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Development of Alginate-Based Artificial Plant Tissues for Trapping *Pythium aphanidermatum* Causing Vegetable Root Rot in Hydroponic Growing Systems

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Abstract

The objective of this study was to synthesize and evaluate the efficiency of artificial plant tissues made from alginate at various concentrations for trapping *Pythium aphanidermatum*, a fungal pathogen responsible for root rot disease in plants grown utilizing hydroponic systems. This pathogen is commonly introduced into hydroponic systems through multiple contamination sources such as seeds, nutrients, hydroponic solutions, water, and fertilizers, leading to widespread and difficult-to-control outbreaks in such systems. The synthesized artificial plant tissues were designed to closely resemble natural plant tissues, enabling *P. aphanidermatum* spores to germinate and develop germ tubes that penetrate and adhere to the artificial plant tissues in order to absorb nutrients within 24 h. This approach allows for the efficient removal of the pathogen without the use of chemical agents, thus preventing chemical residue accumulation in the hydroponic system. The samples used in this study included artificial plant tissues synthesized from alginate at concentrations of 1%, 2%, 3%, 4% and 5%. These samples were tested for their ability to trap *P. aphanidermatum* spores within 24 h while being placed on the water circulation grid of the hydroponic system. Additionally, the amount of residual *P. aphanidermatum* in the nutrient reservoir of the hydroponic system (prior to re-entry into the circulation loop) was analyzed. The results indicated that the artificial plant tissue synthesized from 3% alginate exhibited the highest average retention of *P. aphanidermatum*, with 9.43 colonies per unit area. Moreover, it resulted in the lowest residual amount of *P. aphanidermatum* in the water reservoir after 24 h, averaging 6 colonies/mL.

Introduction

Plant diseases caused by hydroponic cultivation systems occur in almost every season, especially in the summer. Most of these diseases occur in lettuce such as red oak, green oak. All red oak, green oak or any other varieties of these salad plants are lettuces that are expensive compared to other types of vegetables. In addition, there is a higher demand for these vegetables from consumers. The vegetables produced need to be

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cleaned and made safe for consumers (Kangwarin & Roosae, 2020). Hydroponic cultivation is popular. Since these vegetables originated in cold climates, they are not well adapted or acclimatized to the heat in Thailand, which averages temperatures of over 20°C. Notably, vegetables tend to be weak during hot weather (Goldberg & Stanghellini, 1990). Various plant diseases are caused by pathogens of the genus *Pythium*, especially *P. aphanidermatum*, which is in the form of oospores and is a pathogen that destroys the root and base systems of important vegetables (Postma et al., 2008). It tends to spread rapidly in hot weather. In addition to the pathogens that come with the nutrient solution used for vegetables grown in hydroponic systems, diseases can also be caused by environmental factors, such as the use of circulating water from a whirlpool tank system that has been filled with water previously contaminated with pathogens, or the humidity in the atmosphere and the temperature of the water in the hydroponic system, which may be high enough to allow the spores to grow prolifically and weaken the plants, making disease more likely. Plants with rotten roots will be stunted and wither. The affected roots will rot and peel off the outer layer, leaving only the core, which results in a loss of economic value for vegetable farmers (Borrero et al., 2017; Juber et al., 2014).

Table 1 Pathogen activity period and effects of *P. aphanidermatum* on green oak grown in a hydroponic system

Stage	Pathogen Activity	Host Impact
1. Germination	Germ tube emerges from encysted zoospore	Anchors on root
2. Appressorium formation	Generates turgor & enzymes	Wall softened for entry
3. Penetration	Peg & hyphae invade vascular tissues	Access to water/nutrients
4. Nutrient uptake	Hyphae absorb fluids and kill cells	Rot and wilting
5. Sporulation	Zoospores released into soil	Initiates new infection cycles

Remark: Modified from Hardham (2001) and Pettongkhao et al. (2020)

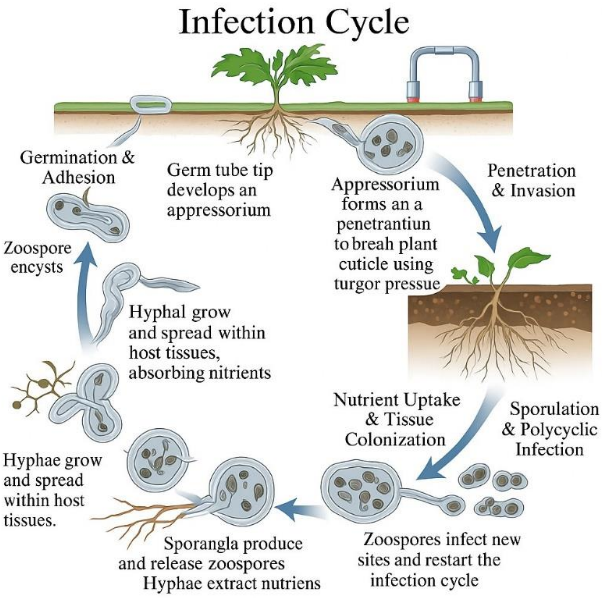


Fig. 1 Pathogenesis cycle of *P. aphanidermatum* on green oak grown in a hydroponic system

Source: Hardham (2001) and Pettongkhao et al. (2020)

Gomes et al. (2025) stated that once the spores enter the planting system, they are difficult to control. They spread well in water and can survive in both soil and water by living in the roots of diseased plants. Therefore, if the roots of plants with root rot disease stick to the planting rails and the grower cannot thoroughly clean the inside of the rails, the pathogens will be able to grow again. It was found that in the early stages of growing plants in a hydroponic system, no pathogens would be found, or there may be such a small amount that observation could not effectively be achieved. But as time passes, the number of pathogens increases in later stages. Root rot problems in the hydroponic system are caused by pathogens such as *Pythium spp.*, especially *P. aphanidermatum*, which is a contaminant in the hydroponic system. From the studies of Lee and Lee (2015) and Utkhede et al. (2000), there were reports of root rot disease in various vegetables such as green oak, lettuce, butterhead, etc., grown in hydroponic systems, which severely affected the yield of the vegetable growth. Sun et al. (2012) remarked that a popular solution is to remove the entire set of vegetables, wash the equipment, and disinfect it with chlorine. However, cleaning the hydroponic system used to grow infected plants with acid or disinfectant with 72.2% propamocarb hydrochloride, which remains in the plant parts grown in the system, will increase cost burdens. Additionally, time is wasted when cleaning the system, which is difficult to achieve and labor intensive, and causes environmental pollution problems from the disinfectant added to the cleaning system that kills *P. aphanidermatum* and other germs that destroy plant roots (Table 1 and Fig. 1). This is just one way to solve the problem of plant diseases in hydroponic systems. There is another way with better outcomes for the health safety of consumers than the first method. Pongpiyadech et al. (2020) reported on a biological method that uses microorganisms to control plant diseases using *Trichoderma harzianum*, a highly effective antagonistic fungus, which collects diseased plants and plant root debris from the hydroponic system and drains the solution in a vortex tank of the hydroponic system to reduce the levels of germs.

In addition to the two methods of disease control, using chemicals and antagonistic fungi respectively, as presented above, another convenient and safe method is to use artificial plant tissue to store germs in hydroponic systems by developing alginate, which is naturally occurring, with a tissue-like appearance similar to that of plants, in order to attract more fungal spores than the actual plant tissue, which would otherwise germinate germ tubes to enter the plant tissue and cause the plant to be infected with *P. aphanidermatum*, which eventually causes damage. Alginate has been used to control various diseases, as seen in research such as Juthamard et al. (2017) who studied the use of chitosan together with sodium alginate to control anthracnose in mangoes, which effectively reduced the incidence of diseases. In addition, the study of Adan et al. (2015) controlled diseases by wrappings with sodium alginate glycol to prevent *T. asperellum* in the field. Yamaguchi et al. (2017) synthesized artificial plant tissue by using an alginate solution with a calcium solution. The calcium ions would bind to the alginate molecular chains, causing the polymer chains to move closer together, forming an outer gel that calcium ions can penetrate less. Then, calcium ions on the outside diffuse through the outer gel into the center of the alginate, making the outer gel stronger than the inner gel, allowing germs that attach to Alginate-based materials to exhibit a structure that closely resembles plant cell fibers and possess functions and properties analogous to natural plant tissues (Sahoo & Biswal, 2021). They grow germ tubes to embed themselves in the artificial plant tissue that is synthesized instead of the plant tissue trapping and preventing from circulating back into the hydroponic system again. This can reduce the problem of pathogens in the hydroponic vegetable system, reduce the time spent on solving problems (Lee & Mooney, 2012), reduce the use of chemicals in the hydroponic system, and reduce the cost of eliminating pathogens in the system. Therefore, bearing in mind the benefits of alginate in controlling diseases mentioned above, this research has 2 aims. 1. To develop synthetic artificial tissues from alginate at different alginate concentrations (1-5%) and examine the physical characteristics and chemical composition of the artificial plant tissues, and 2. to analyze the efficiency of artificial plant tissues at different alginate concentrations (1–5%) in retaining *P. aphanidermatum*, which is a pathogen of vegetables grown in hydroponic systems (Fig. 1) and inhibits the occurrence of root rot disease while also stopping spread from the roots to the leaf base to the top in hydroponic vegetable cultivation.

Materials and methods

1. Development of artificial plant tissue from alginate

The process was as follows: Weigh 1 g of sodium alginate (Lobachemic, India), add 99 mL of water into a beaker containing alginate, put the beaker in a 600 W microwave (MS4295DIS, LG, Japan) for 2–3 min, and stir the alginate until it melts. Let it cool and pour the solution into the mold, spread it evenly, and let it cool at room temperature. Then prepare 5 g of calcium chloride (KC, Japan), and mix it with 250 mL of distilled water. Pour the chilled mixture at 4°C calcium chloride into the Petri dish containing the prepared alginate, leave it to coagulate, and remove the film. The experiment was conducted with variations of weighing 1 g (1%) of alginate to 2 g (2%), 3 g (3%), 4 g (4%), and 5 g (5%), respectively.

2. Physical properties and chemical composition of artificial plant tissue synthesized from alginate

The physical characteristics were tested by scanning electron microscopy (SEM) (LEO 1450 VP, Carl Zeiss, Jena, Germany) and for chemical composition by measuring the energy distribution of X-rays (FEI Quanta 450, USA).

Sample Preparation for SEM Analysis

1) Artificial plant tissue synthesized from alginate at various concentrations were prepared by cutting them into appropriately sized pieces.

2) The surface area of each sample was selected and trimmed to a suitable size for mounting on the specimen stub and adhering it to carbon conductive tape.

3) The samples were then coated with a platinum layer with a grain size of 2–3 nm to provide electrical conductivity under high-vacuum conditions and to reduce thermal damage from the incident electron beam.

After coating, the samples were analyzed using a scanning electron microscope (SEM) in BE/BSE and EDS modes. The SEM system operates at an electron energy range of 0.1–30 keV, and elemental analysis is conducted using characteristic X-ray spectra. A silicon drift detector (SDD) is used to collect the emitted X-rays for energy-dispersive spectroscopy (EDS). Then, the experiment was conducted by placing it in a filter funnel with a mesh under an alginate sheet to retain spores in a hydroponic system (Talbot & White, 2013).

3. Population and sampling at various concentration levels of alginate artificial plant tissue in a hydroponic system

The total population was hydroponic vegetables of Green Oak (Chaitai, Thailand), totaling 150 plants (Fig. 2). The experiment was a completely randomized design (CRD) by experimenting at each concentration level of alginate artificial plant tissue, namely at concentration levels of 1%, 2%, 3%, 4% and 5%, with 10 plants/rail system, arranged in rails 1–5 representing the percentage of alginate artificial plant tissue concentration, totaling 50 plants. Green Oak seeds were obtained from Chia Tai Company, Thailand., for the purpose of containing germs in the hydroponic system. Green oak was grown in the hydroponic system. Each replication was grown for 14 days, The experiment was conducted from April 1 to April 14, 2023, between 08:00 and 17:00. The growing medium used was peat moss provided by Chaitai Company. The hydroponic system applied was the nutrient film technique (NFT). The pH of the nutrient solution was maintained between 6 and 7, with a water temperature of 26°C. The ambient air temperature ranged from 30 to 32°C, with a relative humidity of 75–85% RH. Light intensity was maintained between 40,000 and 60,000 lux, when the plants had dense root growth, and 3 replications were tested (grown in 3 hydroponic systems).

Fig. 2 Hydroponic system for experimental vegetable cultivation

4. Preparation of *P. aphanidermatum* culture

The process was as follows: Weigh 9.6 g of potato dextrose broth (PDB) (Himedia, India), 8 g of agar powder (agar), and 4 g of carboxymethyl cellulose (CMC) (KC, Japan). Dissolve in 150 mL of distilled water and place in a chemical storage bottle. Stir until the mixture is well mixed. Add 250 mL of water and again stir until the mixture is well mixed. Store in a chemical storage bottle. Sterilize the mixture in an autoclave (HP85 serial, Hirayama, Japan) at 100°C under steam pressure for 1 h. Then, let it cool to 50–60°C and remove it. Let it cool down before pouring it into a 90 mm diameter Petri dish, approximately 8 mL/dish. Wait for the culture medium to set and add *P. aphanidermatum* obtained from the Department of Plant Disease Protection. Ministry of Agriculture and Cooperatives. *P. aphanidermatum* was incubated on potato dextrose agar (PDA) by growing it in the medium for 18 h; use a needle to cut a piece into potato dextrose agar (PDA) medium and incubate at 25°C; wait for *P. aphanidermatum* fungus to grow for 5 days (Anon et al., 2017). *P. aphanidermatum* is grown on potato dextrose agar (PDA) + carboxymethyl cellulose (CMC) medium (used instead of natural cellulose to form *P. aphanidermatum* spores). Add 10 mL of sterile water and pour it over. Take the loop that has been sterilized by burning and cooling the mold sufficiently to scrape the *P. aphanidermatum* fungus and take the sample to test under a light microscope (SteREO Discovery.V8, ZEISS, Germany) to see the spores of *P. aphanidermatum*. Confirm the presence of spores, as in Fig. 3a, showing a group of *P. aphanidermatum*, Fig. 3b, showing *P. aphanidermatum* in isolation. Precultures are prepared at a spore concentration of 10/mL, and 10 mL of the solution containing *P. aphanidermatum* spores are aspirated into a 100-mL volumetric flask.

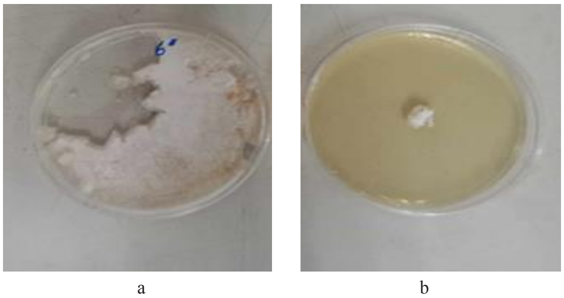


Fig. 3 Spores of *P. aphanidermatum* grown on potato dextrose agar (PDA) and carboxy methyl cellulose (CMC) media, showing a group of *P. aphanidermatum* (a) and showing *P. aphanidermatum* in isolation (b)

5. Performance testing of alginate artificial plant tissue and performance testing of synthetic alginate artificial plant tissue

The process was as follows: Weigh 1 g of sodium alginate, add 100 mL of water, pour into a beaker containing alginate, then put the beaker in a 600W microwave for 2–3 min and stir until the alginate dissolves. Let it cool and pour the solution into the mold, spread it evenly, and let it cool at room temperature. Prepare 5 g of calcium chloride (calcium chloride 2-hydrate) mixed with 250 mL of distilled water and refrigerate. Then pour the chilled calcium chloride (Calcium chloride 2-hydrate) into the prepared alginate culture dish, let the substance coagulate and remove the film, then change from weighing 1 g of alginate to 2 g, 3 g, 4 g, and 5 g, respectively, then put the artificial plant tissue at all concentrations into the filter funnel; inside the funnel. For this experiment the artificial plant tissue made from alginate was placed in a filter funnel at 09:00 AM, and the hydroponic system was operated continuously for 24 h. Samples from the artificial plant tissue were collected at 09:00 AM on the following day for further analysis as described in subheadings 6. There was a net supporting the alginate sheet, and it was installed in the wastewater system of the hydroponic system. Collected spores from artificial plant tissue were made to alginate into the filter funnel and the culture samplings were collected. The culture samples were collected for analysis by growing them on potato dextrose agar (PDA) and carboxymethyl cellulose (CMC) and then using the count plate method to calculate the number of colonies.

6. Collection and analysis of water samples in hydroponic systems

The process was as follows: Take a sterile glass pipette to collect a 10 mL sample of water from the hydroponic system tank containing artificial plant tissue at concentrations of 1%, 2%, 3%, 4% and 5% in the system. Collect into sterile screw-cap test tubes and close the lid tightly. Start collecting at 0 h, 6 h, 12 h, and 24 h, respectively. Prepare the culture medium: Dichloran rose bengal chloramphenicol agar (Himadia, India) 15.82 g/500 mL. Mix the test tubes with the water sample in a shaker and burn the top of the bottle. Use a micropipette (Pipet-Lite LTS Pipette L-2XLS+, Mettler Toledo, Switzerland) to suck the sample from the test tube into dichloran rose bengal chloramphenicol agar (to test for the presence of *P. aphanidermatum* remaining in the water in the hydroponic system) in the volume of 100 µL. Spread the burnt triangular glass rod over the Petri dish (Bioscan, China). Incubate in a 25°C incubator for 6 days and count the bacterial colonies using the count plate method. The efficiency of *P. aphanidermatum* retention in the artificial plant tissue at each concentration level and the remaining in the hydroponic water was analyzed using ANOVA statistical analysis.

7. Statistical analysis

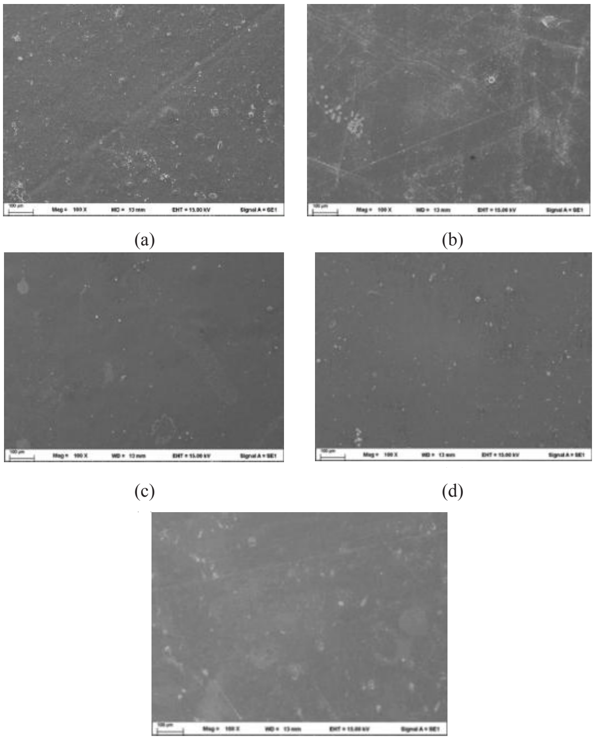
The differences were compared statistically using the analysis of variance (ANOVA, SPSS Version 20, IBM Corp., USA) for 1) number of *P. aphanidermatum* infections of synthetic alginate-based artificial plant tissue 2) residual *P. aphanidermatum* colony count in the hydroponic system.

Results and discussion

1. Physical characteristics of artificial plant tissue using scanning electron microscope (SEM)

Fig. 4, shows the results of the analysis of the physical characteristics of the synthesis of artificial plant tissue from alginate at concentrations of 1%, 2%, 3%, 4% and 5% with the results of the study observed from the images utilizing a scanning electron microscope (SEM) with a magnification of 100 times, a scale of 100 µm and a distance from the tip of the lens to the objective of 13 mm, revealing the synthesis of artificial plant tissue from alginate as having a rough surface. The artificial plant tissue from alginate at a concentration of 1% shows that the artificial plant tissue has a smoother surface with fewer air bubbles (O_2 permeation into the tissue occurred during the synthesis of alginate derived from natural algae, leading to bubble formation) on the surface of the tissue than at concentrations of 2%, 3%, and 4%. At a concentration of 5% (Fig. 4), the tissue has better tissue cohesion at all concentrations, including the smoothness of the surface, as judged by the air bubbles on the surface of the artificial plant tissue (Fig. 5). An ideal artificial plant tissue should possess pores of various sizes, contain fibers with uniform distribution, and have a surface with fine roughness to facilitate the attachment and growth of fungal hyphae. Additionally, it should exhibit cellulose-like characteristics.

The justification for using only 100×magnification is that it allows clear visualization of the overall surface morphology of the film, such as smooth areas, folds, or large pores, without distorting the sheet. This is important for preliminary assessment. In contrast, using a higher magnification, such as 1000×, would cover only a very small area and may fail to capture the general surface characteristics of the film.



(a) (b)

(c) (d)

(e)

Fig. 4 Physical characteristics of the synthetic alginate artificial plant tissues at concentrations of 1% (a), 2% (b), 3% (c), 4% (d), and 5% (e)

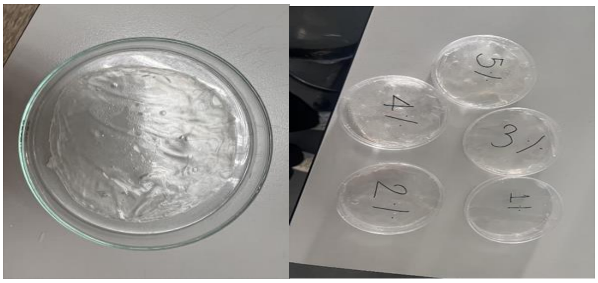
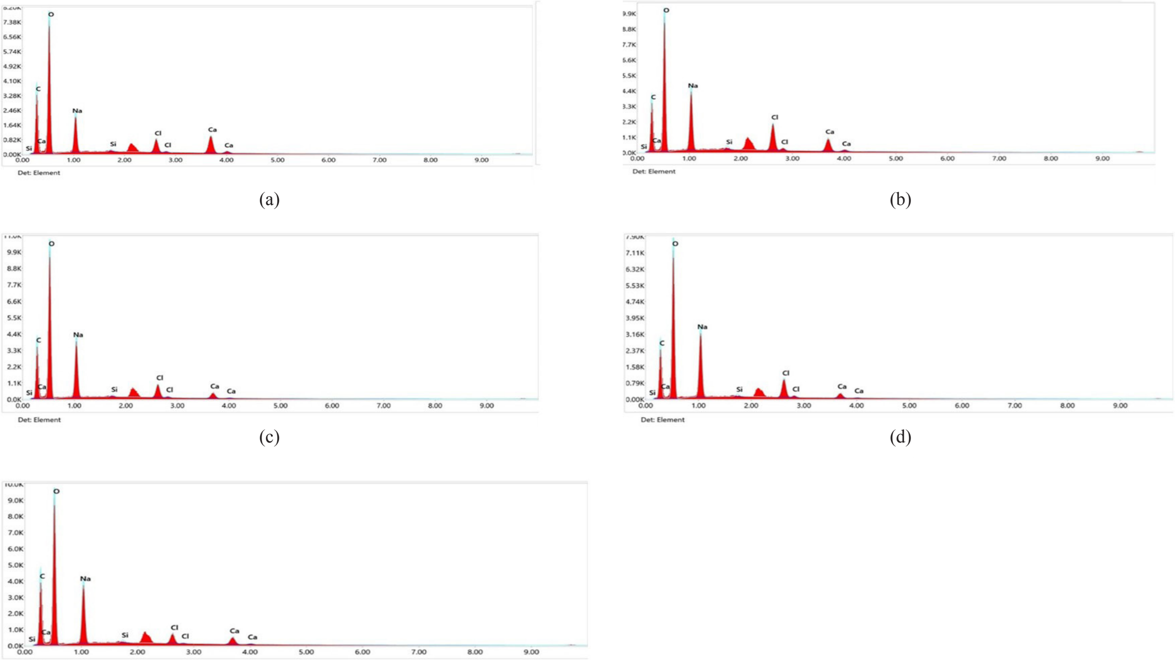


Fig. 5 Physical characteristics of the artificial plant tissues developed from alginate at concentrations of 1, 2, 3, 4, and 5%

As shown in Fig. 5, the artificial plant tissues synthesized with 1% and 2% alginate concentrations exhibited smooth surfaces with few air bubbles. In contrast, the tissues prepared with higher alginate concentrations of 3%, 4%, and 5% showed increased surface roughness and a greater number of air bubbles on the surface. The synthetic tissues also displayed higher porosity. Notably, at 5% alginate concentration, the surface roughness was the highest, but the structure appeared to be the most rigid and brittle.

2. Results of chemical characterization of synthetic alginate artificial plant tissue using an energy dispersive X-ray spectroscopy (EDS/EDX) technique

Fig. 6(a), the obtained EDS spectrum shows the relationship between the Y-axis, which is the number of



(a) (b)

(c) (d)

(e)

Fig. 6 Chemical characteristics of the synthetic alginate tissues at 1% (a), 2% (b), 3% (c), 4% (d) and 5% (e) concentration examined by the energy dispersive X-ray spectroscopy (EDS/EDX) technique

X-ray signals measured, and the X-axis, which is the energy of the X-rays in keV, in which the peaks indicate the elements that are components of the artificial plant tissue from alginate that need to be studied. The analysis results at a concentration level of 1% showed that the chemical characteristics were carbon (C) 42.24%, oxygen (O) 45.65%, sodium (Na) 5.77%, silicon (Si) 0.20%, chlorine (Cl) 1.81%, calcium (Ca) 4.32%, and oxygen (O) yielded the highest value.

Fig. 6(b) The obtained EDS spectrum shows the relationship between the Y-axis, which is the number of X-ray signals measured, and the X-axis, which is the energy of the X-rays in keV units. The peaks that occur indicate the elements that are components of the alginate artificial plant tissue that need to be studied. The analysis results at a concentration level of 2% showed that the chemical characteristics were carbon (C) 40.75%, oxygen (O) 43.66%, sodium (Na) 8.69%, silicon (Si) 0.30%, chlorine (Cl) 3.56%, calcium (Ca) 3.04%, and oxygen (O) was the highest value.

Fig. 6(c) The obtained EDS spectrum shows the relationship between the Y-axis, which is the number of X-ray signals measured, and the X-axis, which is the energy of the X-rays in keV units. The peaks that occur indicate the elements that are components of the artificial plant tissue from alginate that need to be studied. The analysis results at a concentration level of 3% showed that the chemical characteristics found there were carbon (C) 41.08%, oxygen (O) 46.78%, sodium (Na) 8.75%, silicon (Si) 0.19%, chlorine (Cl) 1.86%, and calcium (Ca) 1.34% as components, and it was found that oxygen (O) was the highest component.

Fig. 6(d) The obtained EDS spectrum shows the relationship between the Y-axis, which is the number of X-ray signals measured, and the X-axis, which is the energy of the X-rays in keV. The peaks indicate the elements that are components of the alginate artificial plant tissue to be studied. The analysis results at a concentration of 4% showed that the chemical characteristics were carbon (C) 40.89%, oxygen (O) 45.48%, sodium (Na) 9.71%, silicon (Si) 0.21%, chlorine (Cl) 2.58%, calcium (Ca) 1.13%, and oxygen (O) was at the highest.

Fig. 6(e) The obtained EDS spectrum shows the relationship between the Y-axis, which is the number of X-ray signals measured, and the X-axis, which is the energy of the X-rays in keV, where the peaks indicate the elements that are components of the artificial plant tissue from alginate that need to be studied. The analysis results at a concentration level of 5% showed that the chemical characteristics found were carbon (C) 43.60%, oxygen (O) 44.77%, sodium (Na) 8.47%, silicon (Si) 0.23%, chlorine (Cl) 1.27%, and calcium (Ca) 1.65% as components, and it was found that oxygen (O) was the highest component.

With respects to the results of the chemical characterization of the synthetic alginate artificial plant tissue using the technique of measuring the energy distribution of X-rays (Energy Dispersive X-ray Spectroscopy /EDS/EDX) at all 5 concentration levels, by analyzing as a percentage ratio or percentage for easy reading of the quantity, the chemical composition analysis reveals the composition of elements in the synthetic alginate artificial plant tissue where the main chemical elements are carbon (C), oxygen (O), sodium (Na), silicon (Si), chlorine (Cl), and calcium (Ca), and where oxygen (O) is found the most, which is a component of the elements.

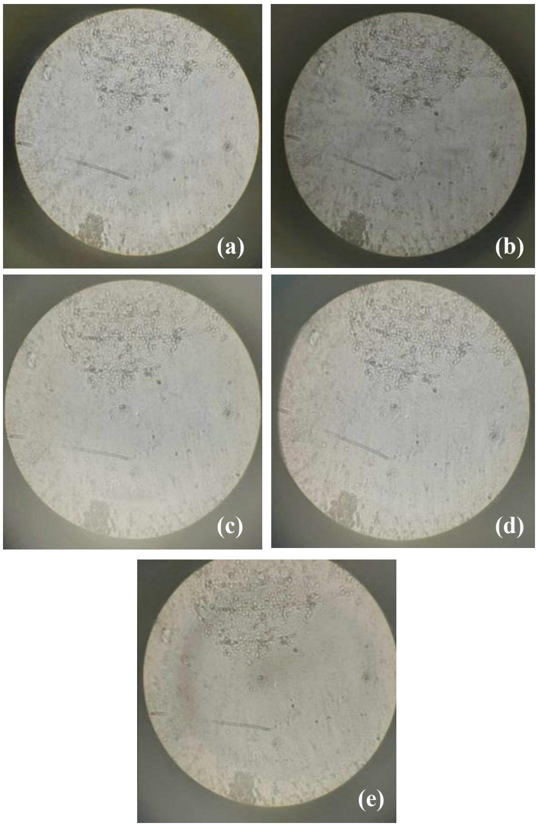


Fig. 7 Appearance of *P. aphanidermatum* spores entrapped in hydroponic alginate-based artificial plant tissue at concentrations of 1% (a), 2% (b), 3% (c), 4% (d), and 5% (e) under a microscope with a magnification of 100x

3. Efficiency of the development of artificial plant tissue from alginate at different concentrations in retaining *P. aphanidermatum* colonies

The efficiency of the developed alginate artificial plant tissue at different concentrations in harboring *P. aphanidermatum* colonies of the synthetic alginate artificial plant tissue at concentrations of 1%, 2%, 3%, 4% and 5% (Fig. 7) was obtained, as shown in Table 2.

Table 2 Number of *P. aphanidermatum* infections of synthetic alginate artificial plant tissues

Alginate concentration (%)	Number of colonies per area** of synthetic artificial plant tissue (colonies/cm²*)
1	3.44±0.12^{e}
2	5.60±0.22^{c}
3	9.43±0.41^{a}
4	3.95±0.11^{d}
5	8.92±0.16^{b}

Remark: * cm³ by finding the area of alginate from the formula for finding the area of a circle with a radius of 5 cm. The area of alginate is equal to 78.5 cm².

$^{a-e}$ Each alphabet character showed there is a statistically significant difference at the 0.05 level.

** Spore counting units are counted in colony units, where 1 spore is equivalent to 1 colony forming unit (CFU).

From Table 2, it was found that the synthetic artificial plant tissue from alginate at a concentration of 3% had the highest number of *P. aphanidermatum* colonies, with a value of 9.43 colonies/cm² in the duration of 24 h (evaluation of the fungal retention efficiency of synthesized artificial plant tissues at various concentrations within 24 h), which showed the colony retention of *P. aphanidermatum* in the synthetic artificial plant tissue from alginate on the left side with the majority of spores. *P. aphanidermatum* collected on the grid supporting the synthetic artificial plant tissue came with the water flowing through the hydroponic system. The synthetic artificial plant tissue from alginate at a concentration of 1% had the least number of colonies, with a value of 3.44 colonies/cm². In addition, the retention of *P. aphanidermatum* from the synthetic artificial plant tissue from alginate at a concentration of 5%, 2%, and 4% was at a moderate level, with a value of 8.92, 5.60, and 3.95 colonies/cm², respectively. When taking the average values of the colony retention of *P. aphanidermatum*, the results showed that the retention values of *P. aphanidermatum* at all concentration levels were significantly different from each other at all concentration levels.

Water samples were collected from the reservoir tank of the hydroponic system and cultured to determine the number of fungal colonies at different time intervals (0 h, 6 h, 12 h, and 24 h). This was done to compare the ability to retain *P. aphanidermatum* that had entered the circulating nutrient solution of the hydroponic system. Table 3. The results of the analysis of the amount of *P. aphanidermatum* colonies in the artificial plant tissues synthesized from alginate showed that the artificial plant tissue synthesized from 3% alginate was able to retain the highest amount of *P. aphanidermatum* colonies, while the artificial plant tissue synthesized from 4% alginate was able to retain the second highest amount of *P. aphanidermatum* colonies, followed by the artificial plant tissue synthesized from 5% alginate and alginate at a concentration of 2%, and the artificial plant tissue that was able to retain the least amount of *P. aphanidermatum* colonies was alginate at a concentration of 1%.

This artificial plant tissue synthesized from alginate is consistent with the study of Yamaguchi et al. (2017), who synthesized artificial plant tissue from cellulose, which can effectively retain *P. aphanidermatum* in a hydroponic system, especially the synthetic tissue at 3%, which has a tissue similar to that of plant root tissue. The higher percentage is harder, making it difficult for the germ tube to penetrate the tissue. Using 3% tissue will make *P. aphanidermatum* misunderstand the artificial plant tissue as vegetable tissue, so it will grow a germ tube to penetrate the wall of the cultivated vegetables, which is the cause of vegetable wilt and eventual death. The embedment of spores on the roots is mediated by saccharides residual on the root surface mucus by releasing plant tissue attractants in the hydroponic system (Li et al., 2014), so it can retain the germ well. In addition, Rashedy et al. (2021) developed sodium alginate artificial plant tissue, a polysaccharide derived from seaweed and phenolic compounds, as an artificial plant tissue similar to real plant tissue, as a source of *P. aphanidermatum* retention. In the hydroponic system, Saberi et al. (2022), which is consistent with the study of the efficiency test results of the alginate artificial plant tissue membrane, were able to retain the highest average of *P. aphanidermatum* at a concentration of 3%. Each hour, the number of bacterial colonies decreased, and the microscopic images clearly showed that the spores were retained on the alginate artificial plant tissue. As for the amount of *P. aphanidermatum* colonies remaining in the hydroponic system, the average of the 3% alginate artificial plant tissue had the least amount of *P. aphanidermatum* colonies.

Table 3 shows the results of water sample analysis with the amount of *P. aphanidermatum* colonies remaining in the hydroponic system in the experiment using synthetic alginate tissue at concentrations of 1%, 2%, 3%, 4% and 5% compared to no artificial plant tissue used to contain *P. aphanidermatum* colonies at 0 h, 6 h, 12 h, and 24 h. It can be seen that the experiment conducted without using synthetic alginate tissue had the highest average amount, equal to 165 colonies/mL. At concentrations of 3 to 5% the *P. aphanidermatum* colonies were significantly reduced from the original. The least amount of *P. aphanidermatum* colonies remaining in the hydroponic system was found in the synthetic alginate tissue at concentrations of 3%, equal to 6 colonies/mL. The synthetic alginate tissue at 1 and 5% had the second-lowest amount of *P. aphanidermatum* colonies remaining in the hydroponic system. The average amount of *P. aphanidermatum* spores remaining in the hydroponic system of the artificial plant tissues from 4% and 2% alginate concentrations was 12.5 and 20 colonies/mL, respectively. The average amount of *P. aphanidermatum* spores remaining in the hydroponic system of all experiments was statistically different.

From You et al. (2015), it was explained that *P. aphanidermatum* was found to contaminate nutrient solution given to plants grown in hydroponic systems, brand O'tsuka A' (Agrioco, Japan), causing problems for the roots of plants grown in said hydroponic systems. It was observed that the pathogen had a morphology and growth of 10 µm mycelium, mostly terminal sporangia, which clearly shows that there is *P. aphanidermatum* occurring in the system. Even though it is a closed system and the material is screened for pathogens, although there are various methods to solve the root rot problem, both chemically and biologically, there is still a problem regarding the outbreak of root rot from *P. aphanidermatum*. This is because there are still other factors that promote the outbreak of this disease in hydroponic systems, emanating from different growing conditions. For example, Sánchez-Montesinos et al. (2019) conducted a study in 2019 and found with the use of biological products from Trichoderma to control *P. aphanidermatum* in hydroponic vegetable cultivation during spring and summer that biological products can control the outbreak of *P. aphanidermatum* in hydroponic vegetable cultivation well and are highly effective at temperatures between 20 and 31°C. However, if the air temperature is higher than 31°C, the effectiveness of controlling the outbreak of *P. aphanidermatum* will decrease and cause the outbreak to continue in the hydroponic cultivation system. Most hydroponic vegetable cultivation in Thailand is at a temperature higher than 31°C, which is at risk of the spread of *P. aphanidermatum* in hydroponic vegetable cultivation.

Table 3 Residual *P. aphanidermatum* colony count in the hydroponic system at different concentration levels

Alginate concentration (%)	Duration (h)	Spore count (colony/mL)	Average (colony/mL)
Control	0	92	165.00±12^{a}
	6	123	
	12	202	
	24	243	
1	0	30	7.5±0.76^{b}
	6	0	
	12	0	
	24	0	
2	0	50	20.0±0.60^{b}
	6	20	
	12	0	
	24	10	
3	0	4	6.0±0.26^{b}
	6	20	
	12	0	
	24	0	
4	0	40	12.50±1.1^{b}
	6	0	
	12	10	
	24	0	
5	0	30	7.5±0.56^{b}
	6	0	
	12	0	
	24	0	

Remark: ^{a,b} Each alphabet character showed there is a statistically significant difference at the 0.05 level

Conclusion

The artificial plant tissues synthesized from alginate at concentrations of 1%, 2%, 3%, 4%, and 5% exhibited distinct surface characteristics. The tissues synthesized using 1% and 2% alginate displayed smooth, flat surfaces with minimal roughness, low porosity, and fewer air bubbles. In contrast, tissues prepared with 3%, 4%, and 5% alginate concentrations showed noticeably rougher surfaces, the presence of obvious air bubbles, and increased surface porosity. Elemental analysis of the artificial plant tissues synthesized from different alginate concentrations revealed the presence of identical elements—C, Na, Si, Cl, and O—with only slight variations in their proportions. Oxygen (O) was found to be the most abundant element, likely due to the diffusion of O_2 into the tissues during synthesis. This is attributed to the reaction of alginate, derived from algae, being composed of mannuronic acid and guluronic acid units.

The synthesis of artificial plant tissues from alginate can retain *P. aphanidermatum* colonies in the hydroponic system, and at a concentration of 3% it can retain bacteria the best. It can be observed that *P. aphanidermatum* on the alginate sheet has the highest amounts of bacteria, and from the result of the water analysis it has reduced amounts of bacteria remaining in the hydroponic system when compared to alginate with concentrations of 1%, 2%, 4% and 5%. In addition, the analysis of the water found a low number of spores at a concentration of 3%, where the spores were of *P. aphanidermatum*. The fastest adhesion of the synthetic tissue from alginate and the time of the fastest retention was 6 h at a concentration of 5%. Furthermore, the alginate artificial plant tissue can retain microorganisms in the hydroponic system and has the best efficiency in retaining microorganisms at 3%. This phenomenon results from the differences in the structural characteristics of artificial plant tissues synthesized with varying concentrations of alginate. Each concentration produces artificial plant tissues with different degrees of similarity to real plant tissues. Among them, the artificial plant tissue made with 3% alginate exhibited surface morphology most closely resembling that of natural plant tissue. As a result, *P. aphanidermatum* germinated and penetrated this artificial plant tissue via germ tubes more effectively than others. Consequently, the fungal structures were most concentrated and retained in the artificial plant tissue synthesized with 3% alginate.

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