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Valorization of sawdust and water hyacinth for mycelium-based Thai Krathongs with embedded seeding plants

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Abstract

The Loy Krathong Festival, a cherished cultural event in Thailand, poses significant environmental challenges due to the use of non-biodegradable materials in traditional krathongs. This study presents the development of *mycelium-based krathongs* (MBKs) using *Pleurotus pulmonarius*, lignocellulosic sawdust, and ground water hyacinth in two substrate ratios (50:50 and 80:20), designed as biodegradable floating offerings embedded with *Ipomoea aquatic*a (morning glory) seeds to enhance ecological benefits. MBKs exhibited low density (0.30–0.35 g/cm^3) for buoyancy and compressive strengths of 6.18 MPa (50:50) and 5.83 MPa (80:20). Water contact angle tests revealed higher hydrophobicity in the 80:20 composite (127.13°) vs 50:50 (70.73°). Seed germination showed high viability, and soil burial tests confirmed significant biodegradation within 28 d. SEM imaging displayed uniform mycelial networks, enhancing structural integrity. This study underscores the potential of MBKs as sustainable alternatives for traditional krathongs, offering a regenerative solution that aligns cultural heritage with environmental restoration and waste valorization. By integrating biodegradable materials and seeding plants, MBKs contribute to ecosystem recovery post-festival, setting a benchmark for eco-conscious cultural practices in Thailand.

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Introduction

The Loy Krathong Festival, celebrated annually on the full moon of Thailand's twelfth lunar month, holds profound cultural significance, symbolizing gratitude to the water goddess, Phra Mae Khongkha[1−3]. However, the festival has evolved into an environmental concern due to the widespread use of non-biodegradable materials in modern krathongs (floating decorations shaped like lotus blossoms). Traditionally crafted from banana leaves and stems[4,5], many krathongs are now made from Styrofoam, or plastic—materials that disrupt aquatic ecosystems, deplete oxygen levels, and contribute to water pollution[6,7].

Although efforts have been made to introduce 'eco-friendly' alternatives, such as bread or plant-based krathongs, these often still pose ecological risks or lack full biodegradability. Some designs, reinforced with metal pins or synthetic adhesives, inadvertently harm marine life and hinder decomposition. As a result, there is a pressing need for fully biodegradable, environmentally restorative alternatives[6]. This shift in materials and scale has turned Loy Krathong into a double-edged sword, balancing cultural beauty with ecological challenges[3].

This study addresses the environmental challenges posed by the Loy Krathong Festival by developing mycelium-based krathongs (MBKs) using *Pleurotus pulmonarius*[8,9], lignocellulosic sawdust[10−12], and ground water hyacinth[13]—abundant agricultural wastes in Thailand. *Pleurotus pulmonarius* was selected for this study due to its considerable economic and scientific importance. It ranks among the most widely cultivated mushroom species in Thailand, primarily because of its excellent adaptability to hot and humid climates, resilience to environmental stressors, and ease of cultivation with relatively low production costs[14−15]. Notably, *P. pulmonarius* shares partially overlapping and complex morphological traits with *P. ostreatus*, as evidenced by mating compatibility tests, RAPD-PCR analyses, and mitochondrial small subunit ribosomal DNA sequence analyses[16−17]. Furthermore, the availability of local cultivation materials reduces costs and minimizes dependency on imported fungal strains. Although *P. ostreatus* has been more extensively utilized in mycelium-based composite research, *P. pulmonarius* offers specific advantages such as accelerated mycelial growth, robust adaptability to various tropical agricultural residues, and efficient degradation of lignocellulosic substrates[18−20]. These characteristics underscore its substantial potential for sustainable composite material development, aligning with previous research findings demonstrating its effectiveness in utilizing agricultural waste streams[21−23].

Uniquely, the MBKs incorporate embedded seeding plants (*Ipomoea aquatica*), enabling post-festival ecological restoration. The study evaluates the physical, mechanical, and biological properties of the MBKs, emphasizing their biodegradability, buoyancy, and potential to promote biodiversity. By valorizing agricultural waste and integrating ecosystem services, this research offers a sustainable alternative that harmonizes cultural traditions with environmental conservation[24].

This novel approach not only mitigates the ecological impact of the Loy Krathong Festival but also transforms waste into value-added products, fostering both cultural preservation and ecological regeneration. The proposed MBKs serve as a model for sustainable practices in cultural events, demonstrating how environmentally conscious innovations can support biodiversity, reduce pollution, and promote long-term ecological balance.

Materials & methods

Source of mushroom mycelia/preparation of feeding and growing substrates

Pure cultures of *Pleurotus pulmonarius* (MU code 00021) were sourced from AnonBiotec's mushroom farm, Talaad Thai Market, Phahonyothin Road (km 42), Khlong Luang District, Pathum Thani Province, Thailand (https://anonbiotec.co.th, accessed February 21, 2025). The isolate was initially cultured on potato dextrose agar (PDA; BD Difco™, USA) and incubated at 30 °C for 7 d to establish a working culture. Sawdust was used as the primary growth substrate, providing a lignocellulosic base for mycelial colonization as a modification method by Kohphaisansombat et al.[25]. Ground water hyacinth (WH; *Eichhornia crassipes*) was introduced as an additive to enhance porosity and nutrient content[26].

Two substrate ratios were tested: 50% sawdust:50% WH and 80% sawdust:20% WH. The percentage was indicated by volumetric percentage. Both sawdust and WH were sourced from local sawmills and nearby water bodies in Nonthaburi Province, Thailand. After thorough washing to remove impurities, the materials were oven dried at 103 °C until weight stabilization. Dried samples were ground to a fine powder (0.1–0.2 mm particle size) and stored in airtight containers until use. Prior to inoculation, the substrates were supplemented with 4% wheat flour as an additional carbon source. The mixtures were sterilized at 121 °C for 30 min to eliminate microbial contaminants and cooled to room temperature under sterile conditions[25].

Mycelial growth rate assessment

Pleurotus pulmonarius inoculum was isolated from mushroom spawn and cultured in potato dextrose agar (PDA). The inoculum was then cut from the mycelia growth tips. The circular inoculum pieces (diameter = 0.8 cm) were placed on four different media to monitor the influence of media on mycelium growth. Growth was monitored by measuring radial expansion on PDA, PDA supplemented with water hyacinth (PDA + WH), and the two substrate ratios over a 5 d.

Mycelium-based composite fabrication

The experiment commenced with the preparation of dried water hyacinth, rice bran, flour, and minerals, which were placed in plastic bags and sterilized alongside all necessary equipment. Following sterilization, the spawn of *P. pulmonarius* and water were incorporated into the sterilized mixture, adapting the method described by Kohphaisansombat et al.[25]. The plastic bags were then securely sealed with adhesive tape to prevent contamination and transferred to a mushroom cultivation chamber, where humidity, temperature, and light conditions were carefully regulated to optimize mycelial colonization over a period of 5 to 7 d. The growth conditions for mycelium-based composite were maintained at 80% RH, 28 °C in a dark greenhouse. Once the mycelium had fully colonized the substrate, the mixture was extracted for further processing into mycelium-based krathongs (MBKs). The production process began with sterilizing all equipment, followed by the application of a food-grade plastic wrap inside cylindrical molds to facilitate easy removal of the MBKs. The mycelium-colonized mixture was then placed into the molds, which were covered with an additional layer of food-grade plastic wrap to prevent contamination. The molds were subsequently returned to the mushroom cultivation chamber for 14 d, where environmental conditions continued to be carefully controlled to support further fungal growth. Following this period, the MBKs were removed from the molds, flipped, and transferred to plastic bags, which were placed back in the cultivation chamber for another 7 d incubation under identical conditions. Finally, to halt mycelial growth and enhance structural stability, the MBKs were subjected to drying in a hot air oven at 70 °C for 24 h.

Chemical composition and growth behaviour analysis

ATR-FTIR spectroscopy

The chemical composition of the mycelium and composites was analyzed using Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy (iS50, Thermo Fisher Scientific, USA). Spectra were recorded over a wavenumber range of 4,000–650 cm^{-1} with a resolution of 4 cm^{-1} and 64 scans. Comparative analyses were conducted for pure sawdust, pure water hyacinth (WH), and the 50:50 and 80:20 composite ratios to identify functional groups and assess lignocellulosic integration[27].

Scanning Electron Microscopy (SEM)

The structural morphology and mycelial distribution within the composites were examined using Scanning Electron Microscopy (SU-8010 FESEM, Hitachi, Japan). Samples were coated with a thin layer of platinum using a sputter coater and imaged at magnifications ranging from 1,500× and 5,000×. SEM images were used to assess the bonding between the mycelium and the lignocellulosic substrate, as well as fiber distribution and pore structure[28].

Physical and mechanical properties

Density

The density of the mycelium-based krathongs (MBKs) was measured following ISO 9427:2003 standards. Fully grown and dried MBK samples were weighed using an analytical balance (accuracy ±0.001 g), and their volume was calculated based on sample dimensions. Density (D) was calculated using the formula: $D = M/V$, where M is the mass (g) and V is the volume (cm^3). Each treatment was replicated three times, and results were reported as mean ± standard deviation[25]. These measurements provided insights into the structural uniformity and lightweight nature of the MBKs—critical parameters for their potential applications in eco-friendly krathongs or other biodegradable products.

Compressive strength

Compressive strength tests were performed using a Universal Testing Machine (Model 5569, INSTRON, USA). MBK samples (5 cm × 5 cm × 5 cm cubes) were tested at a maximum load of 50 kN. The compression speed was set at 10 mm/min and applied until either a 50% strain was reached or until structural failure occurred. The test was conducted at room temperature (23–25 °C). The compressive strength (F) was calculated using $F = P/A$, where P is the maximum load (N) and A is the cross-sectional area (mm^2). Three replicates were tested for each condition, and the results were reported as average values with standard deviations (SD). Compressive strength values were recorded in MPa[25].

Water contact angle

The surface hydrophobicity of MBK samples was determined using a contact angle goniometer (SL200KS, Kino, USA). Each sample was fixed on the instrument platform, and 10 μl of distilled water was dropped on the surface of the material using a syringe. The image of the droplet was taken, and the water contact angle (WCA) was determined using the CAST3 program (version 3.22.0.2678).

Each sample was tested in triplicate, and three samples of each mycelium bio-composite were measured. Finally, the average value was taken[29].

Water absorption

Three dried MBK cubes (5 cm × 5 cm × 5 cm) were collected, and their weight was recorded as the initial weight (W_0). The samples were placed in containers filled with distilled water maintained at a constant temperature of 23–25 ± 1 °C. The cubes were immersed for 0, 3, 10, 20, 30, 40, 50, 60, 90, 120, 240, 480, 720, 960, 1,200, and 1,440 min. At each time point, the samples were removed, and their post-immersion weight (W_t) was recorded. Water absorption was calculated by determining the weight difference relative to the initial dry weight[30]. Each sample underwent triplicate testing, with water absorption (%) calculated using the specified formula. Water Absorption (%) = $W_t - W_0/W_0 \times 100$, where W_0 is the initial dry weight and W_t is the weight after immersion in water. Volume expansion and density changes were also recorded.

Fabrication of Mycelium-Based Krathongs (MBKs) and biological properties

Seed germination performance

Ipomoea aquatica (water convolvulus) seeds were embedded within MBKs during fabrication. Seed germination tests were conducted in a growth chamber maintained at 25 °C, 70% relative humidity, and a 12 h light/dark cycle. Watering was performed daily at 7:30 am with 500 mL of water. Germination rates were recorded daily for 21 d. The chamber provided a 12 h light and dark cycle to simulate natural environmental conditions. Control experiments were conducted by planting seeds from the same batch directly into sterile soil, without the mycelium-based material. Germination rates were monitored daily for 21 d, recording the percentage of seeds that successfully germinated and the time to first sprout. Growth parameters such as number of shoots, number of leaves, shoot length, and shoot width were measured after 21 d to assess the impact of the composite material on seed viability and early-stage plant development[31].

Degradation analysis

Soil burial tests were conducted to assess the biodegradability of the MBKs in compliance with ASTM D5988-18 standards[32]. Six MBK samples from both 50:50 and 80:20 conditions were buried in natural soil at a depth of 10 cm in a controlled outdoor environment. Soil moisture was maintained at 60% of field capacity by periodic watering every morning at 7:30 am with 500 mL of water, and the temperature was monitored to remain within 20–30 °C. Each MBK sample was retrieved at intervals of 7, 14, 21, and 28 d. The degree of degradation was assessed visually and quantitatively by measuring weight loss using a digital scale and taking a picture of each MBK sample.

Results

Growth characteristics of *P. pulmonarius* on water hyacinth

The growth characteristics of *Pleurotus pulmonarius* were assessed across different substrates, including potato dextrose agar (PDA), PDA supplemented with water hyacinth (PDA+WH), and two composite formulations comprising sawdust and water hyacinth in 50:50 and 80:20 ratios. PDA was used as the control medium for comparison. As shown in Fig. 1a, mycelial growth was monitored daily by measuring colony diameter. The fastest growth was observed on PDA + WH, with a rate of 1.86 cm/d, followed by PDA alone at 1.55 cm/d (Fig. 1b). This indicates that water hyacinth

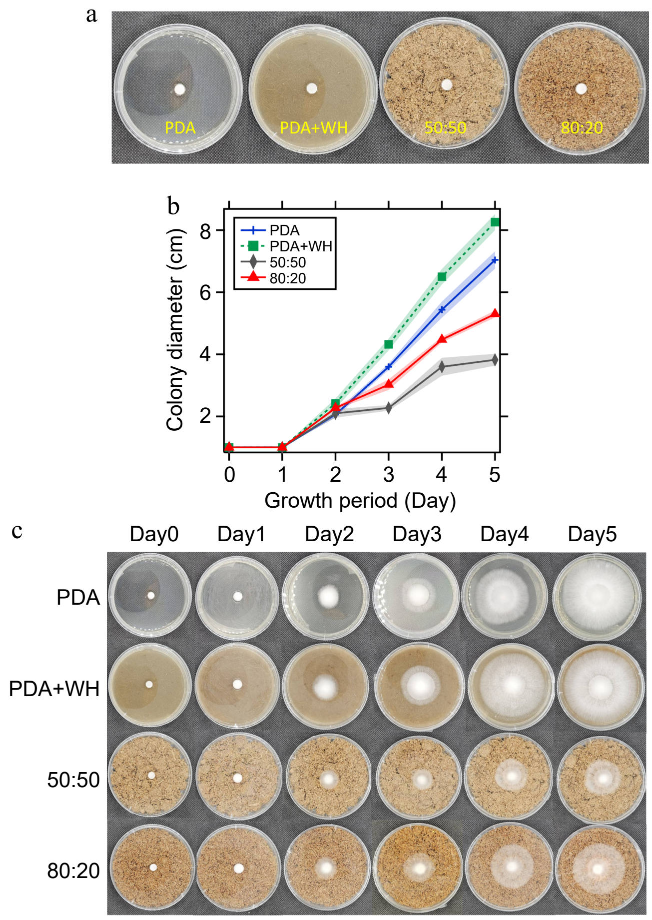

Fig. 1 (a) Growth of *P. pulmonarius* on PDA, PDA + WH, 50:50 composite, and 80:20 composite substrates. (b) Measurement of colony diameter across different media over a 5 d period, with the shaded area representing the standard deviation of mycelial growth. (c) Photographic documentation of *P. pulmonarius* cultured on various media from day 0 to day 5.

supplementation enhances nutrient availability or alters the substrate texture to favor faster colonization. In contrast, the 50:50 and 80:20 composites exhibited slower growth rates of 0.72 cm/d and 1.08 cm/d, respectively, likely due to differences in substrate density and water retention. The higher sawdust content in the 80:20 composite appears to support better mycelial network formation compared to the 50:50 mixture, which may retain more moisture but offer less structural integrity. Moreover, the different compositions of 50:50 and 80:20 composites also affected water retention ability, which led to different moisture conditions for mycelium growth. Not only was there a different growth rate between PDA-based media and water hyacinth composite, but the characteristics of the mycelium were also distinguished.

Morphological differences were also observed (Fig. 1c). Mycelium grown on PDA and PDA+WH adhered uniformly to the substrate surface, whereas the water hyacinth composites developed extensive aerial mycelia. Notably, the mycelial fibers on water hyacinth were thinner than those on PDA, possibly due to higher moisture content and differences in substrate degradation rates, consistent with prior findings[33].

Mycelium chemical composition and growth behavior

The chemical composition of mycelium grown on sawdust-water hyacinth composites (SWH) with sawdust-to-water hyacinth ratios of 50:50 and 80:20 was analyzed using ATR-FTIR and compared to pure sawdust and pure water hyacinth. As shown in Fig. 2a, the FTIR spectra of both composites displayed prominent peaks at 3,340 and 1,026 cm^{-1}, indicative of polysaccharides present in the mycelium. These peaks, also found in the pure sawdust and water hyacinth spectra, confirm the lignocellulosic nature of the substrates. Distinct peaks at 2,917 and 2,850 cm^{-1}, characteristic of water hyacinth, were more intense in the 50:50 composite, verifying its higher water hyacinth content. A key peak at 1,317 cm^{-1}, corresponding to C–N stretching of amide III in chitin, was observed in both composites, with higher intensity in the 80:20 composite. Similarly, the peak at 1,622 cm^{-1}, linked to hydrogen bonding in chitin chains, was more pronounced in the 80:20 composite, indicating a greater chitin content. The presence of glucan was confirmed by the peak at 1,026 cm^{-1} (C–O–C and C–C stretching) in both composites. The higher chitin content in the 80:20 composite suggests enhanced structural rigidity, while the 50:50 composite, with its higher water hyacinth content, may offer greater porosity and faster biodegradability. These differences in chemical composition likely influence the physical and mechanical properties of the resulting mycelium-based krathongs.

Mycelium growth behavior on different composites

The mycelial growth behavior on different composites was evaluated using optical microscopy and Scanning Electron Microscopy (SEM). As shown in Fig. 2b, distinct differences were observed between the 50:50 and 80:20 sawdust-to-water hyacinth composites. In the 50:50 composite, mycelial fibers were loosely deposited on the water hyacinth surface, displaying a fluffy texture indicative of suboptimal growth conditions. This resulted in reduced structural cohesion and a lower mycelial growth rate. In contrast, the 80:20 composite supported more homogeneous mycelial growth, with fibers fully penetrating the substrate and forming a dense, interconnected network. SEM analysis (Fig. 2c) confirmed these observations, revealing that mycelial fibers in the 50:50 composite had irregular diameters ranging from 300 to 2,000 μm and exhibited a bumpy surface, indicative of uneven growth. In the 80:20 composite, the fibers displayed smoother surfaces with a more uniform diameter averaging around 1,500 μm. The denser and more consistent growth in the 80:20 composite suggests improved nutrient distribution and aeration, fostering optimal mycelial development. Additionally, the presence of some spores within the 80:20 composite indicates active fungal colonization, further enhancing structural integrity. These findings highlight that a higher sawdust content creates a more favorable environment for robust mycelial growth, likely resulting in superior mechanical properties and enhanced durability in the final composite.

Effect of water hyacinth on the physical properties of mycelium composites

The physical properties of sawdust-water hyacinth (SWH) composites were evaluated to assess the impact of water hyacinth content on material structure. As shown in Fig. 3a, the 50:50 composite exhibited a smooth, fluffy mycelial skin on its surface, while the 80:20 composite demonstrated homogeneous mycelial penetration throughout the material, resulting in a rougher, more rigid texture. Density measurements (Fig. 3b) revealed that the overall density of SWH composites ranged from 0.30 to 0.35 g/cm^3, indicating their porous nature and ability to float on water (density of water = 1.0 g/cm^3). The 50:50 composite, with a higher water hyacinth content, exhibited a lower density compared to the 80:20 composite. This difference is attributed to the lower intrinsic density of water hyacinth and its impact on mycelial growth patterns, which led to less compact mycelial networks in the 50:50 composite. In contrast, the 80:20 composite, with increased sawdust content, supported more extensive mycelial colonization, contributing to its higher density. To further explore the internal structure, BET analysis was performed to assess surface area and pore volume (Fig. 3c). Both composites exhibited similar pore volumes — 1.018 cm^3/g for the 50:50 composite and 1.027 cm^3/g for the 80:20 composite — indicating that porosity was largely influenced by the sawdust content. However, the BET surface area of the 80:20 composite (1.28 m^2/g) was 1.5 times greater than that of the 50:50 composite (0.85 m^2/g). This increase is attributed to the formation of a denser mycelial network, creating numerous micro-pores within the composite structure. The higher surface area in the 80:20 composite suggests enhanced mechanical strength and potential water resistance, while the lower surface area in the 50:50 composite reflects the dominance of water hyacinth, resulting in a more open and porous structure. These findings highlight the critical role of substrate composition in determining the physical properties and potential applications of mycelium-based composites.

Mechanical properties of SWH composites

The mechanical properties of the sawdust-water hyacinth (SWH) composites were assessed through compression tests, with the results presented in Fig. 3d. The compressive modulus for the 50:50

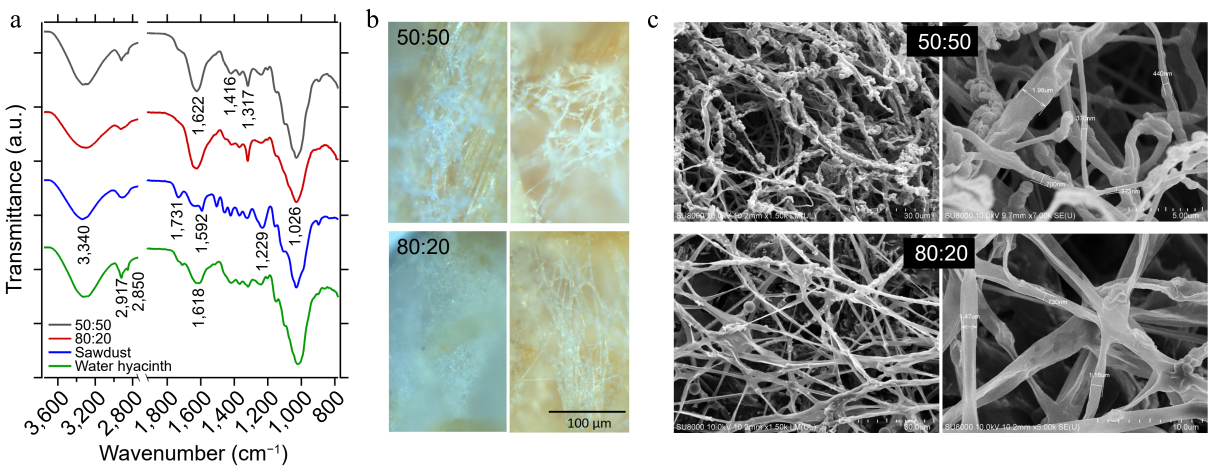

Fig. 2 (a) FTIR spectra comparing the 50:50 and 80:20 composites with pure sawdust and water hyacinth. (b) Micrographs of mycelial fibers within the 50:50 and 80:20 composites captured at 20× magnification. (c) SEM images of mycelial fibers in the 50:50 and 80:20 composites, displayed at 1.5 k (left) and 5 k (right) magnifications.

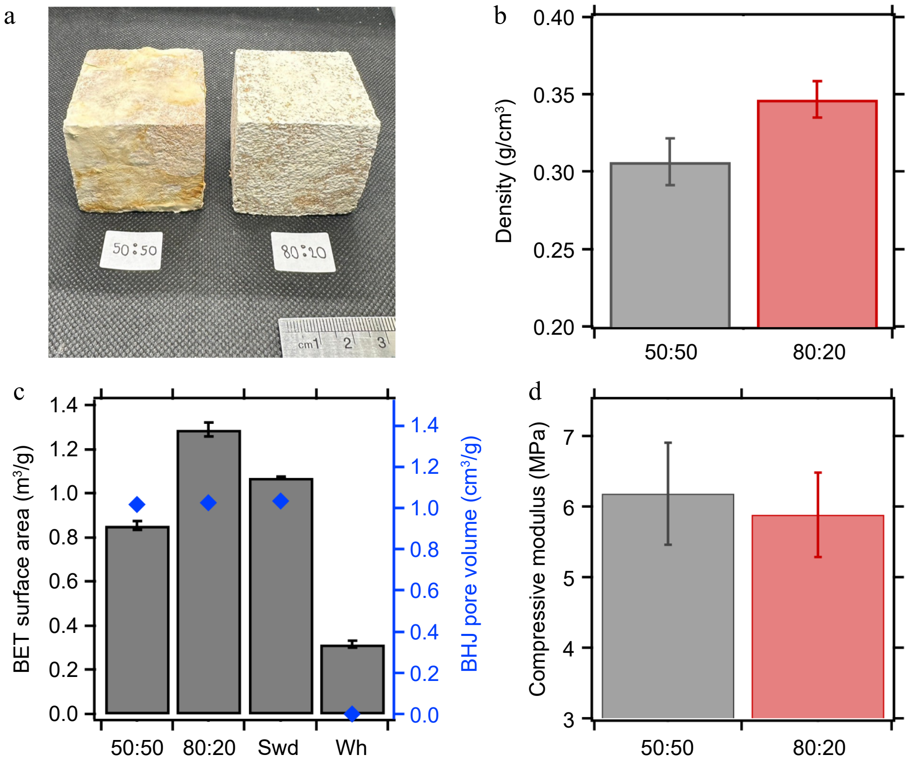

Fig. 3 (a) Photographic representation of the 50:50 and 80:20 composites. (b) Measured density of both composite formulations. (c) BET surface area and BJH pore volume analysis. (d) Compressive modulus comparison of the 50:50 and 80:20 composites.

composite was 6.18 MPa, slightly higher than the 5.83 MPa observed for the 80:20 composite. However, statistical analysis revealed that this difference was not significant ($p > 0.05$), indicating that the variation in substrate ratios did not substantially affect the compressive strength of the composites. Both composite types exhibited approximately 10% variability in compressive modulus, likely due to natural inconsistencies in the distribution of water hyacinth fibers and the extent of mycelial colonization. The slightly higher compressive modulus observed in the 50:50 composite may be linked to its denser water hyacinth content, which could provide localized reinforcement. Conversely, the 80:20 composite benefited from a more uniform mycelial network, contributing to consistent load distribution. Overall, these findings suggest that while substrate composition influences certain physical properties, the mechanical integrity of the composites is primarily governed by the mycelial matrix. The comparable compressive moduli across both composites underscore the potential for flexible substrate formulation without compromising structural strength.

Waterproof properties of SWH composites

The waterproof properties of sawdust-water hyacinth (SWH) composites were evaluated through water contact angle measurements, as shown in Fig. 4a. The 50:50 composite exhibited a water contact angle of 70.73° ± 15.79°, classifying it as hydrophilic (contact angle < 90°). In contrast, the 80:20 composite displayed a significantly higher contact angle of 127.13° ± 5.65°, indicating strong hydrophobicity. These differences highlight the influence of substrate composition on the surface characteristics of the composites, with the higher sawdust content in the 80:20 composite promoting a denser mycelial network and reduced surface wettability.

The water absorption behavior of the composites over 24 h is presented in Fig. 4b. The 50:50 composite exhibited rapid water uptake, reaching 52.9% absorption within the first 3 min and doubling to 103.0% by 10 min. The absorption plateaued at 189.8% after 120 min. Conversely, the 80:20 composite absorbed water at a slower rate, with 37.3% absorption at 3 min and 124.7% at 120 min. By 24 h, the total water absorption reached 220.9% for the 50:50 composite and 162.0% for the 80:20 composite.

Water absorption rates were modeled using exponential growth functions (Fig. 4c), revealing that the 50:50 composite absorbed water at a faster rate (7.9%/min) compared to the 80:20 composite (5.8%/min). These results align with the water contact angle data, where the more hydrophilic 50:50 composite absorbed water more readily than the hydrophobic 80:20 composite.

Volume expansion and density changes due to water absorption were also recorded. The 50:50 composite showed a peak volume expansion of 13.3% at 6 h, stabilizing at 12.6% by 24 h. The 80:20 composite exhibited a slower expansion, reaching 8.6% at 12 h and 8.8% at 24 h. Despite these changes, both composites maintained densities below 1 g/cm^3 (0.85 g/cm^3 for 50:50 and 0.78 g/cm^3 for 80:20) after 24 h, ensuring buoyancy.

Floating tests (Fig. 4d) demonstrated that the 50:50 composite floated with its surface level at the waterline, while the 80:20 composite remained partially elevated above the water, consistent with its lower water absorption and higher hydrophobicity. These findings suggest that the 80:20 composite offers superior waterproofing and structural stability, making it more suitable for prolonged exposure in aquatic environments.

Fabrication of Mycelium-Based Krathong (MBK)

The characterized 50:50 and 80:20 sawdust-water hyacinth composites were fabricated into plate-like shapes to create mycelium-based Krathongs (MBKs) suitable for use in the Loy Krathong festival. As shown in Fig. 5a, the 50:50 composite exhibited a smooth, fluffy surface due to surface-dominant mycelial growth, whereas the 80:20 composite displayed a rougher, more rigid surface resulting from deeper mycelial penetration into the composite matrix. The MBKs were designed with embedded morning glory (*Ipomoea aquatica*)

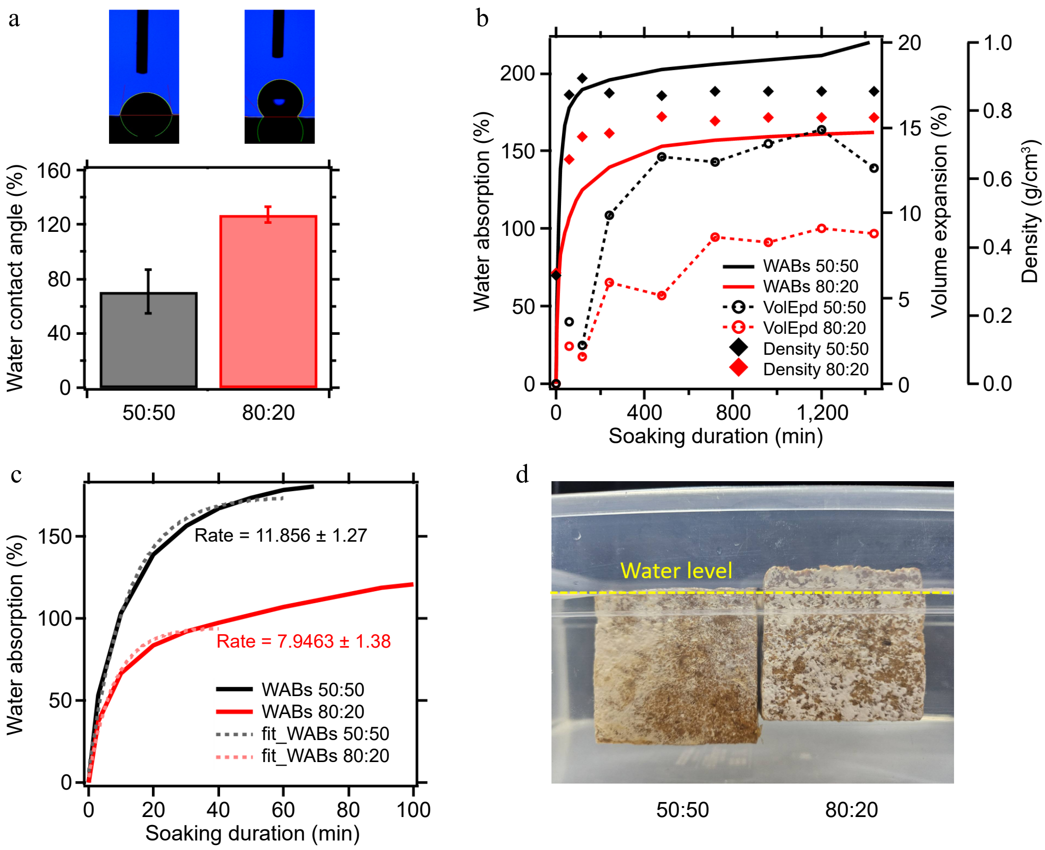

Fig. 4 (a) Water contact angle measurements on the surfaces of 50:50 and 80:20 composites. (b) Relationship between water absorption, volume expansion, and composite density over different soaking durations. (c) Water absorption rate of the 50:50 and 80:20 composites recorded over a 24 h period. (d) Floating demonstration of the 50:50 and 80:20 composites after 24 h in water.

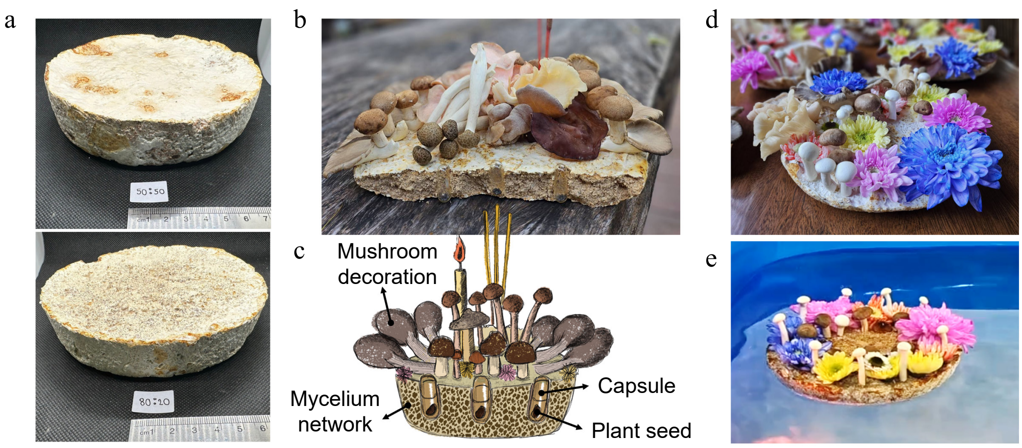

Fig. 5 (a) Photographs of mycelium-based Krathongs (MBKs) made from 50:50 and 80:20 composites. (b) Cross-sectional illustration of a mycelium Krathong. (c) Sketch representation of the Krathong structure. (d), (e) Mycelium Krathongs used during the 2024 Loy Krathong Sai festival.

seeding capsules (Fig. 5b), allowing the Krathongs to contribute to ecological restoration post-festival. The placement of the seeding capsules within the MBK structure is illustrated in Fig. 5c.

The mycelial network formed a rigid base capable of supporting decorative elements such as flowers and mushrooms, maintaining both aesthetic appeal and structural integrity. These MBKs were featured in the 2024 Loy Krathong Sai festival in Tak, Thailand (Fig. 5d), where they successfully floated on the river, as shown in Fig. 5e. The Royal Forest Department facilitated a controlled floating area to collect used Krathongs for post-festival composting and reforestation efforts. This initiative highlights the potential of MBKs as a sustainable alternative to conventional Krathongs, combining cultural traditions with environmental stewardship.

Degradation and seed germination of Mycelium-Based Krathong (MBK) composites

To evaluate the seed germination potential and degradation behavior of the mycelium-based Krathongs (MBKs), both the 50:50 and 80:20 composites were submerged in water overnight to simulate festival conditions before being transferred to soil for further observation. Figure 6a shows the weight loss of 50:50 and 80:20 MBKs after performing the degradation test by being buried in soil. At 28 d in the soil, both 50:50 and 80:20 MBKs showed significant

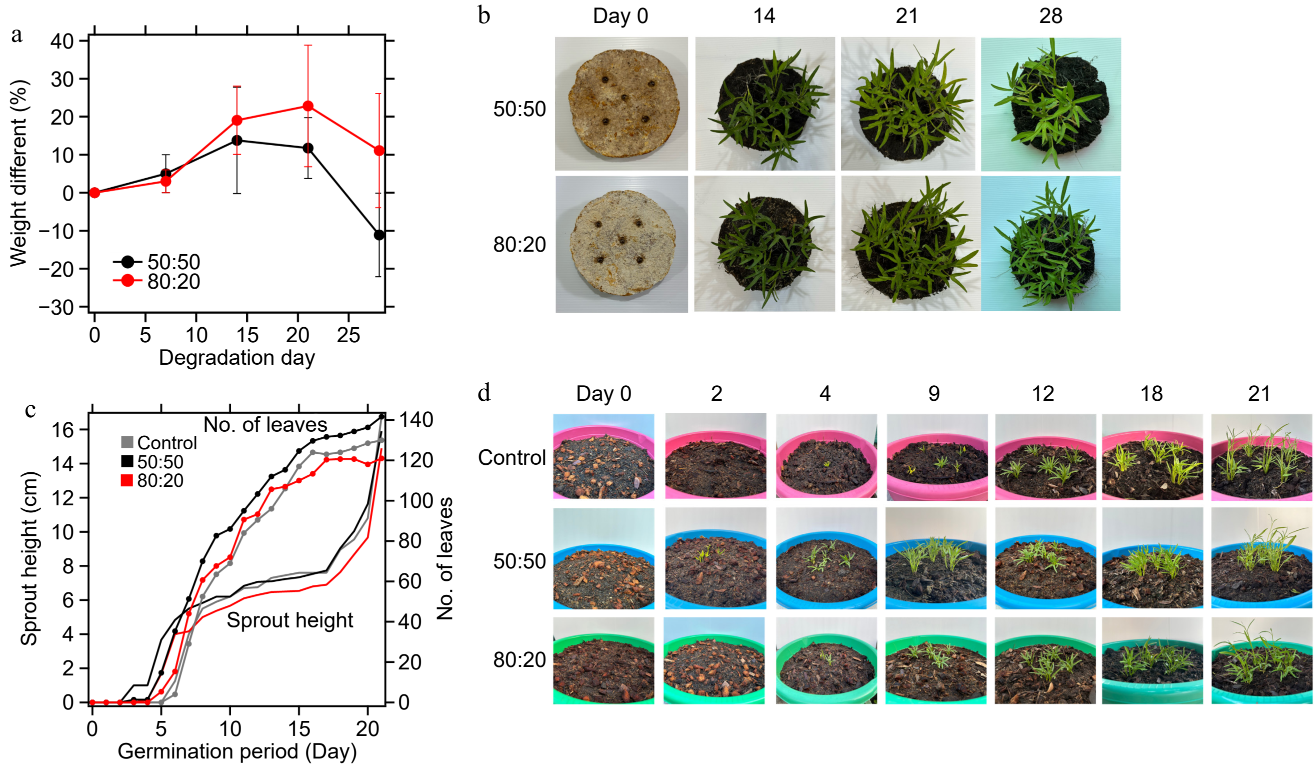

Fig. 6 (a) Mass reduction of mycelium-based Krathongs (MBKs) over a 28 d degradation test. (b) Photographic documentation of MBK degradation at different time intervals. (c) Growth rate of morning glory (*Ipomoea aquatica*) seeds embedded in MBKs after being transferred to soil. (d) Images showing seed germination and plant development in MBKs over time.

structural breakdown, with a recorded mass loss of 100% and 50%, compared to the maximum weight on day 21. The increase in weight of the composites was observed for both 50:50 and 80:20 MBKs until day 21, which was due to the weight of germination seeds and the incorporation of soil microorganisms into the composites. However, the 50:50 MBK showed a dramatic drop in weight during the 28 d periods, which was caused by higher porosity and water retention of 50:50 MBK compared to 80:20 MBK. Conversely, the higher sawdust content in the 80:20 composite resulted in a denser structure, providing greater resistance to biodegradation. These findings highlight the trade-off between durability during the festival and post-use environmental breakdown. Moreover, the higher content of chitin in 80:20 MBK also resulted in the higher hydrophobicity on the surface, which directly influenced the slower degradation by microorganisms. The photographs in Fig. 6b also confirmed the generation of roots and sprouts during MBKs degradation. Since the structure of MBKs were held by plant roots, it ensured the role of MBK to support the growth of the plant during degradation.

Figure 6c shows the sprout height and number of leaves for a 21 d period. The pictures of seed germination were also illustrated in Fig. 6d. Seeds embedded in the 50:50 MBK began germinating within 2 d after soil transfer, achieving a germination rate of 92.0% ± 6.9% by day 21. The higher water retention of the 50:50 matrix likely contributed to faster hydration and seed activation. In contrast, seeds in the 80:20 MBK germinated more gradually, with the first sprouts observed on day 4, reaching a final germination rate of 81.3% ± 6.1% by day 21. Plant growth metrics further supported the impact of substrate composition. Seedlings from the 50:50 MBK exhibited faster shoot elongation as well as promoted more leaves than the 80:20 MBK. Similarly, shoot heights were higher in the 50:50 MBK, likely due to the composite's higher moisture content and faster degradation, which enriched the soil with nutrients from the decomposing biomass.

Discussion

This study demonstrates the feasibility of utilizing lignocellulosic sawdust and water hyacinth as substrates for developing mycelium-based Krathongs (MBKs), providing an eco-friendly alternative to conventional, non-biodegradable floating offerings commonly used during Thailand's Loy Krathong Festival. By incorporating *Pleurotus pulmonarius* mycelium with varying substrate ratios (50:50 and 80:20), this research investigates the physical, mechanical, and biological properties of MBKs, focusing on biodegradability, seed germination potential, and structural performance.

Valorization of agricultural waste for sustainable materials

The use of lignocellulosic agricultural by-products such as sawdust and water hyacinth aligns with circular economy principles, promoting waste valorization and reducing environmental impact[34,35]. Both sawdust and water hyacinth are abundant yet underutilized biomass sources in Thailand[36−38]. Water hyacinth (*Eichhornia crassipes*) is an invasive aquatic plant that threatens native ecosystems and water quality[39]. Converting this problematic biomass into value-added products not only addresses its overgrowth but also contributes to ecosystem management[40,41].

Sawdust, a by-product of the timber industry, offers a rich lignocellulosic matrix conducive to mycelial growth[11,42]. On the other hand, using water hyacinth proved to promote macro-pore structure in the composite, as the density was lower for a 50:50 composite at 0.30 g/cm^3, compared to an 80:20 condition at 0.35 g/cm^3. Apart from the density, the blending of sawdust and water hyacinth in varying ratios provided a comparative framework to assess how substrate composition affects material performance. The 50:50 composite yielded a lightweight, porous structure with enhanced biodegradability, while the 80:20 composite displayed greater structural rigidity and hydrophobicity, reflecting the influence of substrate composition[43].

Mycelial growth dynamics and composite structure

The growth dynamics of *P. pulmonarius* on different substrates revealed that water hyacinth positively influenced initial mycelial proliferation, particularly when supplemented in PDA medium, where the growth rate peaked at 1.86 cm/d. Because the growth of mycelium of *P. pulmonarius* mainly depends upon consuming lignin, cellulose, and hemicellulose in the substrate[44]. According to the microscope images, mycelium in the 80:20 composite showed a tendency to stick to each other and the substrate than in the 50:50 condition. Moreover, mycelium on the 50:50 composite revealed the mycelial collapse, which indicated the lack of cellulose content in the substrate[45]. These different characters of mycelium on 50:50 and 80:20 conditions were also reflected in SEM results. The hypha diameter of mycelium bio-composite derived from the 80:20 condition showed thicker cell wall as well as homogeneity in mycelial diameter. This difference in mycelial morphology was due to the difference in substrate composition, especially cellulose content. While sawdust exhibited approximately 80% of cellulose in its composition, water hyacinth was found to contain much lower cellulose content at 62%[46,47]. Therefore, this study revealed the influence of cellulose in the substrate to facilitate hypha growth and utilization, which correlated with previous reports[48]. This aligns with findings by Peng et al.[45], who reported that the high cellulose and hemicellulose content in corn straw supports fungal metabolism and hyphal extension[45]. However, in composite substrates, the higher water content in the 50:50 formulation resulted in less dense mycelial networks and greater porosity, leading to faster biodegradation post-use. Not only mycelial morphology difference, but the substrate content also dominated mycelial chemical composition. ATR-FTIR analyses confirmed the successful colonization and integration of the fungal matrix within the lignocellulosic mycelium-based composites[49,50]. The presence of characteristic chitin peaks (1,622 cm^{-1} for C=O and 1,317 cm^{-1} for amide III) indicated the formation of robust mycelial networks, particularly in the 80:20 composite, which exhibited stronger chitin signals[51]. This suggests that higher sawdust content enhances the development of chitinous structures, contributing to mechanical strength[52,53]. SEM imaging further revealed the morphological differences between the composites[50]. The 50:50 composite showed loosely structured, irregular mycelial fibers, while the 80:20 composite demonstrated more homogeneous mycelial penetration, leading to smoother, denser fibers. These structural differences and the higher chitin content directly influenced the mechanical and waterproof properties of the composites[54].

Mechanical and physical properties: balancing buoyancy and durability

Buoyancy is a fundamental requirement for Krathongs to ensure they can float effectively during the festival. Both the 50:50 and 80:20 sawdust-water hyacinth composites demonstrated low densities, ranging from 0.30 to 0.35 g/cm^3. These numbers were close to the previous reported of the mycelium composite made from sawdust, rice straw, flax, wheat straw, which their densities ranged from 0.094 to 0.350 g/cm^3[45,55,56]. Even though the observed densities are still higher than those of expanded polystyrene foam (0.01-0.04 g/cm^3), which is the traditional petroleum-based material for making Krathong, MBKs exhibited a significant density below the density of water (1.0 g/cm^3), thus guaranteeing their buoyancy[57]. The 50:50 composite exhibited a lower density and higher porosity, favouring floatation and faster degradation, whereas the 80:20 composite, with its denser matrix, offered enhanced durability.

Compressive strength is another critical parameter, particularly for mycelium-based composites used in applications like packaging or construction, where long-term structural integrity is essential. However, for Krathongs, which are designed for short-term use—floating for a night during the festival before being collected for decomposition—extreme mechanical strength is less critical. In this study, compressive strength tests revealed marginal differences between the two composites, with the 50:50 composite achieving 6.18 MPa and the 80:20 composite reaching 5.83 MPa. Notably, these values exceed those reported for sawdust-only mycelium-based composites[11], indicating that incorporating water hyacinth may enhance the mechanical properties.

Despite the slight advantage in compressive strength, the 50:50 composite's higher porosity could make it less resistant to long-term mechanical stress compared to the denser 80:20 composite. López-Nava et al.[58] emphasized that the compressive strength of mycelium-based foams (MBFs) made from wheat stalks and *Pleurotus* species generally falls below that of synthetic polymer foams of similar density. They highlighted that water absorption significantly affects compressive strength, as both the substrate and the mycelium readily absorb moisture. This observation was consistent with the current findings, where water absorption influenced the mechanical performance of the MBKs, particularly in the 50:50 composite with its higher porosity.

Overall, both composites demonstrated sufficient strength and buoyancy for their intended purpose, with the 80:20 composite offering slightly better structural durability, while the 50:50 composite provided faster biodegradability—an essential factor for post-festival environmental integration.

Water contact angle measurements underscored significant differences in hydrophobicity[59,60]. The 80:20 composite exhibited a contact angle of 127.13°, classifying it as hydrophobic, while the 50:50 composite was hydrophilic (70.73°). The hydrophobic effect caused by the higher chitin content of mycelium in the 80:20 composite, which promoted hydrophobicity[61]. In addition, the smoother surface of 80:20 MBK also created a surface microbubble layer, which acted as a thin barrier between water and the MBK surface[62]. However, the rougher surface and lower mycelial density of 50:50 MBK supported water penetration. Since hydrophobicity is crucial for maintaining structural integrity during flotation, the 80:20 MBK therefore offers extended durability during the festival. On the other hand, the 50:50 MBK supports quicker environmental reintegration post-use due to its higher water absorption rate.

The physical properties, such as compressive modulus, water contact angle, and water absorption of MBKs, were compared with the traditional Styrofoam Krathong. In general, the compressive modulus of commercial Styrofoam ranges from 4.7 to 5.2 MPa[63]. This value is comparable with MBK, which showed slightly stronger than Styrofoam at 5.83 and 6.18 MPa. Moreover, the water contact angle of Styrofoam was reported to be 92.9°, which indicates hydrophobicity of the surface[64]. The water contact angle of 50:50 MBK was 70.73° and 127.13° for 80:20 MBK. Therefore, the surface property of MBKs were tuneable from hydrophilic to highly hydrophobic. The 80:20 MBK exhibits a more hydrophobic surface compared to Styrofoam, thus it can efficiently block water absorption and is suitable for buoyancy. Lastly, the water absorption of Styrofoam is very low. It absorbs less than 5 wt% once soaked in water for a year[65]. However, this property leads to the very slow degradation of Styrofoam, which negatively affects nature. This point is the ultimate drawback for the traditional Krathong.

Although MBKs could absorb water up to twice their original weight, they remain floating on the water surface. This property is good enough for being used as a Krathong. Moreover, the higher water absorption promotes chemical and biodegradation of MBKs.

Biodegradability and seed germination: ecosystem integration

Post-festival biodegradability is a key environmental concern. Even though both 50:50 and 80:20 MBKs showed significantly degraded at 21 d, the 50:50 MBK showed the faster degradation than 80:50 MBKs according to their dramatic weight drop in soil burial tests. This is proved to be faster than the known biodegradability rates of water hyacinth-based composites, which varied from 22 to 121 d[66], and highlights the role of increased porosity in accelerating microbial decomposition[67]. At the beginning of the decomposition period, weights of both 50:50 and 80:20 MBKs were increased due to the germination of seeds and colonization of soil microbes within the composite[68]. Once the seeds were completely germinated, the rooting network also embraced the original structure of MBKs. Therefore, MBK was truly the growth support material as well as a source of nutrients for the sprouts. According to this reason, the degradation of MBK was synchronized with plant growth and could not be degraded in a short time.

The incorporation of morning glory seeds into the MBK structure not only supports cultural aesthetics but also enhances post-festival ecosystem recovery. Seed germination rates were higher in the 50:50 composite (92.0% ± 6.9%), facilitated by its higher water retention and faster degradation, which enriched the surrounding soil. In contrast, the 80:20 MBK exhibited a slower germination rate (81.3% ± 6.1%) due to its reduced porosity and slower degradation. Moreover, compared to the control condition in which the seeds were directly buried in the nourished soil, 50:50 MBK was able to promote a better germination rate, higher sprouts, and a greater number of leaves. Therefore, it proved the efficiency of MBK to facilitate plant growth. This design effectively transforms the Krathongs into vehicles for ecological restoration, where floating offerings become agents of vegetation growth once they settle on land. This aligns with studies on seed-embedded biodegradable materials, which highlight the importance of substrate porosity and moisture retention for seed germination. Furthermore, the integration of biodegradable composites with embedded seeds offers a dual function—cultural expression and ecological benefit—thereby closing the material lifecycle loop.

Cultural Relevance and Environmental Implications

By replacing Styrofoam, plastic, and bread-based Krathongs, which often lead to water pollution and ecosystem disruption, the developed MBKs provide a biodegradable alternative that aligns with Thailand's sustainability goals. The floating Krathongs, which traditionally symbolize the release of negativity and the start of new beginnings, now carry the added symbolism of ecological renewal. The field application during the 2024 Loy Krathong Sai Festival in Tak demonstrated public acceptance of MBKs, as well as their practical performance. The controlled collection and soil transfer of used Krathongs by the Royal Forest Department exemplify an integrated approach to waste management, transforming a cultural event into an environmental restoration opportunity (www.biotec.or.th/home/seeding-rebirth-at-tak, accessed February 21, 2025).

Future Perspectives and Limitations

While this study presents a promising solution, several challenges remain. Scalability of MBK production depends on the availability of raw materials and the optimization of mycelial growth rates[69,70]. Further research could explore the incorporation of other fast-growing fungal species or additional waste materials (e.g., rice straw or corn husks) to diversify substrate sources[9]. Additionally, field studies evaluating long-term environmental impacts, such as the effects of MBK degradation on soil nutrient cycles and local biodiversity, would strengthen the ecological case for widespread adoption. There is also potential for design innovation; integrating local plant seeds for site-specific reforestation, or developing MBKs tailored for different water bodies (e.g., rivers, lakes, and ponds) with varying flow rates and depths. By integrating cultural practices with ecological restoration, MBKs offer a model for sustainable festival materials that actively contribute to environmental stewardship. Future work could explore the use of alternative plant species for seeding and assess long-term impacts on local ecosystems post-degradation.

Conclusions

This study successfully demonstrates the valorization of sawdust and water hyacinth into mycelium-based Krathongs (MBKs), presenting an eco-conscious solution that harmonizes cultural tradition with environmental sustainability. By integrating *Ipomoea aquatica* seeds within biodegradable substrates, MBKs go beyond minimizing aquatic pollution—they actively contribute to post-festival ecosystem restoration, transforming traditional offerings into biodegradable plant carriers that enhance riparian vegetation. The study further highlights a key trade-off between durability and biodegradability, where the 50:50 MBK formulation supports rapid degradation and seed germination, making it ideal for quick ecological reintegration, while the 80:20 MBK offers greater structural integrity and hydrophobicity, ensuring prolonged floatation and resilience in aquatic environments.

Beyond their role in sustainable festival materials, MBKs represent a broader shift toward regenerative biomaterials, demonstrating how mycelium-based composites can be designed for environmental stewardship. The ability to transform agricultural waste into functional, nature-integrated products underscores the potential for expanding this concept to biodegradable floating platforms, eco-packaging, or even water remediation applications. Moving forward, scaling up MBK production will require process optimization, raw material diversification, and strategic collaboration with environmental organizations and local communities. With continued innovation and public engagement, MBKs could become a flagship model for eco-friendly festival practices, inspiring similar efforts worldwide and reinforcing the role of biomaterials in closing the loop between human traditions and nature's resilience.

Author contributions

The authors confirm contributions to the paper as follows: Writing - original draft preparation: Boonyuen N, Wattanavichean N; validation: Boonyuen N, Sangkawanna Supatsorn , Aiemtanakul S, Sangkawanna Supatsara, Koedrith P, Hu Y, Wattanavichean N; supervision, conceptualization: Boonyuen N, Wattanavichean N; resources: Sangkawanna Supatsorn, Aiemtanakul S, Sangkawanna Supatsara, Chandrapatya P, Promfai I, Wattanavichean N; writing - editing: Sangkawanna Supatsorn, Aiemtanakul S, Sangkawanna Supatsara, Koedrith P, Wattanavichean N; methodology, formal analysis, data curation: Sangkawanna Supatsorn, Aiemtanakul S, Sangkawanna Supatsara, Wattanavichean N; project administration, writing - revision: Wattanavichean N. All authors have read and agreed to the published version of the manuscript.

Data availability

The data supporting this study's findings are available from the corresponding authors upon reasonable request.

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Conflict of interest

The authors declare that they have no conflict of interest.

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