Suitability of Simulated Lunar Regolith (TLS-01) for Agriculture

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Abstract
With the advancement of space exploration, scientists have been considering the use of lunar soil as a starting material for agriculture in future moon colonies to ensure food security. Here, Thailand artificial lunar regolith simulant (TLS-01), developed by Space Zab Co., Ltd., was tested for agricultural suitability using sunflower (Helianthus annuus L.) as a test plant. It was found that TLS-01 soil can support plant growth, although it has characteristics that are not suitable. The physical and chemical properties of the TLS-01 were improved by incorporating humic acid microcapsules and coconut coir. The optimal ratio of TLS-01 soil to coconut coir of 1:1 with the addition of double the recommended rate of humic acid was found to result in germination percentage and growth index of sunflower seedlings of 85.00% and 6.62, respectively, which was comparable to results for agricultural soil. Moreover, the release of humic acid from microcapsules using whey protein isolate (WPI) and gum arabic (GA) as encapsulation materials showed conformity to the Fickian diffusion model. The efficiency of WPI and GA as encapsulation materials was found to be 90.37% and 88.36%, respectively.

Keywords: *Simulated Lunar Soil (TLS-01), Sunflower Seedlings, Microcapsule, Coconut Coir, Humic Substances*

I. INTRODUCTION

Over the past years, there have been numerous studies conducted on plant cultivation in space stations. De Pascale et al. (2021) explored the challenges and opportunities of plant production in space environments and found that "microgreen" plants from the Brassicaceae family and sunflowers, which grow rapidly and develop leaves within 1-2 weeks, are suitable for space agriculture. Considering the high costs of transporting resources to space, using lunar soil has been considered as a starting material for astroculture (agriculture on celestial bodies). Chang & Ann (2019) investigated the chemical composition of lunar soil samples collected from the Apollo missions and found the potential for using lunar soil in plant cultivation. Recognizing the significance of this idea, international space organizations have developed their own lunar regolith simulants for studying plant cultivation on the Moon. For example, Japan has developed the JSC-1A lunar regolith simulant, while Thailand has developed the TLS-01 lunar regolith simulant, produced by Space Zab Co., Ltd. Sunflower (Helianthus annuus L.) is a popular fresh vegetable that contains a range of valuable nutrients.

The researchers conducted experiments using TLS-01 soil to cultivate sunflower seedlings. It was found that the plants could grow, but their quality was incomplete due to the physical characteristics of the soil. The TLS-01 soil had a cement-like powdery texture that compacted tightly when mixed with water, resulting in poor water drainage. Additionally, the soil had a relatively low quantity of essential nutrients for plant growth. To address these issues, various methods were considered to improve the TLS-01. For example, Wamelink et al. (2014) added gypsum ($CaSO4$) to lunar regolith simulant to cultivate tomatoes, while Kasiviswanathan et al. (2022) incorporated the cyanobacterium Synechococcus sp. strain PCC 7002 into lunar regolith simulant to grow lettuce, radishes, and alfalfa sprouts. The researchers considered the convenience of transportation in the space industry and application possibilities, leading them to develop humic acid microcapsules using encapsulation techniques and to use coconut coir as an additive. Coconut coir is an agricultural byproduct that is lightweight and can be used as a substitute for imported planting materials, reducing production costs. This serves as the basis for improving the quality of lunar regolith simulants both physically and chemically, making them suitable for cultivating sunflower seedlings to provide a fresh food source for astronauts.

II. METHODS

Characterizing the Properties of TLS-01

Samples of Thailand Artificial Regolith Simulant (TLS-01) were coated with metal powder using a Hitachi E102 Ion Sputter apparatus. Subsequently, the coated samples were analyzed for morphology and particle size comparison using a scanning electron microscope (SEM). The TLS-01 soil samples were analyzed for quantity and distribution of chemical components and properties relevant to plant growth using an Energy Dispersive X-ray Spectrometer (EDX).

Improving the Properties of TLS-01

The experiment was conducted as a 6 x 3 Factorial Experiment in a Completely Randomized Design (CRD) comprising two factors. Factor 1 consisted of five types of planting materials: 1) TLS-01 soil, 2) coconut coir, 3) sandy soil, 4) agricultural soil, 5) a mixture of TLS-01 soil and coconut coir in a 1:1 ratio, and 6) a mixture of TLS-01 soil and sand in a 1:1 ratio. Factor 2 consisted of three levels of humic acid concentration: 1) recommended concentration (1 ml humic acid per 1 liter of water), 2) concentration of twice the recommended rate, and 3) concentration of three times the recommended rate. The seedling trays were covered and each seed watered daily with 20 ml of the humic acid solutions. The experimental design details are presented in Table 1.

The following growth parameters were assessed: 1) stem length, 2) root length, 3) leaf area, and 4) fresh weight of the produce. Subsequently, the growth parameter index was calculated using the formula: Growth parameter = (stem length + root length + leaf area + fresh weight of the plant) / 4. (Chuaboon et al., 2008)

Comparing TLS-01 to Agricultural Soil

The average stem length, average leaf length, and average root length of the sunflower seedlings were measured using ImageJ Fiji software. The number of germinated seeds in each planting box for each day was recorded to calculate the percentage of germination for the first count at 5 days old and the final count at 10 days old. The fresh weight of the sunflower seedlings was measured using a mass balance. Dry weight was measured by oven-drying the seedlings to remove all moisture, and then weighing them.

The sunflower seedling samples were ground to a consistent texture, then weighed samples were placed in conical tubes for analysis of nutrient content using Microwave Plasma Atomic Emission Spectroscopy (MP-AES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The samples were digested by adding HNO_3 and H_2O_2, then the solution was transferred to the MAXI-44 microwave digestion vessel rotor for sample digestion using a microwave digestion system. The resulting sample solutions were diluted and the concentrations of Fe

Code	Treatment details[1/]
T1	TLS-01 + Humic Acid at recommended concentration
T2	TLS-01 + Humic Acid at 2X rec. conc.
T3	TLS-01 + Humic Acid at 3X rec. conc.
T4	Coir + Humic Acid at rec. conc.
T5	Coir + Humic Acid at 2X rec. conc.
T6	Coir + Humic Acid at 3X rec. conc.
T7	Sand + Humic Acid at rec. conc.
T8	Sand + Humic Acid at 2X rec. conc.
T9	Sand + Humic Acid at 3X rec. conc.
T10	Agric. Soil + Humic Acid at rec. conc.
T11	Agric. Soil + Humic Acid at 2X rec. conc.
T12	Agric. Soil + Humic Acid at 3X rec. conc.
T13	TLS-01:Coir (1:1) + Humic Acid at rec. conc.
T14	TLS-01:Coir (1:1) + Humic Acid at 2X rec. conc.
T15	TLS-01:Coir (1:1) + Humic Acid at 3X rec. conc.
T16	TLS-01:Sand (1:1) + Humic Acid at rec. conc.
T17	TLS-01:Sand (1:1) + Humic Acid at 2X rec. conc.
T18	TLS-01:Sand (1:1) + Humic Acid at 3X rec. conc.

Table 1. Experimental conditions. [1/] Liquid humic acid, Golden Seal brand) recommended concentration (1 ml of humic acid per 1 liter of water)

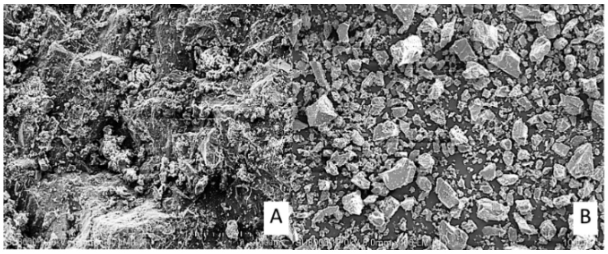

Figure 1. SEM image at magnification X400 of TLS-01 before (A) and after (B) grinding.

and P were measured using MP-AES, while the concentrations of Na, Mg, Al, K, Ca, Cr, Mn, Ni, Cu, Zn, As, Sr, Ag, Cd, and Pb were measured using ICP-MS.

Humic Acid Microcapsules and the Effect of Concentration on Seedling Growth

The microcapsules were prepared through encapsulation using three types of polymers: polyvinyl alcohol (PVA), gum arabic (GA), and whey protein isolate (WPI). Each polymer was dissolved in DI water and left overnight. Tween 80 was added to the solution as a surfactant. Humic acid was mixed with DI water and left for 30 minutes. The polymer solution and humic acid were then mixed in a ratio of 1:2 (v/w) and dried using a freeze dryer at -20°C.

The physical characteristics of the microcapsules, such as color, odor, texture, and visible external features, were examined with the naked eye and a microscope at 40X magnification. The microcapsules' morphological properties were analyzed using a JEOL JCM-6000 scanning electron microscope (SEM) at 200X magnification (Pakthongchai, 2020).

Humic acid was placed in cellophane bags with a solution of Tween 80 at a concentration of 0.1 M. The solution was agitated for different time intervals, and the absorbance at a wavelength of 254 nm was measured to determine the release of humic substances using the Ritger-Peppas equation (Pakthongchai, 2020).

The humic acid was extracted from the microcapsules by filtering through #1 filter paper. The filtered substance was then dried using a rotary evaporator. The total amount of extracted humic acid and the encapsulation efficiency was calculated by mixing the extracted humic acid with

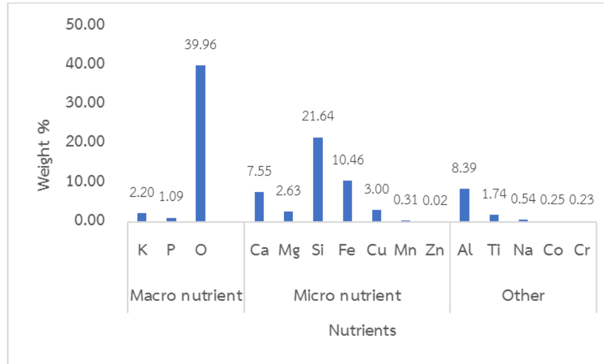

Figure 2. Chemical analysis of TLS-01from Energy Dispersive X-ray Spectrometer.

dichloromethane and methanol, according to Pakthongchai's method (2020).

A completely randomized design (CRD) experiment plan was implemented, consisting of two factors: 1) the type of growth substrate, composed of TLS-01 soil: coconut coir (1:1), and 2) the quantity of humic acid, including 1) the recommended concentration, 2) concentration two times higher than the recommended rate, and 3) concentration three times higher than the recommended rate. The experiment includes 7 treatments, each repeated 6 times with 50 seeds each (Table 6). Growth parameters were measured and analyzed to evaluate the growth of sunflower seedlings under the different conditions.

III. RESULTS AND DISCUSSION

Characterizing the Properties of TLS-01

The analysis of the shape and particle size distribution of TLS-01 using SEM showed that the particles were uniformly dispersed and not clustered (Figure 1), similar to the particle size of simulated lunar soil JSC-1A and lunar soil samples from the Apollo and Luna projects (Neves et al., 2020; Goulas et al., 2019). The particle size of TLS-01 used for cultivating sunflower seedlings in this study was similar to previous research findings. The particle size is related to the soil density, and it was found that the soil density before grinding and after grinding is 1.72 and 2.30 g/cm^3, respectively. Huang et al. (2013) found that particle size significantly affects the growth of sunflower seedlings and can have a significant impact on both mineral elements and physical properties of the soil. Additionally, it did not exhibit as high a water-holding capacity as agricultural soil. These physical characteristics indicate that the physical properties of TLS-01 soil are not suitable for plant growth. The analysis of the shape and particle size distribution revealed that TLS-01 soil has similar physical characteristics to other lunar soils but is not suitable for plant growth. Chemical analysis of TLS-01 soil revealed that it consists of 15 elements, including essential macronutrients and 2 micronutrients for plant growth (Figure 2). Additionally, chemical properties related to plant growth were analyzed, as shown in Table 2. The results of the analysis indicated that TLS-01 soil contains sufficient quantities of essential nutrients for plant growth, particularly a high exchangeable potassium content. These values serve as indicators of soil fertility for cultivation. The pH value of the soil, which is desirable for sunflower seedlings, should be within the range of 5.5 to 6.5. The measured pH value indicates that TLS-01 soil is suitable for cultivating sunflower seedlings (Chinsaen et al., 2018; Siri-Ngam, 2014)

Soil Properties	Analysis Value	Interpretation*
1. pH level	5.6	Moderately acidic
2. Electrical Conductivity	0.09 dS/m	Non-saline, non-hazardous to plants
3. Organic Matter Content	2.25%	Moderate
4. Total Nitrogen Content	1.17%	Moderate
5. Beneficial Phosphorus Content	18.8 mg/kg	Moderate
6. Exchangeable Potassium Content	207 mg/kg	Very high
7. Exchangeable Calcium Content	388 mg/kg	Moderate

Table 2. Results of chemical analysis related to plant growth. **(**Department of Land Development, (2010); Faculty of the Department of Soil Science, (1998))

Soil Type	Ave. Leaf Mass (g)	Ave. Stem Mass (g)	Ave. Total Mass (g)
TLS-01	0.230	0.222	0.452
Mixed	0.216	0.193	0.409
Agricult. Soil	0.363	0.345	0.707

Table 3. The fresh average mass of leaves, stems, and total of sunflower seedlings cultivated in different soil types.

Soil Type	Ave. Leaf Mass (g)	Ave. Stem Mass (g)	Ave. Total Mass (g)
TLS-01	0.036	0.015	0.051
Mixed	0.025	0.019	0.044
Agricult. Soil	0.038	0.037	0.075

Table 4. The dried average mass of leaves, stems, and total of sunflower seedlings cultivated in different soil types.

Comparing TLS-01 to Agricultural Soil

Sunflower seedlings cultivated in agricultural soil have the highest percentage of germination, average plant height, average leaf length, average root length,

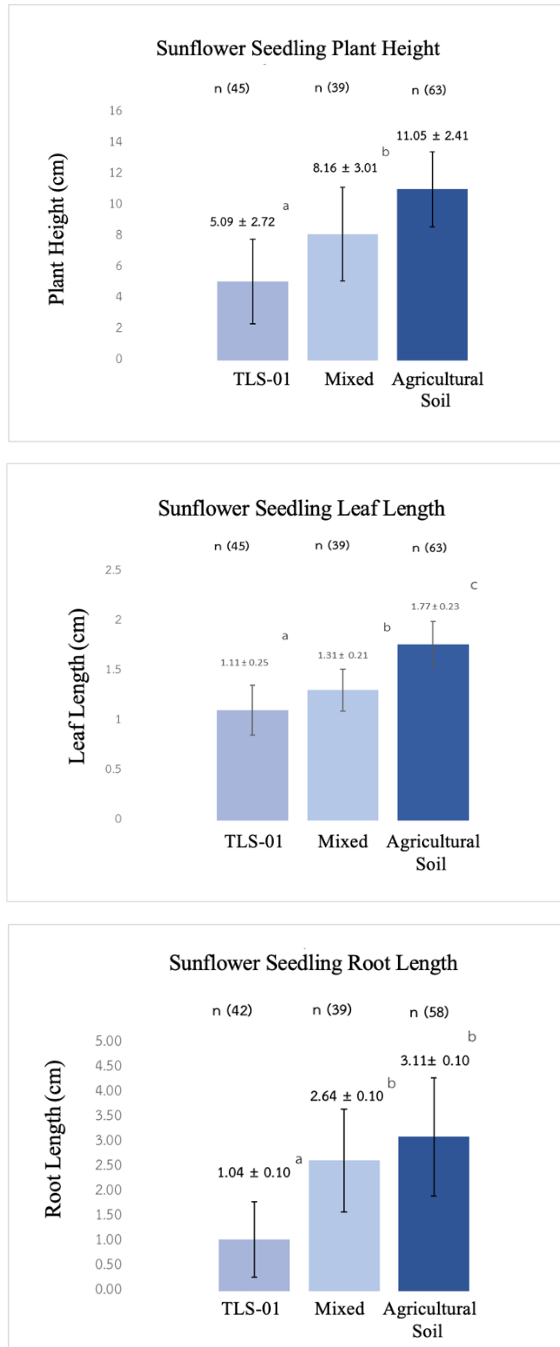

Figure 3. Average plant height, leaf length, and root length of sunflower seedlings for the soil types tested.

and weight (Figure 3, Tables 3 and 4). The subsequent ranking includes sunflower seedlings cultivated in mixed soil and TLS-01. Nanoparticles of SiO_2 in TLS-01 contribute to an increased germination rate and may affect the growth and higher yield of sunflower plants (Sabaghnia et al., 2018; Sabaghnia & Janmohammadi, 2017). However, the effectiveness depends on the concentration of SiO_2 nanoparticles in the cultivating soil (Jurga et al., 2013) and their ability to enhance root and stem elongation. Additionally, the presence of iron (Fe) particles reduces the germination rate of seeds (Verma et al., 2020). In a similar research study by Ilay et al. (2013), sunflower seedlings cultivated in clayey loam soil, which has properties similar to agricultural soil, showed a trend of improved growth over an extended period. Therefore, when sunflower seedlings are cultivated in agricultural soil for a longer duration, they tend to exhibit better growth.

Figure 4. The growth characteristics of sunflower seedlings planted with TLS-01 soil before soil quality improvement (A) compared to agricultural soil (B).

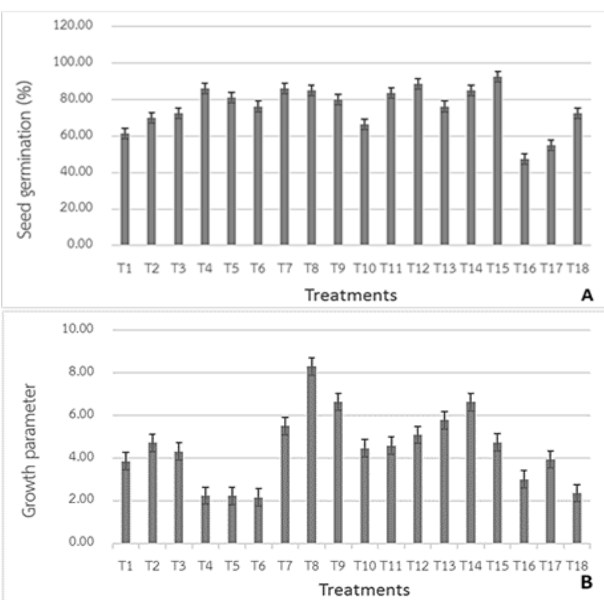

Figure 5. Seed Germination Percentage (A) and Growth Parameter (B) results for the 18 treatment conditions described in Table 1.

Improving the Properties of TLS-01

Sunflower seedlings grown in TLS-01 soil mixed with coconut coir at a ratio of 1:1 and supplemented with a 2-fold concentration of recommended humic acid (recommended rate: 1 ml/1 liter of water) promoted germination percentage and growth index to 85.00% and 6.62, respectively, while agricultural soil had a growth parameter of 8.29 (Figure 5). All conditions were controlled under the same factors, such as light, temperature, water quantity, and the number of seeds sown. After improving the physical and chemical properties of TLS-01, it demonstrated improved characteristics, effectively promoting the growth of sunflower seedlings comparable to agricultural soil ($p \leq 0.05$). Cultivating sunflower seedlings in TLS-01 mixed with coconut coir (1:1) helped reduce the physical limitations of TLS-01 only, such as low water holding capacity and reduced clumping, resulting in increased air-filled porosity of the planting medium. This led to better dissolution and availability of nutrients for plants. Furthermore, the addition of various concentrations of humic acid to the different planting media showed similar effectiveness in promoting the growth of sunflower seedlings, regardless of soil type. The chemical

Soil Type (A)	Concentration of humic substance (B)			Average [2/]
	B1	B2	B3	
1) TLS-01	5.31	6.36	5.88	5.85b
2) Coconut Coir	4.26	3.96	3.94	4.05c
3) Sand	6.82	9.81	7.74	8.12a
4) Agricult. Soil	5.86	6.08	6.01	5.98b
5) TLS-01:Coir (1:1)	7.45	8.54	6.10	7.36a
6) TLS-01:Sand (1:1)	4.33	5.59	3.20	4.37c
Average	5.67	6.73	5.48	
F-Test				
A		*		
B		ns		
AXB		ns		

Table 5. Statistical analysis was performed to determine the correlation between the planting material (A) and the quantity of humic substances. (B) [1/] ns=Not significant, *= Significant at 95% confidence level.

[1/] B1= Recommended Concentration, B2= 2X Recommended Concentration, and B3= 3X Recommended Concentration

[2/] The average values followed by the same letter are not significantly different at $p \leq 0.05$ based on the Duncan's Multiple Range Test (DMRT) when comparing the differences among the types of planting materials.

properties of TLS-01 soil exhibited relatively good essential nutrient components for plant growth, although they were not readily available due to unfavorable physical properties. The supplementation of humic acid facilitated greater nutrient uptake by plants. As a result, plant growth was significantly better compared to pre-improvement TLS-01 soil conditions (Figure 4). Additionally, humic acid enhanced water absorption and soil porosity, while also contributing to the improvement of soil's biological properties as a source of beneficial nutrients for plants and microorganisms (Table 5).

Humic Acid Microcapsules and the Effect of Concentration on Seedling Growth

The physical appearance of microcapsules coated with Gum Arabic (GA) appears as dark brown powder, while microcapsules coated with Whey Protein Isolate (WPI) appear as brown granules (Figure 6). On the other hand, microcapsules coated with PVA have a brown foam-like appearance, which cannot be observed under an electron microscope due to poor molding properties. When examined under a microscope at 4x magnification, it is found that microcapsules coated with WPI and GA have dark brown to black color, resembling separated layers. At 10x magnification, they appear as multi-layered sheets with abnormal shapes. When studied using a scanning electron microscope, microcapsules coated with WPI and GA exhibit physical characteristics of flat sheets with a smooth surface.

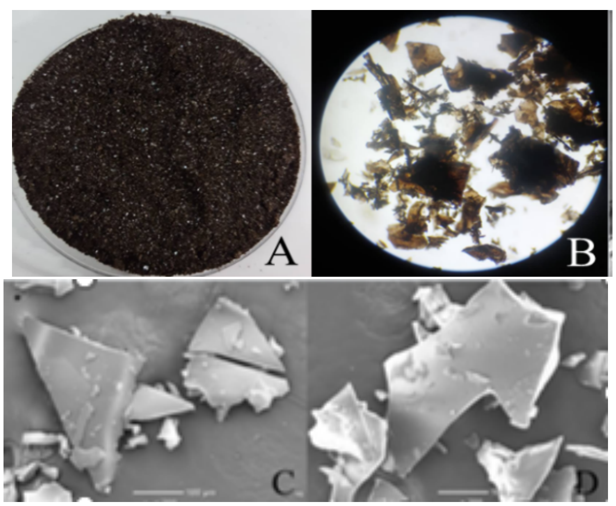

Figure 6. Microcapsules encapsulating humic acid under 4X magnification (A), Microcapsules encapsulating humic acid under a Benchtop SEM at 200x magnification (B), WPI-coated capsules (C) and GA-coated capsules (D).

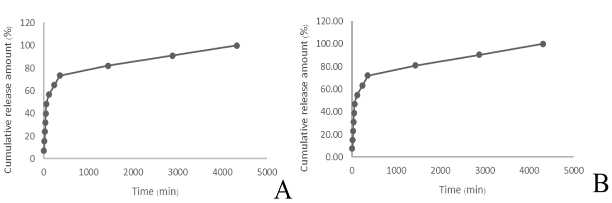

Figure 7. Release of humic acid over time for WPI-coated microcapsules (A) and GA-coated microcapsules (B).

Microcapsules coated with WPI have an R-squared value of 0.6033 and a diffusion exponent value of 0.0027, indicating Fickian diffusion, which is the diffusion from high to low concentration. Within the first 360 minutes, they release 73.50% of the encapsulated humic acid. After that, the release occurs slowly, as shown in Figure 7. As for microcapsules coated with GA, they have an R-squared value of 0.5846 and a diffusion exponent value of 0.0214, also indicating Fickian diffusion. Within the first 360 minutes, they release 72.00% of the encapsulated humic acid, after which the release occurs slowly.

Code	Treatment details [1/]	Germination index [2/]
T1	TLS-01:Coir (1:1) + Humic Acid at 2X rec. conc.	9.49a
T2	TLS-01:Coir (1:1) + WPI Encapsulated Humic Acid at rec. conc.	8.07b
T3	TLS-01:Coir (1:1) + WPI Encapsulated Humic Acid at 2X rec. conc.	8.71ab
T4	TLS-01:Coir (1:1) + WPI Encapsulated Humic Acid at 3X rec. conc.	7.67b
T5	TLS-01:Coir (1:1) + GA Encapsulated Humic Acid at rec. conc.	7.73b
T6	TLS-01:Coir (1:1) + GA Encapsulated Humic Acid at 2X rec. conc.	8.59ab
T7	TLS-01:Coir (1:1) + GA Encapsulated Humic Acid at 3X rec. conc.	8.15b

Table 6. Statistical analysis of germination index using different forms of humic acid microcapsules.

[1/] WPI: Whey Protein Isolate-coated microcapsules. GA: Gum Arabic-coated microcapsules.

[2/] The average values followed by the same letter are not significantly different at $p \leq 0.05$ by Duncan's Multiple Range Test (DMRT) when comparing differences between types of planting materials.

Based on the efficiency testing of microcapsules coated with WPI and GA, it was found that the encapsulation efficiency was 90.40% and 88.30% respectively. This is consistent with the research conducted by Zang, B. et al. (2022) on the encapsulation of capsaicin in whey protein, which stated that GA has the ability to form stable and encapsulated emulsions. Furthermore, it can create a protective layer around the central core, resulting in higher encapsulation efficiency with increasing GA concentration in the coating material. This finding is also in line with the research by Zang et al. (2022) on the fabrication and characterization of whey protein.

In the case of using only WPI as the coating material (comparable to the experimental set of WPI:OS = 10:0 in Zang), the study found that the encapsulation efficiency of microcapsules containing capsaicin from chili using WPI as the coating material ranged from 49.90% to 94.60%. This indicates that the samples in Zang, which had mixtures of WP and OS at weight ratios of 10:0, 9:1, and 7:3, showed significant differences in encapsulation efficiency. Therefore, it indicates that the material has the ability to form excellent coating layers, and WP and OS at weight ratios of 10:0, 9:1, and 7:3 exhibit good interaction.

When improving the cultivation of sunflower seedlings, it was found that using a double concentration of humic acid in the simulated moon soil with coconut fiber at a 1:1 ratio resulted in a maximum germination index of 9.49. The next highest index was achieved using microcapsules coated with WPI with a germination index of 8.71, followed by microcapsules coated with GA with a germination index of 8.59. This shows that the soil quality improvement method mentioned above can alleviate the limitations of TLS-01 soil in terms of physical and chemical properties. By using humic acid microcapsules coated with WPI and GA at double concentration, it promotes the growth of sunflower seedlings, achieving growth rates similar to those obtained using liquid humic acid at double concentration.

IV. CONCLUSION

It was found that sunflower seedlings grown in agricultural soil had the highest germination percentage at 68.00 ± 3.70%, while sunflower seedlings grown in TLS-01 soil had the highest content of organic matter and the highest percentage by weight/volume of Na, Mg, Mn, and Cu elements. Overall, it can be concluded that improving the quality of TLS-01 soil is possible through a relatively simple process. Therefore, enhancing the physical properties and nutrient content of TLS-01 soil for cultivating sunflower seedlings using a combination of coconut coir and humic substances was investigated. It was found that when TLS-01 soil was mixed with coconut coir at a ratio of 1:1 and supplemented with humic substances at 2 times the recommended rate, it promoted germination percentage and growth index comparable to agricultural soil. It also reduced the physical drawbacks of TLS-01 soil, such as low water-holding capacity and clumping.

The study also showed that humic acid in microcapsules, which improves convenience for transportation and ease of use in space, is effective for sunflower cultivation. The results showed that microencapsulated humic acid, with whey protein isolate (WPI) as the encapsulating material at 2 times the recommended concentration, enhanced the germination index of sunflower seedlings close to that of the aqueous humic acid used previously, with 73.50% of the encapsulated humic acid released.
It is possible to apply this approach to modify the properties of lunar soil to make it suitable for cultivation in the future. This could provide fresh food sources for astronauts and contribute to ensuring food security during future space missions or human settlements on the Moon or other planets when the lifespan of our Earth comes to an end.

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