203769-e0203769.

Ren, L., Yan, X., Zhou, J., Tong, J., & Su, X. (2017). Influence of chitosan concentration on mechanical and barrier properties of corn starch/chitosan films. *International Journal of Biological Macromolecules*, *105*, 1636-1643.

Ruamcharoen, J., Munlee, R., & Ruamcharoen, P. (2019). Improvement of water vapor barrier and mechanical properties of sago starch-kaolinite nanocomposites. *Polymer Composites*, 1-9.

Rychter, P., Kot, M., Bajer, K., Rogacz, D., Siskova, A., & Kapusniak, J. (2016). Utilization of starch films plasticized with urea as fertilizer for improvement of plant growth. *Carbohydrate Polymers*, *137*, 127-138.

Sato, K., Azama, Y., Nogawa, M., Taguchi, G., & Shimosaka, M. (2010). Analysis of a change in bacterial community in different environments with addition of chitin or chitosan. *Journal of Bioscience and Bioengineering*, *109*, 472-478.

Savard, T., Beaulieu, C., Boucher, I., & Champagne, C. P. (2002). Antimicrobial action of hydrolyzed chitosan against spoilage yeasts and lactic acid bacteria of fermented vegetables. *Journal of Food Protection*, *65*(5), 828-833.

Sawaguchi, A., Ono, S., Oomura, M., Inami, K., Kumeta, Y., Honda, K., Sameshima-Saito, R., Sakamoto, K., Ando, A., & Saito, A. (2015). Chitosan degradation and associated changes in bacterial community structures in two contrasting soils. *Soil Science and Plant Nutrition*, *61*(3), 471-480.

Saxena, A. K., Kumar, M., Chakdar, H., Anuroopa, N., & Bagyaraj, D. J. (2020). *Bacillus* species in soil as a natural resource for plant health and nutrition. *Journal of Applied Microbiology*, *128*(6), 1583-1594.

Souza, A. C., Goto, G. E. O., Mainardi, J. A., Coelho, A. C. V., & Tadini, C. C. (2013). Cassava starch composite films incorporated with cinnamon essential oil: Antimicrobial activity, microstructure, mechanical and barrier properties. *LWT - Food Science and Technology*, *54*(2), 346-352.

Thakur, R., Pristijono, P., Scarlett, C. J., Bowyer, M., Singh, S. P., & Vuong, Q. V. (2019). Starch-based films: Major factors affecting their properties. *International Journal of Biological Macromolecules*, *132*, 1079-1089.

Torres, F. G., Troncoso, O. P., Torres, C., Diaz, D. A., & Amaya, E. (2011). Biodegradability and mechanical properties of starch films from Andean crops. *International Journal of Biological Macromolecules*, *48*(4), 603-606.

Yuan, G., Chen, X., & Li, D. (2016). Chitosan films and coatings containing essential oils: The antioxidant and antimicrobial activity, and application in food systems. *Food Research International*, *89*, 117-128.