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Research Report

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Title of Research Report

Degraded Peat Swamp Forest Reforestation Innovation with Seed Krathong

and Encapsulated PGPR

Date

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Phattaraphum Suksai, Rhonnakorn Utsaha

Abstract

Thailand's original peat swamp forest exists today in only one location: Phru To Daeng in Narathiwat Province. Other peat swamp forests have become degraded which severely limit plant diversity. One common reforestation method involves planting seedlings, but this method is difficult in the waterlogged environment like peat swamp forests. Therefore, developers introduced an innovative floating device called a "seed Krathong" inspired by the Thai traditional "Krathong" - a floatable tray made up of banana pseudostem from the ancient Thai tradition: "Loy Krathong"- and designed as an improvement over conventional seed balls that often sink and cause seeds to rot.

This innovation constructed from banana pseudostem and other biodegradable materials, applying the principles of Archimedes' buoyancy and metacentric balance to achieve stability and prolonged floatation. Six designs were tested, and the most efficient and stable model was selected based on its ability to remain afloat in swamp water.

To further enhance seed germination, the developers incorporated plant growth-promoting rhizobacteria (PGPR), specifically *Bacillus subtilis*, encapsulated in calcium alginate to form PGPR beads. These beads improved the survival of beneficial bacteria on the surface of the Krathong, remaining viable even after 21 days in the harsh simulated solutions of the degraded peat swamp forest that collected from the real locations.

The synergy between PGPR beads and the floating Krathong structure was evaluated using *Syzygium cinereum* (Waana), a native species of the original peat swamp forest. Results showed that the combination significantly enhanced seed germination, as indicated by higher rates of testa rupture observed 7 days after placement in water, especially when compared with the traditional seed balls. Germination rates were greater when PGPR beads were included alongside the optimized Krathong design.

Keywords: Original peat swamp forest, degraded peat swamp forest, reforestation, Krathong, seed germination, PGPR, Bacillus subtilis, encapsulation, floatable tray, biodegradable material

Acknowledgement

This research project, "Innovation for the Restoration of Degraded Peat Swamp Forests Using Seedling Krathongs and PGPR Capsules", was successfully completed with the kind support and guidance of many individuals. We would like to sincerely thank Dr. Thanasan Nilsu, our project advisor, for his valuable guidance and support throughout the study.

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Commitments on Academic Honesty and Integrity

We hereby declare that we

1. are fully committed to the principle of honesty, integrity and fair play throughout the competition.
2. actually perform the research work ourselves and thus truly understand the content of the work.
3. observe the common standard of academic integrity adopted by most journals and degree theses.
4. have declared all the assistance and contribution we have received from any personnel, agency, institution, etc. for the research work.
5. undertake to avoid getting in touch with assessment panel members in a way that may lead to direct or indirect conflict of interest.
6. undertake to avoid any interaction with assessment panel members that would undermine the neutrality of the panel member and fairness of the assessment process.
7. observe the safety regulations of the laboratory(ies) where the we conduct the experiment(s), if applicable.
8. observe all rules and regulations of the competition.
9. agree that the decision of YHSA(Asia) is final in all matters related to the competition.

We understand and agree that failure to honour the above commitments may lead to disqualification from the competition and/or removal of reward, if applicable; that any unethical deeds, if found, will be disclosed to the school principal of team member(s) and relevant parties if deemed necessary; and that the decision of YHSA(Asia) is final and no appeal will be accepted.

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1. Introduction

1.1. Peat swamp forest

Peatlands are wetland ecosystems characterized by prolonged inundation, which prevents organic plant debris on the soil surface from direct contact with air, thereby slowing down complete decomposition (International Peatland Society, 2020) and resulting in the formation of peat (UNDP Thailand, 2021). As this material accumulates, peatlands develop thick peat layers that serve as carbon sinks. Although they cover only 3–4 % of the Earth's land surface, peatlands store one-third of all soil carbon worldwide (Global Peatlands Assessment, 2022).

In Southeast Asia, peatlands most often occur as tropical forests known as peat swamp forests. Figure 1 shows the distribution of peatlands across this region. Today, peat swamp forests in Southeast Asia face severe threats, primarily from human activities: drainage for agriculture or logging desiccates the peat, making it highly flammable and turning these once–stable carbon stores into some of the largest sources of CO_2 emissions globally.

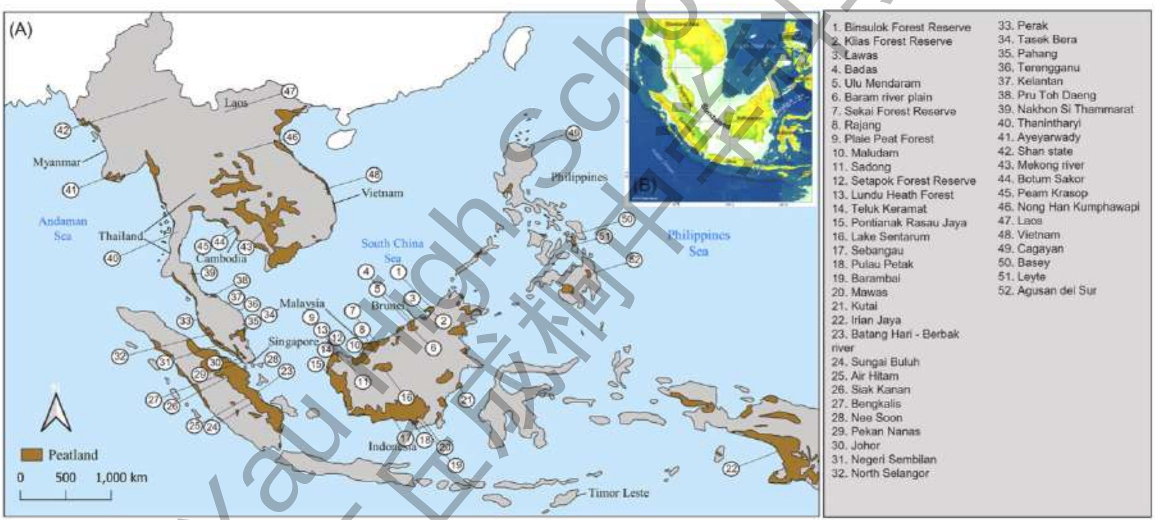


Figure 1. Distribution of peatlands in Southeast Asia (Omar et al., 2022)

Water and soil conditions in peat swamp forests are uniquely distinct from other wetlands. Peat soil falls under the "Histosols" order in soil taxonomy (World Reference Base for Soil Resources, 2022). These soils are defined by an organic-matter content greater than 200 g kg^{-1} in the top 40 cm or more, and are rich in the mineral pyrite (FeS_2). Continuous waterlogging under anaerobic conditions allows organic matter to accumulate as peat. The local water table exerts a controlling influence on both vegetation and soil: if the water level is too high, plant roots may suffocate; if too low, the peat dries out and becomes excellent fuel for wildfires. Re-flooding dried peat can also trigger oxidation of pyrite, producing acid sulfate soils (reaction [1]) that lower pH and release aluminum (Al^{3+}), which further stunts plant growth by disrupting osmotic balance and increasing reactive oxygen species in plant cells (Long et al., 2024).

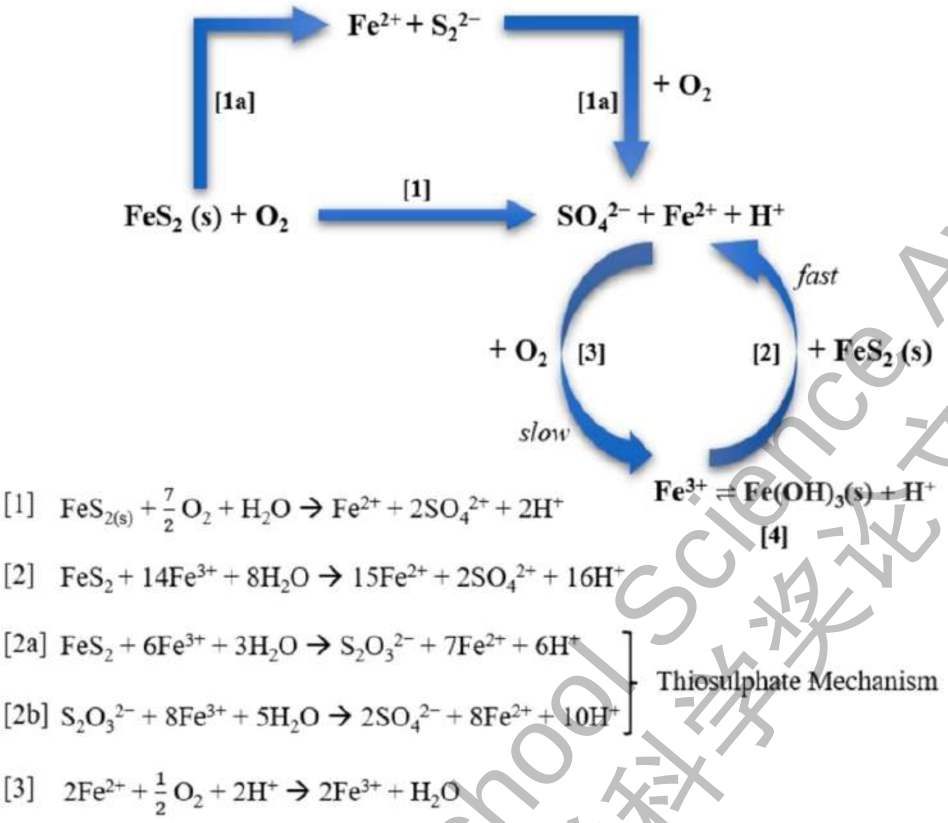


Figure 2. Pyrite oxidation cycle and related reactions (Allman et al., 2021)

1.2. The situation of Peat swamp forest in Thailand

High-resolution satellite imagery indicates that, in 2020, Thailand had just 37 139.56 rai (≈ 59.42 km²) of peatland under the Department of Marine and Coastal Resources—only 0.04 % of the country's total forest area (Suebkarnkaset Foundation, 2022). These peatlands span 12 coastal provinces, with Songkhla Province alone accounting for 12 814.98 rai (20.50 km²), followed by Narathiwat Province at 8 650.15 rai (13.76 km²). Surveys have recorded over 470 plant species in Thailand's peat swamp forests, including 437 flowering plants across 109 families and 33 ferns across 15 families (Kobsak et al., 2020)—far greater diversity than that of mangrove forests, which host only 81 species (Department of Marine and Coastal Resources, 2012).

However, a fully intact peat swamp forest ecosystem now exists only at Toh Daeng (Narathiwat Province). Most other sites have degraded into dense stands of Melaleuca cajuputi, which tolerates the acidic, nutrient-poor conditions that follow repeated fires. The second largest remaining forest, Kuan Kreng, spans Nakhon Si Thammarat, Phatthalung, and Songkhla provinces, but recurrent fires there have severely depleted biodiversity and enriched soil acidity.

To date, numerous restoration projects using nursery-grown seedlings have been attempted. While seedlings ensure higher survival rates and predictable establishment, they are labor-intensive, limited to accessible areas, and often require earth-mounding to raise planting platforms above the water table (Kobsak et al., 2020).

1.3. Seed ball

A seed ball is a traditional reforestation technique in which seeds are encased in a mixture of clay, compost, and sometimes other organic additives. The clay coating protects seeds from environmental stress and predation until conditions are favorable for germination. Seed balls can be broadcast by hand, shot from pneumatic devices, or dropped by helicopter or drone into degraded, inaccessible, or drought-prone areas, as the clay retains moisture around the seed (Atkinson, 2003; Borman et al., 2007).

Compared to seedling outplanting, seed balls offer a low-cost, low-labor alternative. However, peat swamp restoration poses unique challenges: during peak seed-drop seasons, high water tables make ground deployment impossible; conversely, when water levels recede, viable peat swamp seeds are scarce (Rattanavirakul et al., 2019).

Figure 3. Germination from a seed ball (Regenerative Design Group, 2017)

1.4. Plant growth-promoting rhizobacteria (PGPR)

Plant growth-promoting rhizobacteria (PGPR) are a group of beneficial soil bacteria that colonize the rhizosphere and establish significant interactions with plant. These bacteria can promote plant growth through both direct and indirect mechanisms. The direct mechanisms include the enhancement of nutrient availability in soil, such as nitrogen fixation, phosphorus solubilization, and the production of phytohormones including auxins, gibberellins, and cytokinins. Indirectly, PGPR contribute to plant health by producing antimicrobial compounds that inhibit pathogens, and by eliciting induced systemic resistance (ISR), which enhances the plant's defensive capacity. Several genera have been identified as PGPR, including *Acinetobacter, Agrobacterium, Arthobacter, Azotobacter, Azospirillum, Burkholderia, Bradyrhizobium, Rhizobium, Frankia, Serratia, Thiobacillus, Pseudomonas,* and *Bacillus* (Goswami et al., 2016). Some of these strains have been reported to improve seed germination, including *Azospirillum* (Puente & Bashan, 1993), *Pseudomonas*, and *Bacillus* (Widnyana & Javandira, 2016).

Bacillus subtilis is a Gram-positive, spore-forming bacterium widely studied for its plant-beneficial traits.

It has been identified as a native species in peat swamp forests (Su et al., 2007), and specifically in Thailand's To Daeng peat swamp forest (Anurak et al., 2018). This species has demonstrated potential to enhance seed germination in crops such as corn (Li & Hu, 2020) and tomato (de O. Nunes et al., 2023), primarily through mechanisms such as phosphorus solubilization (Swain et al., 2012; Saeid et al., 2018; Ahmad et al., 2020), auxin production (Anguiano et al., 2019), and siderophore production which facilitates iron acquisition (Anguiano et al., 2019; Dertz et al., 2006).

Peat swamp forests are unique ecosystems with high biodiversity and ecological significance. Enhancing seed germination of native plant species in these areas using PGPR such as *B. subtilis* could support reforestation and ecosystem restoration efforts. However, the direct application of bacterial inoculants onto seeds often suffers from low efficacy due to limited bacterial survival during seed storage and after sowing.

To overcome this limitation, encapsulation techniques has been applied to improve bacterial viability and stability.

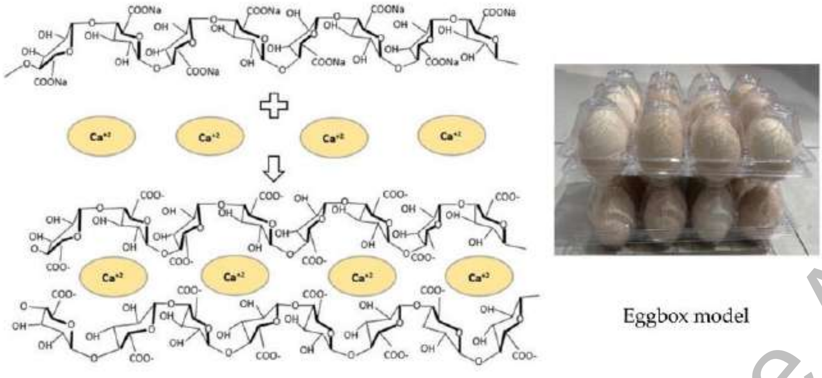
1.5. Encapsulation technique

Encapsulation is a widely accepted technique known for its effectiveness in increasing the viability of bacterial cells. It provides physical protection against hostile environmental stresses, improves nutrient retention, and enhances bacterial survival under unfavorable conditions. The encapsulating material forms a microenvironment that shields the bacteria from harmful factors such as extreme pH, temperature fluctuations, and desiccation, thereby extending the lifespan and functional activity of the bacterial cells. Furthermore, this technique allows for a gradual release of bacteria, facilitating better adaptation to external environments and preventing their rapid loss from the target area (Li et al., 2008).

Encapsulation also offers protection from antimicrobial agents and host immune responses, which further enhances the survival and application efficiency of the encapsulated microbes (Chen et al., 2017).

In this study, the researchers utilize calcium alginate ($C_{12}H_{14}CaO_{12}$), formed by the reaction of sodium alginate ($NaC_6H_7O_6$) with calcium chloride ($CaCl_2$), as the primary encapsulation material for PGPR (plant growth-promoting rhizobacteria), as illustrated in Figure 4.

Calcium alginate was chosen due to several practical and ecological advantages. The preparation process is relatively simple and more accessible compared to other encapsulation materials. It is low-cost, environmentally friendly, and exhibits high biocompatibility with ecosystems. Calcium alginate capsules offer effective controlled release of PGPR, which is beneficial for long-term applications, especially in acidic conditions commonly found in degraded peat swamp environments (Huang et al., 2006).



$$2NaC_6H_7O_6 + CaCl_2 \rightarrow 2NaCl + C_{12}H_{14}CaO_{12}.$$

Figure 4. Mechanism of Calcium Alginate Formation

Sodium alginate ($NaC_6H_7O_6$) reacts with calcium chloride ($CaCl_2$) to form calcium alginate ($C_{12}H_{14}CaO_{12}$). During this reaction, the two chemical compounds undergo rearrangement and cross-linking to create a stable structure. This mechanism is often described using the "egg-box model", where calcium ions coordinate with the guluronic acid blocks in alginate chains to form a three-dimensional network (Shalapy et al., 2020).

1.6. Seed Krathong Innovation

The developers recognized the ecological significance of peat swamp forests and the urgent need to develop sustainable and effective restoration strategies for degraded peatland areas.

Inspired by the concept of seed balls, the developers propose an enhancement of this method by designing seed balls that are capable of floating, allowing reforestation to begin during the flooded season when the seeds of many peatland species naturally mature. The inspiration is drawn from Thailand's Loy Krathong tradition, where banana pseudostems, a low-density and highly biodegradable material, are used to make floating containers. As water levels recede, the floating seed containers will eventually rest on the soil surface, enabling the seeds to germinate and grow into robust plants. The soil contained within the Kratong may act like a planting mound, similar to conventional sapling planting techniques, and the naturally decomposing banana stem may further enrich the soil by acting as an organic fertilizer for the young seedlings.

To improve seed germination rates, the project incorporates the encapsulation technique, using calcium alginate as the main material to encapsulate plant growth-promoting rhizobacteria (PGPR). The PGPR of interest in this study is *Bacillus subtilis*, a species native to peat swamp ecosystems, ensuring that the microbial composition of the soil is not disrupted.

The developers investigate the interaction between encapsulated *B. subtilis* and the germination of selected native peatland tree species. Species selection was informed by expert recommendations and prior studies. The chosen example species is Syzygium cinereum (Waan-Na)

Finally, the effectiveness of the developed Kratong Seed Balls will be tested in controlled tanks simulating peat swamp conditions. These simulations include chemical properties and high acidity levels, based on real soil and water samples collected from four degraded peat swamp locations

2. Research methodology

2.1. Materials and Locations

2.1.1. Biological Materials

- Water from Kuan Kreng peat swamp
- Soil from Kuan Kreng peat swamp
- Water from To Daeng peat swamp
- Soil from To Daeng peat swamp
- *Syzygium cinereum* seeds
- *Bacillus subtilis*
- Banana pseudostem
- Clay
- Coconut coir
- Burnt rice husk

2.1.2. Chemicals

- Luria-Bertani broth (LB broth)
- Nitrogen-free malate with bromothymol blue liquid medium (NFb liquid medium)
- Ammonium chloride (NH_4Cl)
- Hydrochloric acid solution (HCl)
- Aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$)
- Sodium hypochlorite solution (NaClO)
- Sodium alginate powder
- Phosphate buffer solution, pH 7.0, 0.01 M
- 70% Ethanol solution
- Nutrient agar
- Saline solution
- Deionized water (DI water)

2.1.3. Scientific Equipment

- Shaker incubator
- Temperature-controlled incubator
- Hot-air oven
- Spectrophotometer
- Biological safety cabinet
- Magnetic stirrers
- Petri dishes

- 250 ml Erlenmeyer flasks
- Micropipettes
- pH meter
- Freeze-drying vacuum chamber (Lysophilizer)
- Scanning electron microscope (SEM)

2.1.4. Locations

- Kamnoetvidya Science Academy, Wangchan District, Rayong Province
- Vidyasirimedhi Institute of Science and Technology, Wangchan District, Rayong Province

2.2. Experimental procedures

2.2.1. Preparation of simulated peat swamp forest

Peat swamp water samples were collected from two locations: Kuan Kreng peat swamp forest and Toh Daeng peat swamp forest.

- For Kuan Kreng, water was collected from four degraded peat swamp areas: Village 3, Village 4, Village 9, and Village 11 in Kreng Subdistrict, Cha-uat District, Nakhon Si Thammarat Province.
- For Toh Daeng, the water sample was collected from the Sirindhorn Peat Swamp Forest Research and Study Center, located at coordinates 6°04'24.3"N 101°57'56.3"E in Puyo Subdistrict, Su-ngai Kolok District, Narathiwat Province.

Then, the pH of peat swamp water samples was measured using a pH meter. After that, the water samples were sent to Global Utility Services Co., Ltd. (GUSCO) for the analysis of the concentrations of sulfate, aluminum (Al), and iron (Fe).

The simulated peat swamp solutions were prepared by dissolving specific chemicals in deionized (DI) water as follows: (1) Aluminum nitrate nonahydrate ($Al(NO_3)_3 \cdot 9H_2O$) to simulate Al^{3+}, (2) Ammonium iron(II) sulfate ($(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$) to simulate Fe^{2+}, and (3) Ammonium sulfate ($(NH_4)_2SO_4$) to supplement SO_4^{2-} in addition to that provided by ammonium iron(II) sulfate. The quantities of each chemical were calculated to achieve final ion concentrations equivalent to those obtained from laboratory analysis, as shown in Table 1. After complete dissolution, the pH of each solution was measured and adjusted to match the values reported in Table 1 using 1 M HCl or 1 M NaOH as appropriate.

Three different simulated peat swamp solutions (KK123, KK4, TD) were prepared by imitating the pH and metal ion concentrations based on measured values from the following locations

- KK123 : Village 3, Village 4, Village 9 in Kuan Kreng Peat swamp forest.
- KK4 : Village 11 in Kuan Kreng Peat swamp forest.
- TD : Toh Daeng Peat swamp forest.

2.2.2. Preparation of bacterial inocula

Bacterial inocula were prepared by culturing *bacillus subtilis* in separate 250 ml Erlenmeyer flasks with 100 ml sterilized LB broth. Flasks were placed in a shaking incubator at 150 rpm and 37^{o}C for 24 hrs. Then, Bacterial pellet was harvested by repeating the following steps 3 times : First, 50 ml of Bacteria liquid cultures, in centrifuge tube, were centrifuged at 5000 rpm for 10 minutes. After centrifugation, supernatant was discarded. Then, 0.9 % NaCl were added until the final volume was 30 ml. Centrifuge tube was placed on vortex for 30 seconds.

After the three replicates, Bacterial pellet was resuspended in 0.9% NaCl solution and were diluted to adjust an absorbance of 0.5 at 600 nm using spectrophotometer.

2.2.3. Isolation of Bacteria from Peat Swamp Water and Soil

- **Water samples**

Peat swamp water (10 ml) was transferred into a 15 ml centrifuge tube and vortexed for 1 minute. A 100 µl aliquot was spread onto agar plates and incubated at 30 °C for 24 hours. After incubation, colonies with distinct morphologies were selected and streaked onto new plates to obtain single colonies.

- **Soil samples**

Peat swamp soil (20 g) was placed in a 250 ml Erlenmeyer flask containing 200 ml of deionized water. The mixture was shaken at 120 rpm for 1 hour and left to settle for 15 minutes. A 10 ml aliquot of the supernatant was transferred to a 15 ml centrifuge tube and serially diluted to 10^{-1} and 10^{-2}. From each dilution, 100 µl was spread on agar plates and incubated at 30 °C for 24 hours. Distinct colonies were then selected and streaked for single colony isolation, followed by observation under a stereomicroscope.

2.2.4. Encapsulation of bacterial cell (PGPR beads)

The bacterial culture with an OD_{600} = 0.5 was mixed with 2% sodium alginate in a volume ratio of 1:4 (culture:alginate) and shaken for 1 minute with vortex. The mixture was gradually dropped into 10 ml of a 3.5% $CaCl_2$ solution using a 1 ml micropipette and allowed to solidify for 20 minutes. The hardening beads were then collected with a filter cloth and washed with 0.9% NaCl solution.

2.2.5. Survivability of encapsulated bacteria in simulated peat swamp forest water

An experiment was conducted to compare the survival of bacterial cells between two forms: wet cells and bacteria encapsulated in calcium alginate beads, in four different solutions : DI water, KK123, KK4, and TD (as described in section 4.2.1) over incubation periods of 14, 21, and 28 days. For the wet cell group, 0.3 mL of bacterial culture with an OD_{600} of 0.5 was transferred into a centrifuge tube. For the encapsulated group, 1.5 mL of calcium alginate-encapsulated bacteria (beads) was placed into a separate centrifuge tube. Each tube was then filled with one of the test solutions (DI water, KK123, KK4, or TD) to a final volume of 7.5 mL. All samples were incubated in a shaking incubator at 150 rpm and 37 °C for 14, 21, and 28 days. At each time point, 100 µL of the solution was pipetted and plated on an agar plate.

2.2.6. Effect of PGPR beads on seed germination and seed growth

Syzygium cinereum seeds were depulped, then, run with tap water until all fruit residue were removed. The seeds were then sterilized with 1% sodium hypochlorite solution for 10 minutes, then, rinsed with deionized water (DI) three times.

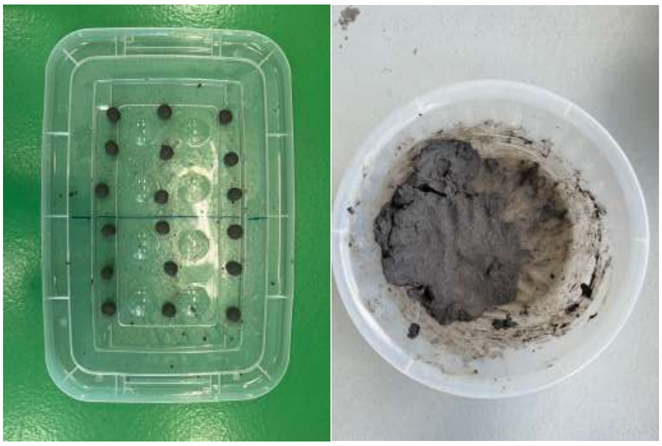
5 Sterilized seeds of *Syzygium cinereum* were cultivated in sterilized plate (Control) , sterilized plate with 5 ml of PGPR beads. Each plate was filled with 20 ml of solution (DI water, KK123, KK4, and TD). Each experiment set up was performed in triplicate. So the numbers of total samples are 24. All plates were placed under a 12-hour light / 12-hour dark cycle.

2.2.7. Buoyancy tests of the Krathong (raft)

Buoyancy tests were conducted on six raft prototypes, each tested in triplicate, in six sets of DI water with a water height of 9 cm. At this stage, no seeds or bacterial capsules were included in the soil balls.

The soil balls were shaped by hand to be as spherical as possible, with an average mass of approximately 5 grams. They were composed of four ingredients: clay, loam soil, burnt rice husk, and coconut coir, in a mass ratio of 27:8:1:1.

Figures 7.1 and 7.2. The four soil components: clay, loam soil, burnt rice husk, and coconut coir.



Figures 8.1 and 8.2. Soil mixing process, in which a small amount of water may be added to ensure homogeneity and form small spherical balls. The resulting 18 soil balls had an average mass of 5.01 grams (SD = 0.198).

Banana pseudostems were sliced into discs approximately 1 cm thick, with diameters ranging from 3.5 to 4.5 cm. Each slice was pierced with a 6.5 cm wooden skewer to prevent the leaf sheaths from separating.

The density of the banana pseudostem was calculated by this formula:

$$\rho = m / V$$

Where:

ρ = Density of the object (g/cm^3)

m = Mass of the object (g)

V = Volume of the object (cm^3)

The mass was measured with a balance, and the volume was determined using the water displacement method. Five banana discs were used, yielding an average density of 0.69 g/cm^3 (SD = 0.201).

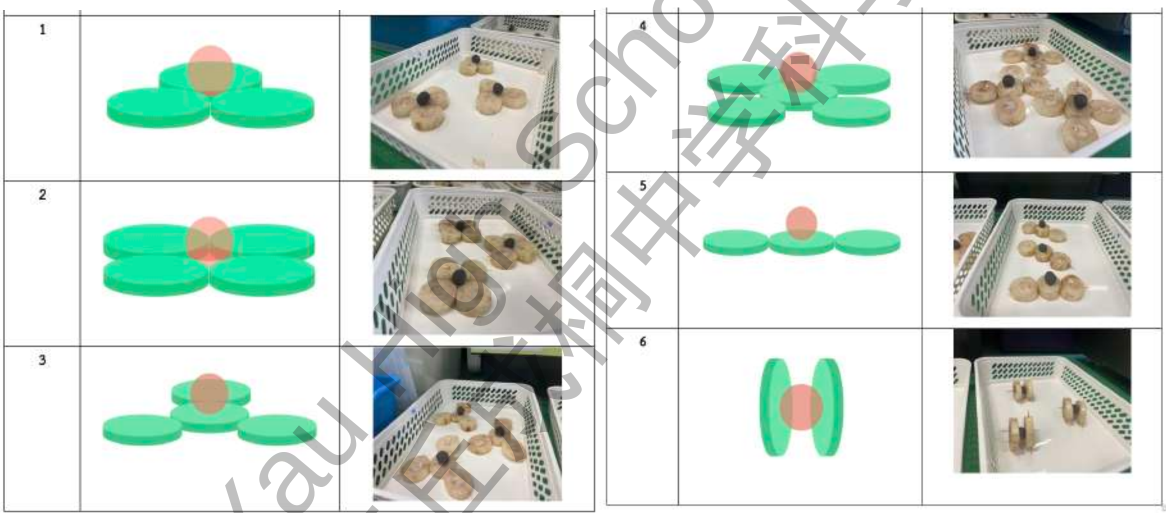
Figures 9.1 and 9.2. (left) Show the water displacement method for determining volume and the weighing process, respectively. Figure 9.3. (right) Shows the 1 cm thick banana pseudostem discs (n = 69), with an average mass of 15.10 grams (SD = 0.321).

The rafts were assembled by attaching the soil balls to the banana discs using wooden skewers, following each prototype's specific design aimed at maximizing the metacentric height (GM).

Table 1.1. Quantities of materials used for each raft prototype

Prototype no.	1	2	3	4	5	6
Number of banana pseudostem disc	3	4	4	5	3	2
Number of wooden skewers	3	4	4	5	3	3

Table 1.2. Characteristics of each raft prototype



Note: Raft prototypes 3, 4, and 5 required additional wooden skewers to support the soil balls and prevent them from falling into the water.

The floating test was conducted in plastic containers with dimensions of 57.5 × 38.5 × 31 cm^3. Equal compartments were created using foam boards (future boards), and the floating behavior of each raft was observed and recorded daily according to the schedule.

Figure 10. Floating test of all six raft prototypes in plastic containers (57.5 × 38.5 × 31 cm^3) with a consistent water height of 9 cm in each container.

2.2.8. Effectiveness of Rafts and PGPR Capsules on Seed Germination Under Simulated Peat Swamp Water Conditions

Raft prototypes of design type 2 were constructed, totaling 24 rafts, following the previously described procedure. Soil balls containing *Syzygium cinereum* seeds (4 seeds per ball) were prepared, totaling 48 balls. These were divided into four groups:

1) 12 seed balls without PGPR capsules (standard seed balls)

2) 12 seed balls with PGPR capsules

3) 12 seed balls without PGPR capsules placed inside rafts

4) 12 seed balls with PGPR capsules placed inside rafts

Three seed balls and three seed-ball rafts from each group were placed in plastic containers filled with simulated water conditions. Eight containers were used in total:

- 2 containers with DI water

- 2 containers with KK123 water

- 2 containers with KK4 water
- 2 containers with TD water

Each container was divided in half: one side for seed balls, the other for seed-ball rafts. Containers with and without bacterial treatment were kept separate. Seed germination rates were recorded for each group.

3. Results and Discussion

3.1. Chemical Properties

According to the pH measurements and the results from laboratory analysis of dissolved substances (Table 1), water samples from all four degraded areas of the Kuan Kreng peat swamp forest exhibited extremely strong acidity (pH 2.76–3.16), whereas water from the Toh Daeng peat swamp forest showed milder acidity (pH 5.34).

Another noticeable finding is that water from the degraded Kuan Kreng areas contained significantly higher concentrations of dissolved sulfate, aluminum, and iron than water from Toh Daeng. In particular, sulfate concentrations in the degraded Kuan Kreng water were dramatically higher—approximately 100 times greater in Villages 3, 4, and 9, and up to 300 times higher in Village 11. Similarly, the concentrations of dissolved aluminum were about 10 times higher in Villages 3, 4, and 9, and 40 times higher in Village 11 compared to Toh Daeng.

However, in the case of dissolved iron, the water from Villages 3 and 9 in Kuan Kreng showed levels comparable to that of Toh Daeng. Additionally, the water collected from Village 11 of the degraded Kuan Kreng peatland contained significantly higher concentrations of all dissolved minerals compared to the other three villages.

Table 2. Chemical properties of peat swamp water from various locations.

Location the sample collected		pH	Sulfate (mg/L)	Aluminium (mg/L)	Iron (mg/L)
Kreng Subdistrict, Cha-uat District, Nakhon Si Thammarat Province (Kuan Kreng peat swamp forest)	Moo 3	3.16	164	6.44	4.65
	Moo 4	2.96	232	5.53	16.8
	Moo 9	2.76	155	5.71	4.35
	Moo 11	2.81	597	22.5	171
Puyo Subdistrict, Su-ngai Kolok District, Narathiwat Province		5.34	2.00	0.583	3.04

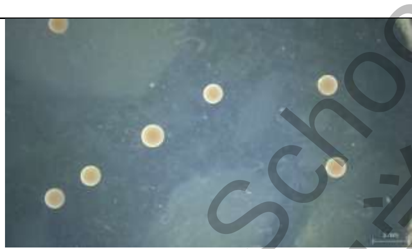
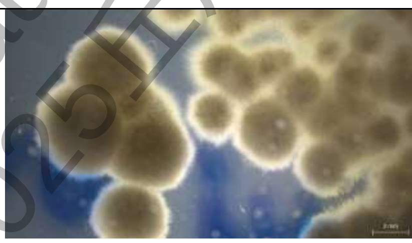
(Toh Daeng peat swamp forest)

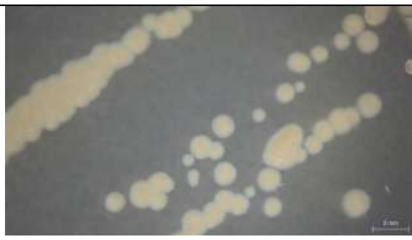
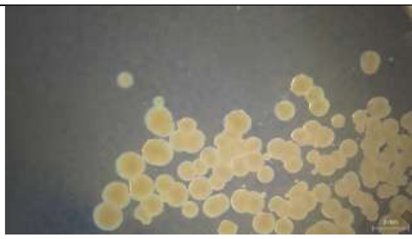
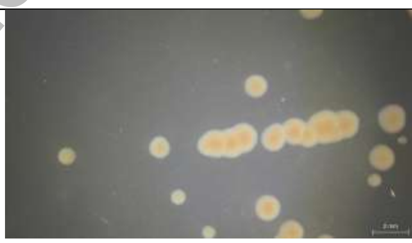
In Thai address system, Moo is equivalent to village.

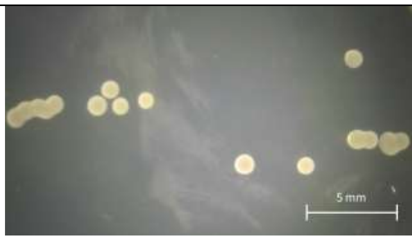
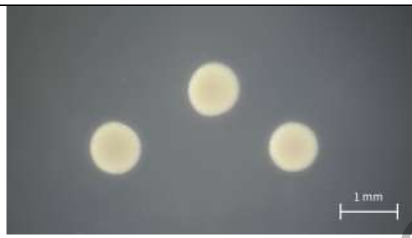
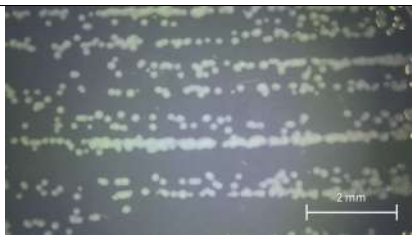
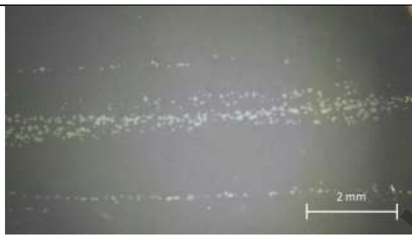
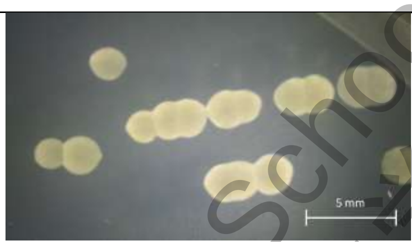
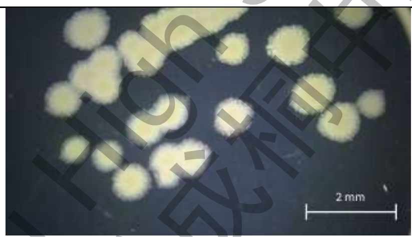
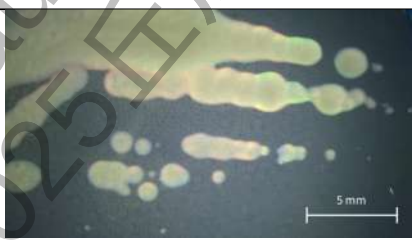
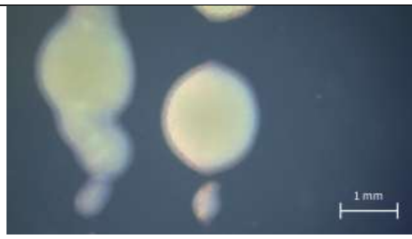
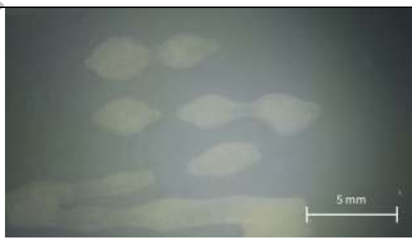
This experiment clearly demonstrates the differences in pH levels and mineral content between water samples from the two peat swamp forests. The degraded condition of Kuan Kreng, with its lower biodiversity and history of repeated wildfires, likely contributes to a higher rate of pyrite oxidation in the soil compared to Toh Daeng. This process produces sulfuric acid, which lowers the pH of both soil and water and leads to elevated levels of sulfate, iron, and aluminum.

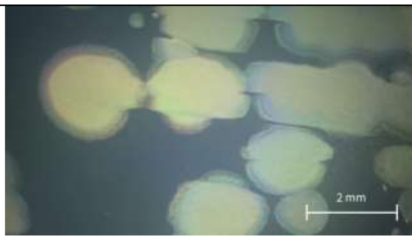
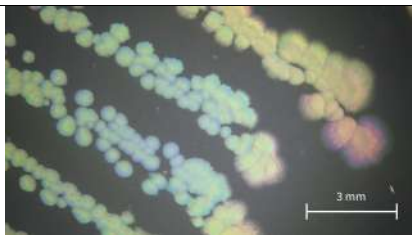
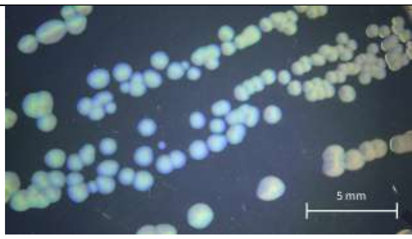
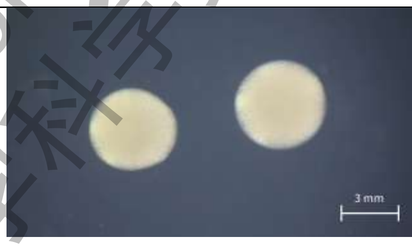
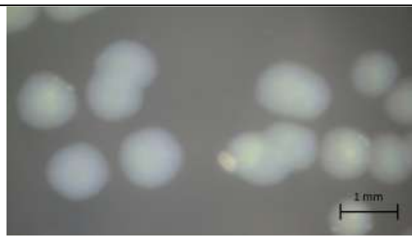
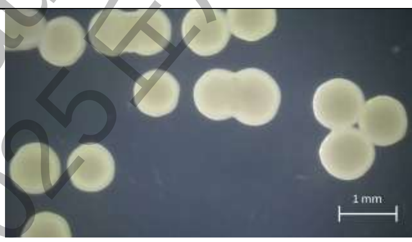
3.2. Biological Properties

Table 3. Colony characteristics of bacteria isolated from water and soil samples from different peat swamp sites.

Colony code	Magnification 0.63x	Magnification 2.00x
KK1 (sampled from W-KK1)		
KK2 (sampled from W-KK1)		
KK3 (sampled from W-KK3)		
KK4 (sampled from S-KK1)		

KK5 (sampled from S-KK1)		
KK6 (sampled from S-KK3)		
KK7 (sampled from S-KK3)		
KK8 (sampled from S-KK3)	Repetition	
KK9 (sampled from S-KK1)		
KK10 (sampled from S-KK2)	Repetition	
KK11 (sampled from S-KK3)		
KK12 (sampled from S-KK3)		

KK13 (sampled from S-KK4)		
KK14 (sampled from S-KK4)		
KK15 (sampled from S-KK4)		
TD1 (sampled from W-TD)		
TD2 (sampled from W-TD)		
TD3 (sampled from S-TD)		
TD4 (sampled from S-TD)		

TD5 (sampled from S-TD)		
TD6 (sampled from W-TD)		
TD7 (sampled from W-TD)		
TD8 (sampled from W-TD)		
TD9 (sampled from S-TD)		
TD10 (sampled from S-TD)		

W = Water sample from peat swamp

S = Soil sample from peat swamp

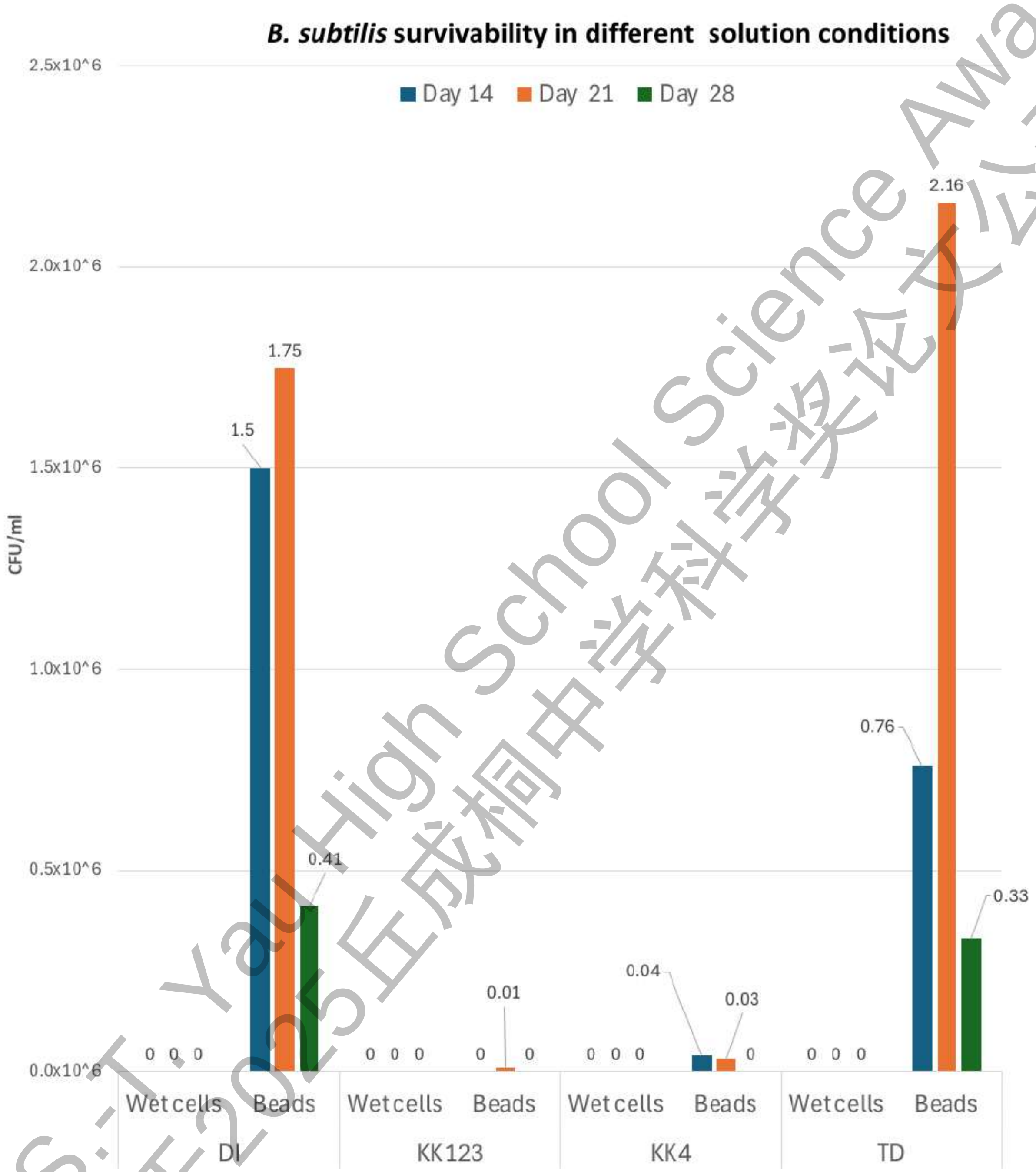
TD = Toh Daeng peat swamp forest

KK = Kuan Kreng peat swamp forest, with samples collected from four areas: KK1, KK2, KK3, and KK4

Based on bacterial isolation from water and soil samples using the method described in Section 4.1.2.3, a total of 13 distinct bacterial species were identified from Kuan Kreng and 10 from Toh Daeng. This study further revealed that all 23 bacterial species were capable of growing well under the acidic conditions of their source environments, as well as on acidified agar media, where the pH was adjusted to be more acidic than normal.

These findings may pave the way for discovering more effective PGPR candidates—both in terms of enhancing seed germination and surviving in harsh acidic environments—which could be applied to further strengthen the innovation proposed in this project.

3.3. Survivability of encapsulated bacteria in simulated peat swamp forest water



The results showed that wet cells were unable to survive in any of the simulated peat swamp water solutions. In contrast, bacteria encapsulated in calcium alginate beads remained viable in all types of simulated solutions after 21 days. Moreover, the encapsulated bacteria were still viable in the TD simulated solution after 28 days.

These findings indicate that encapsulation in calcium alginate beads effectively enhances the survival rate of bacteria in simulated peat swamp water conditions.

3.4. Effect of PGPR beads on seed germination and seed growth

Table 4.1. Seed Germination Percentage and Seed Germination Index of *Syzygium cinereum* under Different Solution Conditions.

Experiment		% Seed germination	Seed germination index
DI		40.0	0.265 ± 0.018
	+ Bacterial beads	20.0	0.083 ± 0.078*
KK123		60.0	0.325 ± 0.031
	+ Bacterial beads	20.0	0.093 ± 0.132*
KK4		60.0	0.272 ± 0.124
	+ Bacterial beads	73.3	0.321 ± 0.109
TD		53.3	0.266 ± 0.124
	+ Bacterial beads	40.0	0.195 ± 0.086

Values represent Means ± Standard deviation. * indicates a statistically significant difference compared to the control group (without bacterial beads) within the same type of solution condition according to t-test ($p<0.05$)

Table 4.2. Average Root Length, Average Shoot Length, and Seedling Vigor Index of *Syzygium cinereum* under Different Solution Conditions.

Experiment		Average Root Length (mm)	Average Shoot Length (mm)	Seedling Vigor Index
DI		7.00 ± 6.19	1.97 ± 1.93	7.79
	+ Bacterial beads	1.83 ± 0.62	0.50 ± 0.71	1.93
KK123		1.54 ± 0.87	0.94 ± 1.42	2.11
	+ Bacterial beads	0.43 ± 0.09*	1.50 ± 0.41	0.73
KK4		0.80 ± 0.39	1.40 ± 0.66	1.64
	+ Bacterial beads	0.49 ± 0.37	1.95 ± 1.03	1.92
TD		2.13 ± 2.91	2.00 ± 1.39	3.19
	+ Bacterial beads	1.30 ± 1.11*	2.17 ± 1.21	2.17

Values represent Means ± Standard error. * indicates a statistically significant difference compared to the control group (without bacterial beads) within the same type of solution condition according to t-test ($p<0.05$)

Based on the results, The addition of PGPR beads led to a decrease in both germination index and seedling vigor index in treatments using DI water, KK123 simulated solution, and TD simulated solution. In contrast, the addition of PGPR beads increased both indices in seeds treated with the KK4 simulated solution.

One possible reason for the inhibitory effect of PGPR beads on seed germination and growth is the excessive number of beads applied (more than 150 beads in 20 ml of solution), which may have caused the bacteria to compete with seeds for essential nutrients, such as sugars converted from starch stored in the endosperm. On the other hand, in the KK4 simulated solution, which presents harsher conditions such as high acidity and elevated levels of heavy metal ions. Most bacteria released from the calcium alginate beads could not survive. Only a small number of bacteria remained, which might represent an optimal concentration that supports seed germination and seedling development.

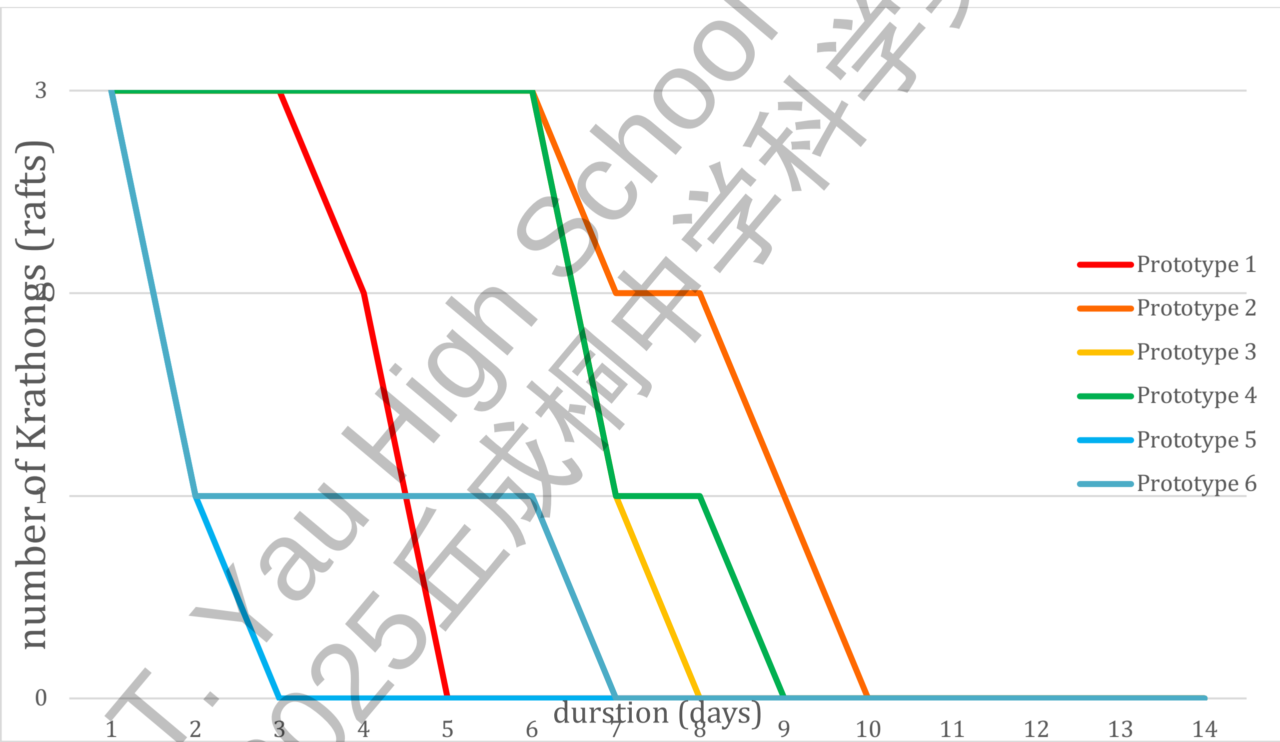
3.5. Effectiveness of the raft prototypes

Table 5. Floating behavior of each raft prototype after 2, 5, 7, and 14 days

Prototype no.	Floating behavior of the raft			
	2 days	5 days	7 days	14 days
1				
2				
3				
4				

5				
6				

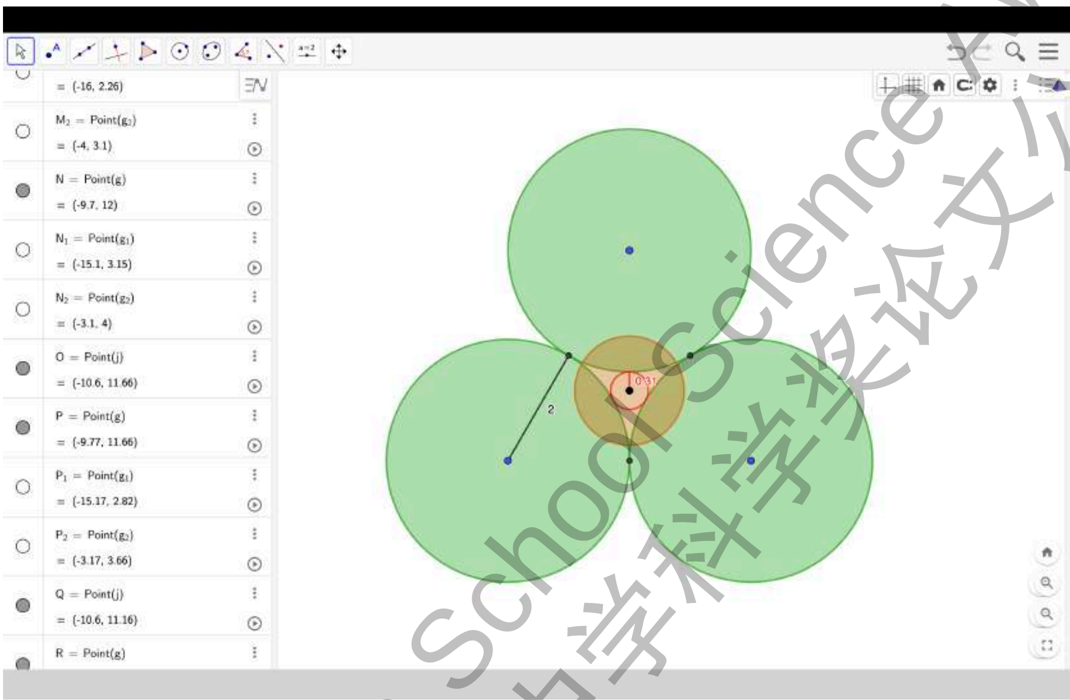
Table 6. Number of rafts with soil balls still floating above water after different durations



From the observation of floating behavior across the six raft prototypes (Prototype 1–6), it was found that Prototypes 5 and 6 experienced a rapid decrease in the number of floating soil balls—from three to one—within just two days. This indicated a tendency for the soil balls to sink completely in less than 11 days, which is significantly below the expected duration of 14–21 days.

Although Prototype 1 featured symmetrical geometry in the horizontal plane, its design positioned the soil ball at the center of a groove formed by three banana discs. However, the area holding the soil ball was comparatively shallow, offering less structural support than Prototype 2. As a result, even minor imbalances in buoyant force could easily cause the soil ball to tilt and fall off the raft.

To analyze this, 2D geometric modeling was performed using GeoGebra Classic 6 to estimate the planar radius at which a spherical soil ball (radius 0.9 units) is embedded and supported within a groove formed by banana discs (radius 2 units). The calculated embedding radius for Prototype 1 was 0.31 units, whereas for Prototype 2 it was 0.83 units, as illustrated in Figures 14.1 and 14.2.



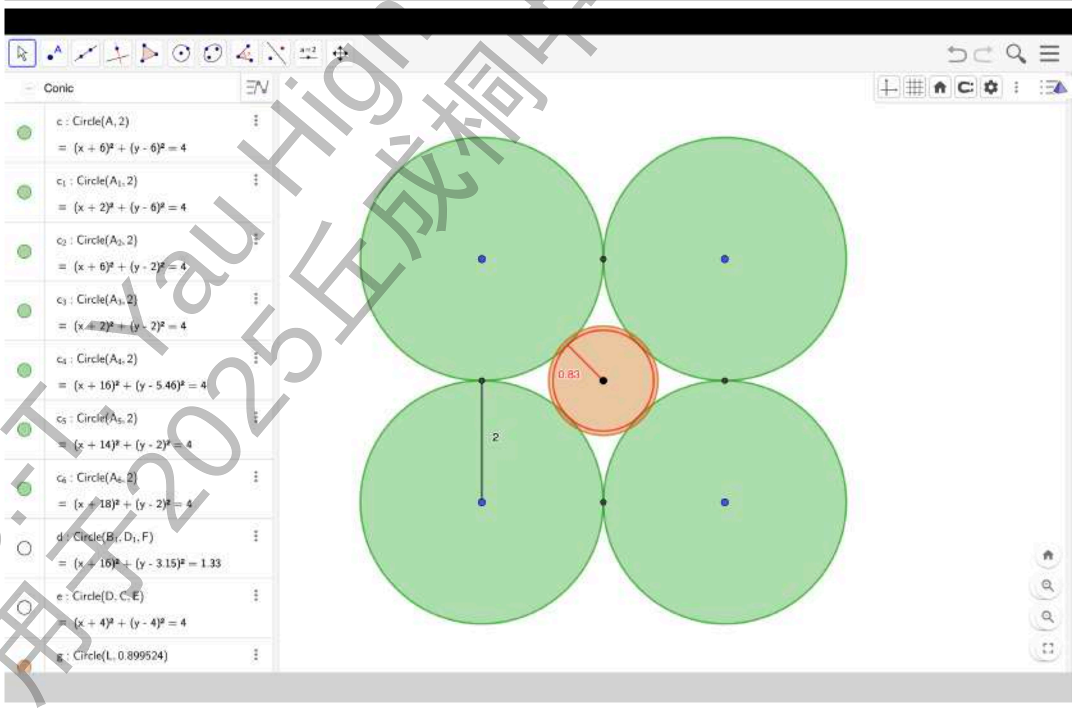


Figure 14.1 (top) and Figure 14.2 (bottom) show the geometric analysis using GeoGebra Classic 6 to estimate the embedded planar radius of the soil ball.

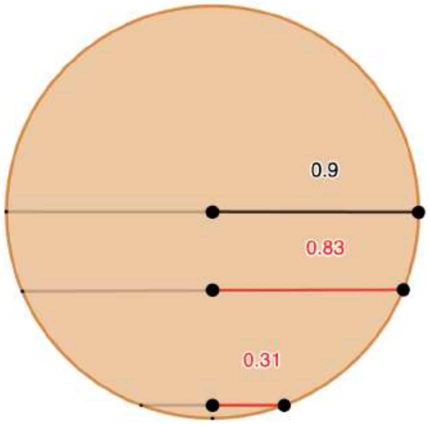
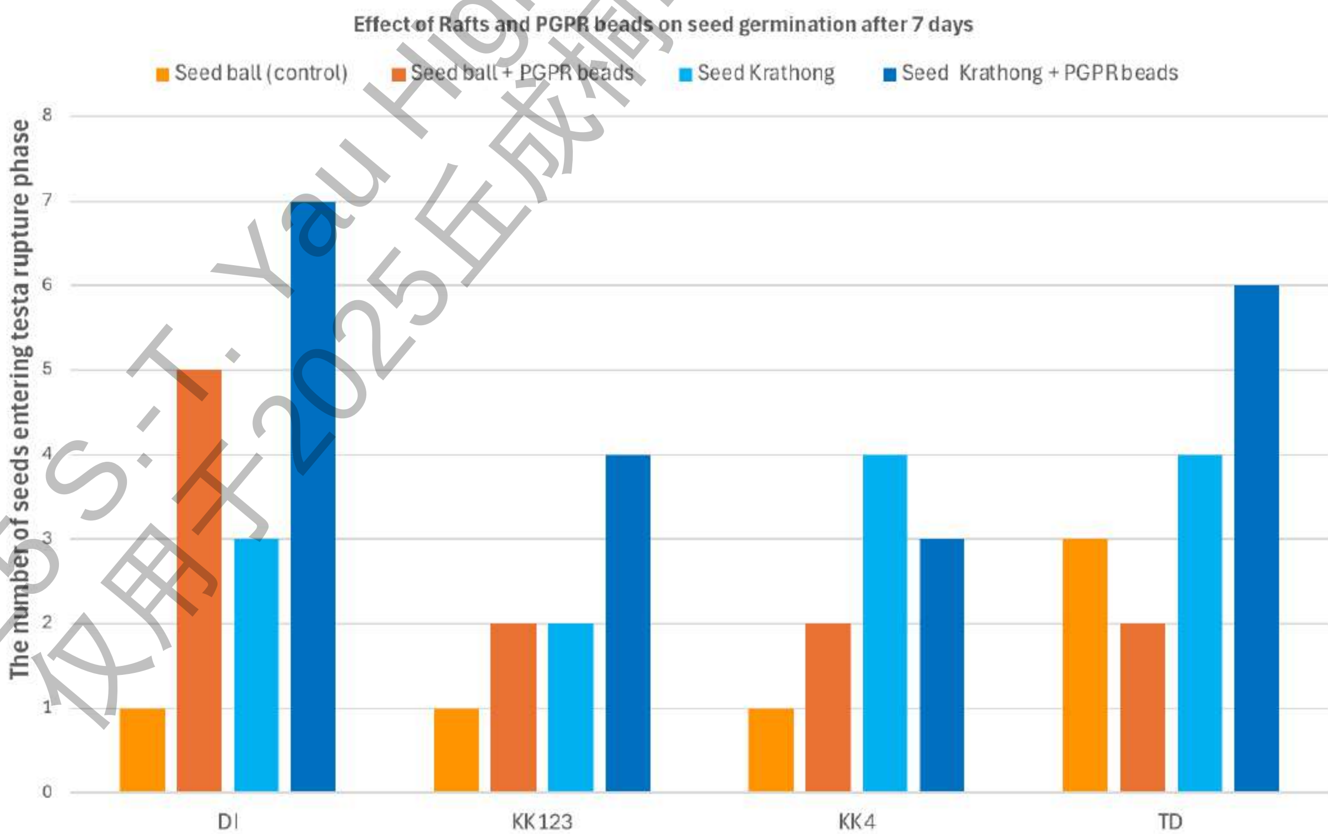


Figure 15. Height of the circular plane intersecting a spherical soil ball of radius 0.9 cm: 0.31 for Prototype 1 and 0.83 for Prototype 2

Prototype 5 was observed to frequently capsize during testing, despite being designed with a high metacentric height (GM). Prototype 6, while not showing capsizing behavior, included a design where a wooden skewer was inserted through the center of the soil ball, which often caused structural failure of the ball and made it unsuitable for use with seeds.

3.6. Effectiveness of Rafts and PGPR Capsules on Seed Germination Under Simulated Peat Swamp Water Conditions



The addition of bacterial beads tended to enhance seed germination rates under both conditions, experiment with and without Raft. However, the addition of bacterial beads did not significantly improve germination in the treatment with Raft cultivated in KK4 solution, nor in the treatment without Raft cultivated in TD solution.

Furthermore, a comparison between treatments with and without Raft demonstrated that the presence of Raft consistently increased germination rates across all experimental conditions. And the greatest enhancement in germination was observed when both PGPR beads and Raft were applied.

These results suggest that both bacterial beads and seedling cones exhibit the potential to promote seed germination.

4. Conclusion

From the experiments, it can be concluded that applying the physical principles of buoyant force according to Archimedes' principle and using the metacentric point in the design and testing of the rafts enabled the development of an optimal raft shape among six prototypes—one that maintained the soil-ball portion afloat for the longest time without capsizing or fracturing.

Encapsulation of *Bacillus subtilis* in calcium alginate markedly increased bacterial survival under simulated water conditions, especially in water from the Toh Daeng peat swamp forest, whereas bacteria in wet-cell form failed to survive under any of the tested conditions.

The study of the relationship between bacterial treatment and the germination rate of *Syzygium cinereum* seeds from Toh Daeng showed positive results in the simulation corresponding to site 4 of the Kuan Kreng peat swamp forest (KK4), while the other simulation sets showed slight negative effects—possibly due to competitive stress from high bacterial concentrations.

Furthermore, when using the optimal Krathong design together with bacterial capsules, the seed germination rate at the testa-rupture stage after seven days was higher than in submerged seed-ball controls, and even higher with bacterial encapsulation—a highly promising result.

The team aims to further extend the flotation duration of the rafts by optimizing both the design and the banana-pseudostem material, making the method easy to deploy rapidly and effectively in the field. Additional studies will focus on long-term storage of PGPR capsules via freeze-drying and, if feasible, field trials in actual peat swamp forests to validate these results and applications.

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