

Sphere Encapsulated Lunar Soil Seed Germination: A Comparative Study of Sodium Alginate Spherification Techniques as a Growth Media Substitute for Loose Lunar Regolith

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Introduction

Reliable agriculture and maintaining life on the Moon are proving to be extremely important in the advancement of space technology, particularly the ability to grow crops in media that are found in space, such as lunar regolith. Research interest was amplified further after 2022, when NASA announced successful plant growth in lunar regolith samples. This advancement inspired research determining which plants grow best in lunar regolith, as well as potential amendments to improve growth. Previously studied methods include seed coatings, pill capsules, and hydrogel blocks, among others, which have been researched and implemented countless times to help address issues with transporting seeds and maintaining them in harsh conditions; however, challenges in water and nutrient retention have remained prevalent. This experiment aims to explore new methods of replacing and carrying growth media for seeds, utilizing methods of spherification to control lunar dust, and allowing for greater water retention. Furthermore, it explores the growth of the nutrient-rich and fast-growing plant, Mizuna Mustard, in the harsh lunar regolith. Mizuna mustard grows within 2-3 months and is commonly used in small salads for a quick, nutrient-dense food source. Exploring the growth of mizuna mustard within a growth media substitute can help to identify if it has potential as a source of food for astronauts.

Background

Lunar regolith is composed of various materials, such as silicones and types of metals, such as iron and aluminum, but it has very few nutrients for vegetation to thrive in. Alternatively, it has been determined that growing plants in lunar regolith is possible, but due to its lack of vital nutrients, the incredibly coarse and rough texture, its poor water retention, and the lack of air circulation, growing vegetation is extremely difficult. (Wieger Wamelink, et. al., 2014) Along with these rough conditions, lunar regolith tends to stick together and form blocks of lunar soil when exposed to moisture from plant watering or environmental conditions. Many experiments aim to compare the lunar regolith with various growth substrates or to add materials to the lunar regolith to improve growth (e.g., fertilizers, coconut coir, and bacteria). With most of these experiments being successful in identifying potential improvements or contributing to research, the issue of water retention and sources of water to the plant are still prominent. To improve water retention, there have been various approaches, including hydrogels. Hydrogels allow for both nutrient and water retention. Indeed, these approaches do help, with the addition of nutrients and water retention, but the components of transportation, efficiency, and longevity for space still stand as a hole in research. This leads the focus to shift onto increasing water retention in harsh conditions, such as lunar soil, without sacrificing the need for sufficient nutrient amounts. Additionally, these approaches do not address the underlying issues with lunar regolith. Loose lunar regolith is also commonly known to damage equipment. It is a material that can be airborne easily, thus allowing it to separate from its pot/container, disrupting growth, and damaging surrounding equipment. However, spherification encapsulates these properties to prevent damage and dust mobility. It is able to contain the lunar regolith particles without allowing them to disperse since they are held in a membrane. In any case, watering plants in space also faces many obstacles, as the water separates from the plants without gravity.

Not only are there issues with the transportation of soil and methods for watering, but the type of plant used within these experiments also plays a crucial role. It needs to be taken into consideration that the plant needs to be fast-growing and highly resilient to harsh conditions for efficacy when grown in space. It also needs to be nutrient-rich and easy to incorporate into various types of meals. An example of this type of crop would be any crop from the mustard family. Crops in the mustard family tend to be nutrient-dense, fast-growing, and also

resilient to harsh conditions. Mustard plants can begin growing within 15-30 days, and can be used as a simple green or incorporated into a mix of greens similar to a salad (Morsi et al., 2024). Mizuna Mustard's tolerance to low-nutrient and low-water environments can be attributed to its shallow, but fibrous roots, which allow the plant to extract nutrients from topsoil levels and allow the plant to thrive in places where the environment is harsh. (Garrido-Ruiz, 2026) Experiments being done with Mizuna Mustard in space conditions helped to form a basis for how Mizuna Mustard grows in lunar soil. Research shows that Mizuna Mustard seeds can be reliable for 2-3 months. (Doughty, 2024). With this in mind, Mizuna is a key component of this experiment and is the optimal plant for the suggested growth setting.

Additionally, transportation and early plant growth stages are two of the key components for growing healthy plants, an example of which is seed coatings. Some seed coatings have used polyacrylate, which is classified as a superabsorbent polymer or SAP, in order to retain water. (Amirkhani, et. al., 2023) Superabsorbent polymers allow for large amounts of water retention, which is extremely important in the case of harsh and infertile soil, such as lunar regolith, which is subject to rapid desiccation in low-humidity environments. It is important to maximize water retention to allow for the most efficient plant growth. Similar to hydrogels, these SAP polymers are used to increase water retention for crops. One of the most effective natural SAP polymers is sodium alginate. Sodium alginate has been largely recognized for its diverse usage, and when incorporated into soil or with various nutrients, it has been shown to improve plant growth in the early stages. Similar to sodium alginate as a seed coating, it can also be used as a coating to encapsulate nutrients to allow for early plant growth and health.

Sodium alginate is often used in Direct and Reverse spherification, which is typically incorporated into food or drug delivery. Reverse spherification consists of submerging a liquid or paste containing calcium chloride(2%) into a bath with a 0.5% sodium alginate mixture, while Direct spherification consists of adding a paste of liquid containing 2% sodium alginate, dropped into a 0.5% calcium chloride bath. (Sen, 2017). Spherification is a method of utilizing sodium alginate and a calcium chloride or calcium lactate bath to form solid or liquid-centered sphere shapes. The sodium alginate, when in contact with the calcium chloride or lactate, forms a solid texture through the replacement of calcium ions. Reverse spherification involves using calcium chloride or calcium lactate and dropping it into a sodium alginate bath. On the contrary, Direct spherification consists of a sodium alginate solution being dropped into a calcium chloride or calcium lactate bath. With these processes, Reverse spherification forms a liquid center while Direct spherification results in a solid center. These methods have been applied in the pharmaceutical field, especially in drug delivery, to provide slow-release of medicines (Pet et al., 2025). Along with the release of nutrients, the outer coating also affects the membrane and sphere itself. A thicker membrane means a slower release of the material inside, while a thinner membrane will dictate a faster release of the material in the center. Thicker membranes also allow for longer water retention, which in turn allows more nutrient retention for the crops being grown. They have also been used in nutraceuticals for the delivery of turmeric (curcumin) supplements and have been seen to be effective as a delivery method (Jagtap, 2025). These benefits can be easily transferred to lunar regolith spheres. Instead of slowly releasing medicines, they slowly release fertilizer, preventing it from running out immediately and allowing it to be used over longer periods of time. Another major benefit of spherification is the ease with which it is handled due to the fact that the medicine or regolith is conveniently encapsulated. Small, free-floating particles are a major liability in zero gravity, but lunar regolith contained in a membrane is not able to disperse and damage or abrade equipment the same way loose regolith would.

Hypothesis

The goal of this experiment is to demonstrate how a new method of growing plants in lunar regolith, Reverse and Direct spherification, can optimally support plant growth in terms of biomass, chlorophyll, nitrogen uptake, and overall plant length. Encapsulating the regolith will create a unique growth environment to combat the challenges posed by the lunar regolith. With the regolith encapsulated, it also indicates a slower release and movement of water, along with longer storage of nutrients. Foremost, regolith tends to form clumps (Jung et al., 2025), and by forming even spheres of regolith, there will be consistent pore spaces. This is essential to plant growth and nutrient intake. Plants are unable to thrive then soil is compacted with no airspace. These pockets of space will give roots better access to oxygen, which is vital to their growth (Arablousabet & Povilaitis, 2024). Moreover, lunar regolith has extremely poor water retention, but the regolith spheres' membrane will hold water in for longer periods of time, as shown through capillary action tests and spherification properties, and will also hold the moisture near the roots. Hence, the regolith spheres are more manageable for astronauts since it contains the regolith rather than allowing it to move freely and damage the equipment.

Encapsulating the lunar regolith provides a unique opportunity to include other materials in the spheres. This experiment will include powder fertilizer containing Nitrogen, Phosphorus, and Potassium in a 14:14:14 ratio (Kratsch, 2010). Because of the previously mentioned benefits, plants grown in regolith spheres will demonstrate more growth and data that support harvesting after the 8-week growth period compared to plants grown in loose regolith with the same additives. Between Reverse and Direct spherification, Reverse spherification will likely be more effective because it tends to yield thicker and longer-lasting membranes. It is shown through the capillary action test that the Reverse spherification spheres, when dehydrated, will soak up the water better and rehydrate more quickly due to the thicker membrane. This, in turn, will allow for more water retention but also protect the roots of the plant as it grows out. This can be seen through the final results, with the final bubble structure, plant growth, nitrogen amounts, and chlorophyll. The group hypothesizes that if Reverse spherification of lunar regolith is used to transport mizuna mustard seeds, then the overall growth rate of the plants within that group will perform better because Reverse spherification will allow for a thicker, water-retaining membrane and slower nutrient release.

Experimental Design & Controls

The experiment includes two experimental groups and two control groups, negative and positive. The negative control group consisted of pots with loose lunar regolith and all materials used during the spherification process, but were kept loose and did not go through any spherification processes. This demonstrates whether the difference in growth was caused by the additives or the process of spherification. The positive control group consisted of pots with only conventional clay pellets to house and grow the seeds. Comparing the experimental sodium alginate spheres to clay pellets establishes a baseline to determine the spheres' moisture-retention and nutrient-delivery capabilities compared to a widely accepted hydroponic growth medium (Sarrocco et al., 2004). The first experimental group contained one of the delivery methods for lunar regolith, created through sodium alginate spheres using Direct spherification. The second experimental group had the delivery method of lunar regolith, produced through sodium alginate spheres formed using Reverse spherification. As opposed to the gelled centers of the spheres made using Direct spherification, the spheres made using Reverse spherification have an ungelled liquid center, which is better suited for plant root and shoot expansion (Patel & Madhan, 2021). There were two components for each of the two experimental groups: the mixture and the bath. The ingredients for these components stayed the same between groups, with the exception that sodium alginate was

used in the bath and calcium chloride was used in the bubble mixture to make spheres for the Direct spherification group, and vice versa for the Reverse spherification group. For preliminary testing, a capillary action test was done on dehydrated bubbles to test the water absorption and rehydration rates. This was done in a short period of time, where the spheres can be dehydrated, placed in a tube, and with added water at the bottom to test how the water spreads upwards. The results determine how the watering system will work in a zero-gravity environment. Since the experiment is testing the efficacy of a planting method rather than soil amendments, the loose regolith used in the negative control groups was amended with the same ingredients used to make the spheres for the experimental groups.

All pots were grown in the same environment and area, without any variation. This area was inside a growth tent with the light intensity set to $230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The light intensity, however, was changed from $230 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ to $148.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the start of week 4. There was also a watering schedule that watered each group for 5 minutes every 15 minutes using a water supply and a timed pump. There were 8 pots for each group, with the bottom-center tray containing two pots of the Direct spherification, Reverse spherification, and negative control groups. The Positive Control also ended up using one-third of an empty tray to fulfill the requirement of 8 pots per group. Additionally, each group, Negative Control, Positive Control, Direct, and Reverse, had its own tray of six pots. Each pot was 4.5 cm in height and width, with a base of 4 cm. Each pot was also 100 mL in volume, totaling to 800 mL of volume for each group.

To ensure all pots get equal and adequate water throughout the 7 weeks, an ebb-and-flow hydroponic system was used. To assemble this system, the pots were placed on inverted plastic containers to sit above the water supply, which was poured into the large white tray and cleaned weekly. The ebb and flow system had a timed water pump, placed in the water supply, which turned on and flooded for 5 minutes and turned off for 15 minutes while the water drained. This pump was connected to a tube separator, which sent 2 tubes to each of the 6 trays. Small holes in the trays allowed for draining, while larger holes above them prevented overflowing. This system was cleaned, and the water was replaced every week.

Variables

The independent variable was the method used to grow the seeds (Direct spherification spheres, Reverse spherification spheres, loose regolith, and clay pellets). The dependent variables included a variety of qualitative and quantitative measurements, with some taken throughout the growth period and others taken solely at the end of it. These measurements were plant height (with ImageJ, weekly), nutrient levels, leaf number (recorded weekly), final root length, final biomass, final plant length, and final leaf chlorophyll content.

Materials

The materials used for the creation and set up of lunar regolith spherification methods included: 36 100 mL pots (six count trays containing six pots each), 1872 g lunar regolith simulant, 79.68 g sodium alginate, 38.4 g calcium chloride, 3.456 g powdered 14-14-14 fertilizer, 14.4 g xanthan gum, 392.2 distilled water, 300 g clay pellets, 1 large white tray, 7 flexible plastic tubes, 1 tube separator, 1 water pump, 1 outlet timer, 1 growth tent with adjustable light settings.

Procedure

Control Groups:

To make the negative control group, a mixture of 624 g of lunar regolith simulant, 26.56 g of sodium alginate, 12.8 g of calcium chloride, 4.8 g of xanthan gum, and 379.2 mL of water mixed with 1.152 g of powdered Osmocote Classic 14-14-14 fertilizer (Carolina Biological Supply) was created. A fertilizer ratio of 14-14-14 (nitrogen to phosphorus to potassium) was selected over other ratios to ensure plants have equal access to nitrogen, phosphorus, and potassium. Fertilizer was put into the spheres to supply seedlings with adequate nutrients and allow their roots to enter nearby spheres to access more nutrients (Shaviv, 2001). 100 mL of the mixture was added to each of 8 pots. 3 Mizuna Mustard seeds were planted into each of the 8 pots, 1 cm apart and around 0.5-1 cm deep.

To make the positive control group, 100 mL of clay pellets was added to each of the eight 100 mL pots. 3 Mizuna Mustard seeds were planted into individual clay pellets in each of the 8 pots.

Direct Spherification:

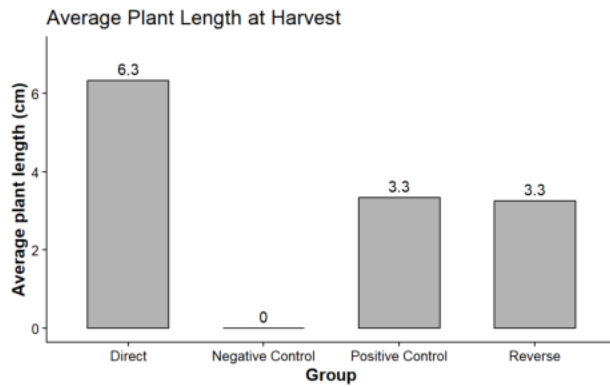
A 2% calcium chloride (CaCl_2) bath to fill 800 mL of a 1000 mL beaker was created by combining 12.8 grams of CaCl_2 with 281.9 mL of water, then mixing until fully combined. In a separate mixture, 26.56g of Sodium Alginate, 4.8g Xanthan Gum, 281.9 mL distilled water, 1.152g of Osmocote Classic 14-14-14 fertilizer (Carolina Biological Supply), and 624g lunar regolith simulant were combined. 2 mL at a time of the regolith mixture was measured out, then placed into the CaCl_2 bath for one minute. Next, they were removed, then left to sit for 1 minute before being rinsed with distilled water for another 1 minute. All of the spheres were removed from the water and placed into the dehydrator. Once they were dehydrated overnight, a small hole was poked in the center of 24 bubbles. In each bubble, a seed was placed inside, and finally, 8 wells were filled with Direct spheres, and 3 of the seed-containing bubbles were placed in each.

Reverse Spherification:

A 2% sodium alginate solution was prepared by mixing 26.56 g of sodium alginate with 800 mL of distilled water on a magnetic stirrer. In a separate mixture, 12.80g of CaCl_2 , 4.8g of Xanthan Gum, 281.9 mL of distilled water, 1.152g of Carolina Osmocote Classic fertilizer, and 624g of lunar regolith simulant were combined. 2mL of this was measured out at a time, then dropped into the sodium alginate solution and left for 1 minute. They were then removed and left to sit for another 1 minute. Then, they were rinsed with distilled water for 1 minute. All of the spheres were removed from the water and placed into the dehydrator. Once they were dehydrated overnight, a small hole was poked in the center of 24 bubbles. In each bubble, a seed was placed inside, and finally, 8 wells were filled with Reverse spheres, and 3 of the seed-containing bubbles were placed in each.

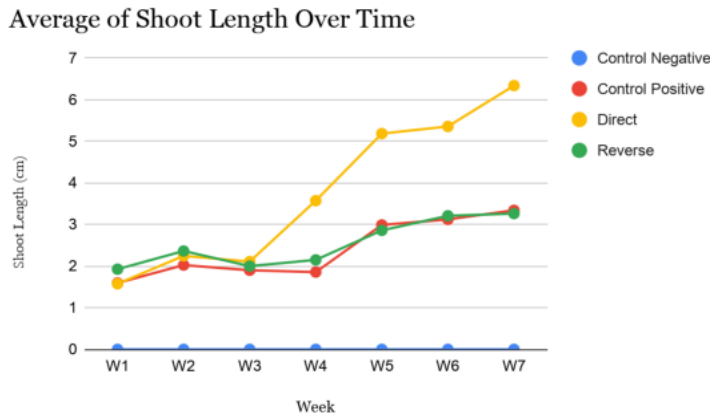
Results

Finalized plant growth was measured across the four experimental groups. Growth and health were evaluated by using the primary variables: shoot length, shoot-to-root ratio, and leaf count.



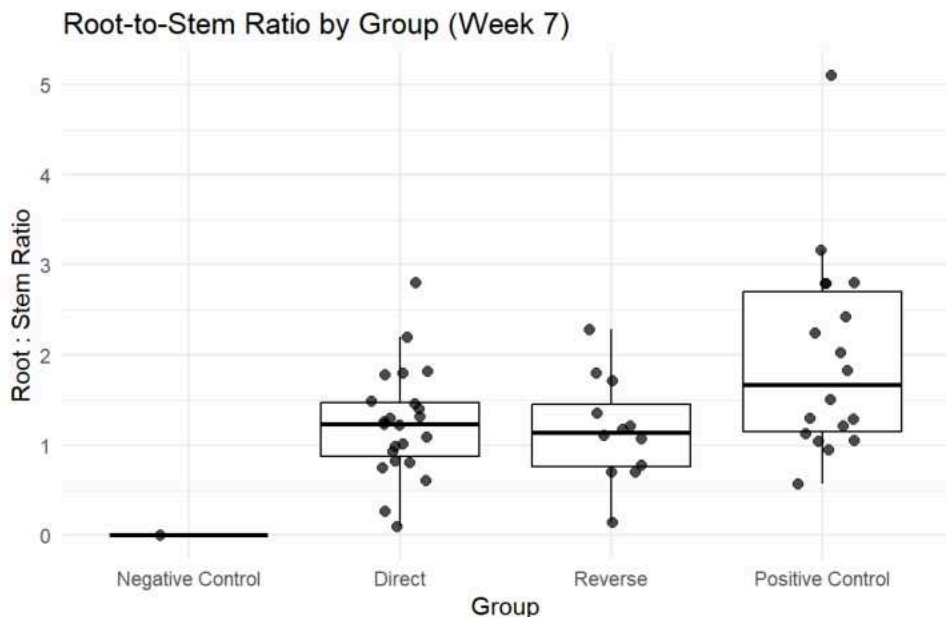
Graph 1: Bar Chart Showcasing the Sum of All Plant Lengths per Group at Harvest. *The average length after seven weeks of growth is measured in centimeters. Groups were measured separately and averaged.*

The average plant length in the final varied. The diameter had an average of 6.34 cm. Plant shoot length for the Positive Control had an average of 3.34 cm, with the Reverse group having 3.26 cm



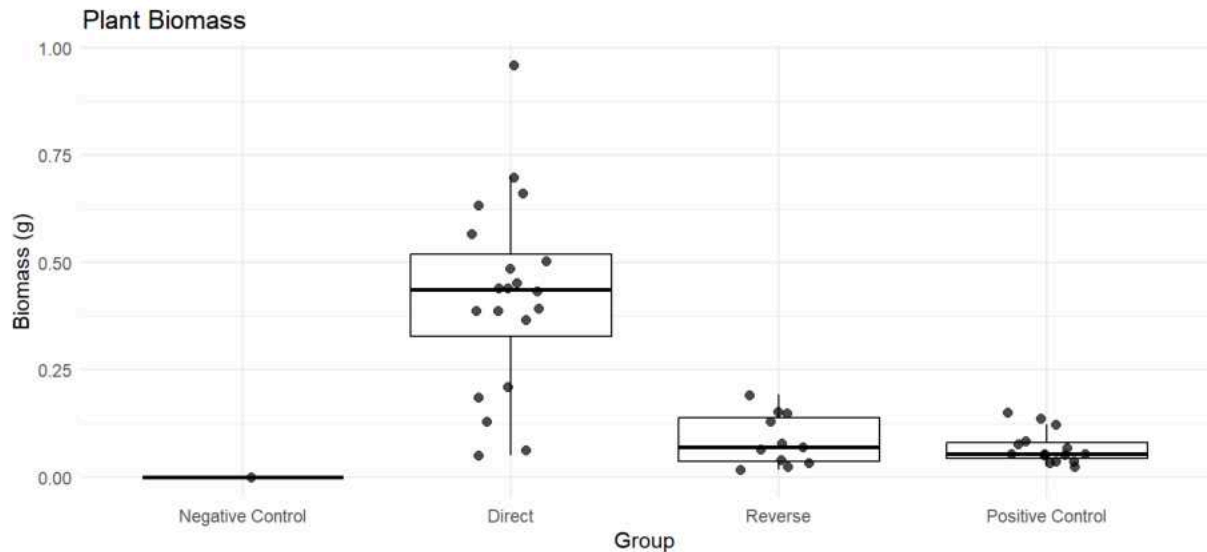
Graph 2: Line Graph Showcasing the Average Length of Shoots Each Week Over Time *Average weekly shoot length for each group over 7 weeks*

During the experiment, data on the shoot length of each group's plants were also recorded weekly. It was tracked for 7 weeks of the 8-week growth period. Negative Control had no growth throughout the whole experiment. Reverse had a decline in week 4 but had an upward trend since then. Direct had a dip in shoot length in week 3, but had a rapid linear growth afterwards. Notably, Positive Control declined in weeks 3 and 4 but gradually rose in length afterwards.



Graph 3: Box Plot Showing the Root-to-Stem Ratio of Each Group *The root-to-stem ratio was calculated by dividing the root length by the shoot length for each plant, represented by one dot*

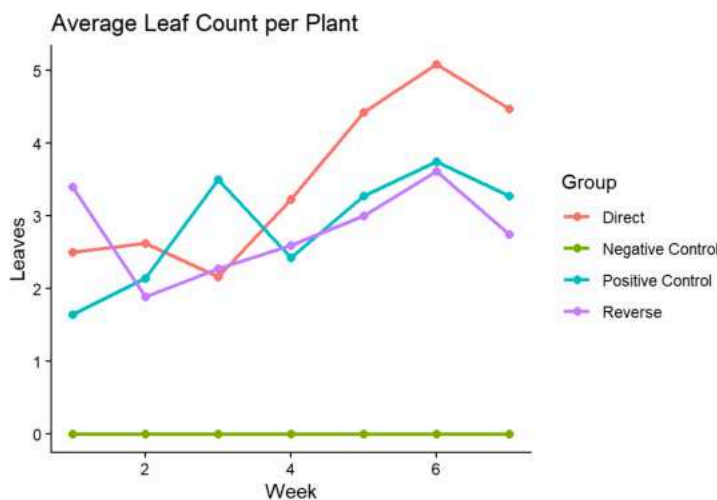
In addition to the shoot length, the ratio of shoot length to root length was calculated to show the amount of root allocation. A lower ratio indicates longer shoots than roots, therefore indicating less root allocation, healthier plants, and more nutrient availability. The Direct group had a ratio of 1.19. Reverse had a ratio of 1.23. The Positive Control Group showed a ratio of 1.79. The Negative Control had no growth in either the shoot or the root, so there is no ratio to be measured.



Graph 4: Box Plot Showing the Biomass of Each Plant at Harvest

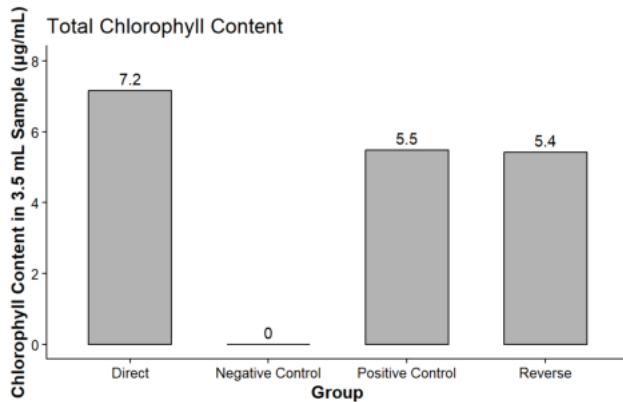
Biomass was measured after seven weeks of growth. Each group was measured and averaged as a gram measurement.

Furthermore, these growth patterns are mirrored in the plants' biomass at harvest. Direct had an average biomass of .422 grams per plant and a sum of 8.443 grams. Subsequently, Positive Control has an average of .069 grams and a total of 1.033 grams. The Reverse group had an average biomass of .086 but a whole of .949 grams. Since Negative Control had no growth, there was no biomass to weigh for this group.



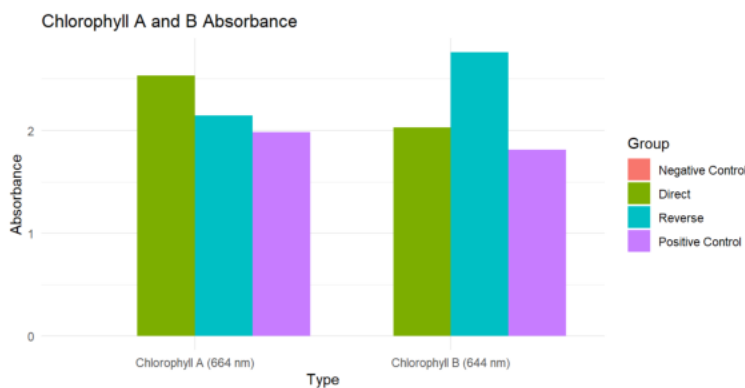
Graph 5: Line Graph of the Average Leaf Count for Each Group

True leaves were counted on each plant after each week of the growth period. An average was taken for each group every week.



The number of leaves in each group was counted weekly to create an average number of leaves per plant for each group. The leaf count for Reverse initially decreased, then followed a linear trajectory until week 6, when it had 3.61 leaves, then it decreased again in week 7. The Positive Control increased slightly from week 1 to week 2, then increased significantly more the following week. Then it decreased again before decreasing again from week 6 to 7. Direct increased slightly leading up to week 2 before decreasing in week 3. After this, it increased considerably until week 6, where it reached 5.18

leaves on average, then had a final decrease leading up to week 7. Negative Control showed no growth; there were no leaves either.



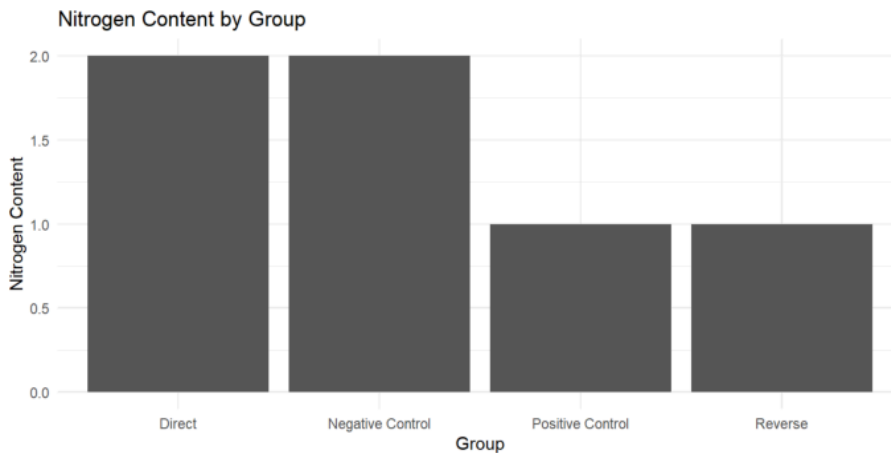
Graph 6: Bar Chart Showing the Leaf Chlorophyll Content at Various Wavelengths

Chlorophyll A and B were detected by extracting leaf pigments using 90% acetone and utilizing a spectrophotometer to measure Chlorophyll.

Graph 7: Bar Chart Showing the Total Chlorophyll Content per Group

Raw data from the absorbance rates were taken to calculate the chlorophyll concentration in each group.

Chlorophyll concentration was taken as a cumulative average for each group when being measured. The absorbance was first measured using the wavelengths of 664 nm (nanometers) for Chlorophyll A and 644 nm for Chlorophyll B. The absorbance measured on average for all chlorophyll types was 1.8985 Absorbance Units (AU) for the Positive Control, 2.278 AU for the Direct group, and 2.4515 AU for the Reverse group. Using the measurements or averages, the numbers were plugged into an equation to find out the actual concentration of the chlorophyll. The Positive control had 6.56 µg/ml of chlorophyll, the Direct group had 8.37 µg/ml of chlorophyll, and the Reverse group had 7.07 µg/ml of chlorophyll.



Graph 8: Bar Chart Showcasing the Nitrogen Content in the Soil
This data was taken using the Nitrogen Luster Leaf Rapitest capsule detector kit and lunar regolith companies with water used in the EB and Flow watering system.

Utilizing a pill soil test kit, the average concentration of nitrogen in each of the groups' soil was measured. The concentration of nitrogen was measured on a scale from 0 to 5, with 0 indicating little to no nitrogen and 5 indicating a surplus of nitrogen in the soil. Sourced from the pill kit, a range of 2-3 is the most desired amount of nitrogen for plant growth. From the data collected, Positive Control and Reverse had a deficient amount of nitrogen in the soil, with a measurement of 1 from the kit. Conversely, the groups Negative Control and Direct had an adequate amount of nitrogen, with them scoring a 2.

Analysis

The data within the results section is analyzed based on growth, chlorophyll content, biomass, and nutrient measurements, and the methodology can be efficient depending on the data. The graphs and data presented represent the averages of plant growth over the last 7 weeks, which can be further applied and analyzed.

Growth Analysis:

The shoots were measured every week for 7 weeks, and had different averages for each group. The Negative Control had no growth over the 7 weeks and showed no progress, meaning the regolith, when kept as the control, did not support the growth of Mizuna Mustard. From Graph 1, some trends stood out, including a collective decrease in length in Week 3. This was due to the light being too harsh on the newly growing plants, causing them to yellow and wilt. The light was then changed, which could have altered the data in some form, but no qualitative or quantitative differences between the Midpoint growth date and final growth date were observed. As seen in Graph 3, the shoot to root ratio for Direct is much lower than that of the Positive Control group, showing adequate growth. The Reverse group, however, showed optimal growth rates when comparing the shoot to root growth ratio. The ratio for Reverse is the closest to the optimal plant growth ratio, being 1:5 or 1:6 for root to shoot, respectively (Harris, 1992). Although the Reverse, Direct, Negative Control, and Positive Control groups showed no relative accuracy to the optimal growth ratio, the Reverse group presented the closest results. The results showed a different group to be sufficient for harvesting and consumption rather than for growth rate. With the data presented in Graph 2 of the results, it is seen that the shoot lengths between groups differ and would impact the rate of harvest and consumption. The Direct group showed a total growth of 145.7 cm, which is 142.43% greater than the Positive Control. The next most growth was shown by the Positive Control group with 60.1 cm. The next largest growth was shown in the Reverse group with 39.1 cm, or 53.71% less than the Positive Control. The Negative Control had no germination; therefore, there were no shoot lengths

to measure. With all of the data in consideration, when it comes to harvesting, the shoot length and amount of leaves are important in comparison to the roots. This is because the roots will not be harvested or consumed. The results show that the shoots within the Direct group grew twice as much as both the Reverse and Positive Control groups. This demonstrates that the Direct group would be the most efficient with harvesting and consumption being the primary focuses during the growth period. The leaf count also presented similar relations to those in the shoot and root growth data. The number of leaves in each group was counted weekly to create an average of leaves a plant would have at the time taken. All groups showed a general increase in leaf count throughout the growth period. The Reverse group initially had the most leaves, but the average dropped significantly leading up to week 2 due to the initial harsh lighting and the loss of smaller true leaves. Although the plant length decreased for two of the groups during week 3, the leaf count increased on its normal trajectory during this time, except for the Direct group. Intriguingly, the Direct group was the only group that did not yellow during week 3. Throughout the growth period, Direct showed the highest count of leaves due to quick maturity. The next highest was the Positive Control, followed by the Reverse group. In the final week, all groups showed fewer leaves. This is mostly likely due to smaller leaves shriveling up, as bigger, stronger leaves grew and restricted light for them. The Positive Control had an average of 3.278 leaves, and the Reverse group had an average of 2.75 leaves. The Direct value showed the highest leaf count of 4.48. When considering the growth as per the null hypothesis, the sample values from the four groups were analyzed. With this sample data, a Tukey-Kramer ANOVA test was run on all sample data within the groups. The resulting p-value was 0.00001106 regarding total plant growth, and the corresponding p-value regarding the number of leaves was 0.0002643. This data from both total plant length and leaf count showed a significant difference between the Direct group and the positive control, as well as the Direct group and the Reverse group. However, the Reverse group data and Positive Control data did not show a significant difference. This allows for the conclusion that the null hypothesis was rejected, providing significant data

Chlorophyll Analysis:

The chlorophyll concentrations and absorption levels for each group provide results sufficient for analysis of which group is the healthiest and produces the most chlorophyll. The Reverse group experienced the highest amount of absorption, which was most likely due to the small but strong sample size, which was also clouded by material from the spheres. While looking at the concentrations, the ideal chlorophyll concentration of 12.6 $\mu\text{g/mL}$ was compared to the measured values (Flores et al 2024). This reference value would not be accurate, as there is no studied reference value for mizuna mustard grown in lunar soil. With the actual chlorophyll concentration, however, Reverse was deficient in comparison to the four groups. The group with deficient absorption and adequate chlorophyll was the Positive Control group, suggesting that the plants had a higher concentration of chlorophyll compared to the Reverse and would serve better for harvesting. However, results suggested that the Direct group would be best for harvest and growth based on the chlorophyll content and absorption. With adequate absorption and more than optimal chlorophyll concentration, the Direct group showed to be the best growth setup for growth, optimal chlorophyll, and future harvests.

Biomass Analysis:

The results from biomass collection were used to determine the quantity of growth rather than the quality of growth within the four groups. The four groups were compared, considering that the Negative Control had no growth, and the best group for harvest was determined. The results indicate varying biomasses for each group, but present the large difference within the Direct group, showing the largest amount of biomass, with this

measurement being 8.433 grams. This data suggests that when it came to the amount of growth, Direct was the most successful, therefore making it better for harvesting, getting maximum plant product, and observations. When observing the p-value of this test, it was concluded that it was 1.249×10^{-8} , determining the rejection of the null hypothesis, proving its significance.

Nitrogen Analysis:

The analyzed amounts for nitrogen determine how much nitrogen there is within the soil post-harvest, from a rating of N0 (Depleted) to N5 (Surplus). The Reverse and Positive Control groups had adequate amounts of nitrogen, while the Direct and Negative Control groups were almost deficient in nitrogen. The nitrogen likely varies between groups because of excess material such as the sodium alginate cover, the amount of nitrogen the plant took up, or the base amount of nitrogen when the soil was first implemented into the growth setup. The nitrogen levels could not be measured when first introduced in the growth setup, limiting the ability to persist if the nitrogen was taken up by the plants or the nitrogen was low when the experiment started. The plants in the Direct group likely used up the nitrogen in the soil, while the Reverse and control groups did not use much of the nitrogen within the growth medium. The low nitrogen levels in the Direct group were likely used to produce more chlorophyll (UCIPM, 2026), contrasting with the Reverse and control groups, which had higher levels of nitrogen not used up by the plants to make chlorophyll, complementing the data retrieved from chlorophyll collection.

Conclusion

The analysis presents two different conclusions for the experiment, one which answers the research questions, aims to reject or accept the null hypothesis, and is largely applicable to the issue identified in the introduction and background. The p-value within each group tested indicates that the data is statistically significant, leading to rejection of the null hypothesis; this was concluded in the p-values for both total plant growth and biomass. The data and analysis present the optimal growth, chlorophyll, nitrogen content, and harvest material from the group of Mizuna Mustard plants within the Direct spherification group. This goes against the initial hypothesis that the Reverse spherification group will produce the most optimal growth, even though the Reverse spherification group still performed better than the positive control. This indicates that spherification, especially Direct spherification, is a promising method of growing plants in lunar regolith that warrants further research.

Having an intensive setup costs a significant amount of time. Future experiments should be completed with the preparation beforehand so that the entirety of the growth period can be utilized. An additional way of improving the quality of the results is to provide each tray with individual lights. Since only one light was available in the growth tent, trays received varying amounts of light, even though that one light was constantly set to the same light intensity every time it was on (except when the light intensity was permanently decreased during week 4 to mitigate plant damage).

Appendix



Figure 1: Initial Growth Tent Setup

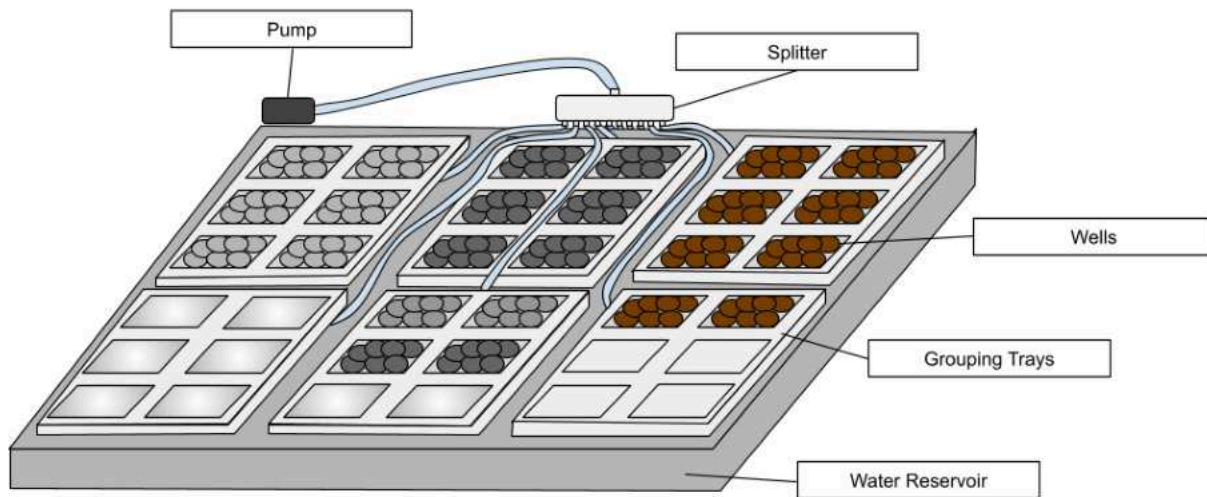


Figure 2: Labeled Model of Water and Placement Setup



Figure 3: Ebb-and-flow Hydroponic System Tube Separator

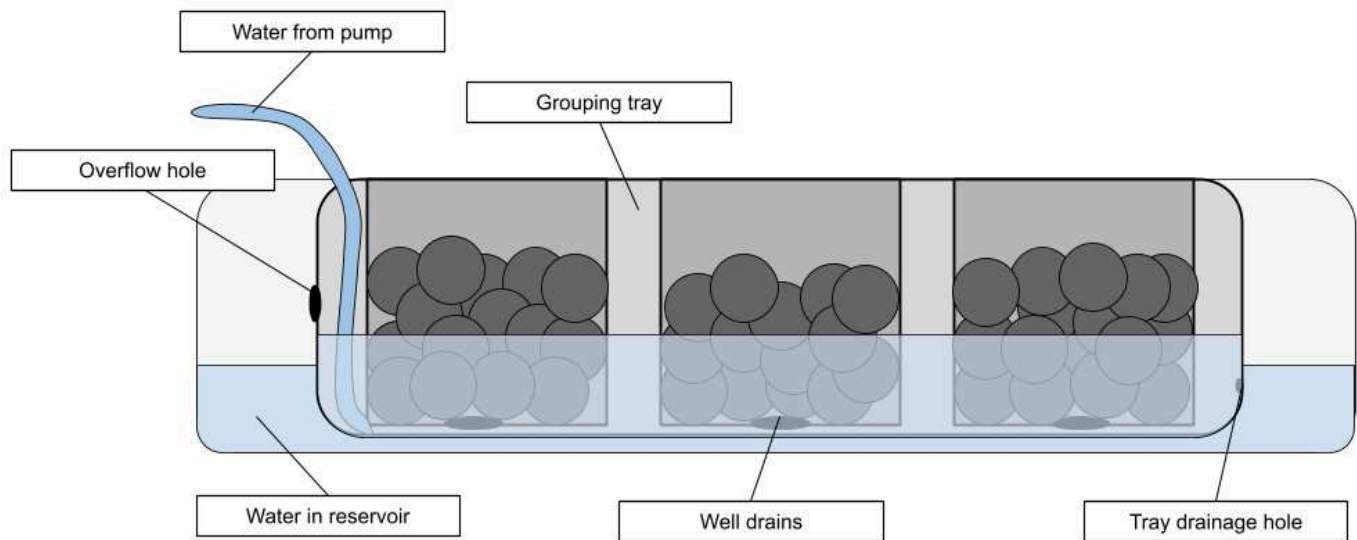


Figure 4: Internal Piping System for Ebb and Flow Watering System

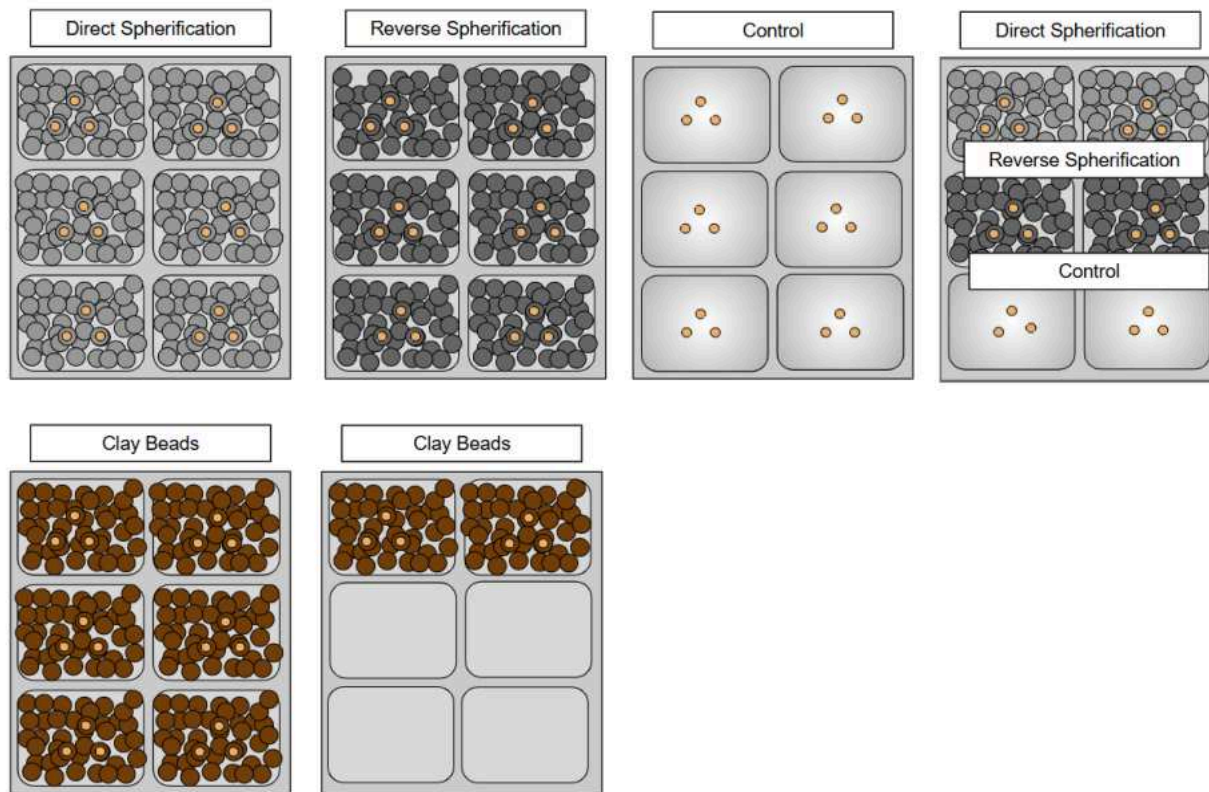


Figure 5: Visual Model of Seed and Pot Setup



Figure 6: Plants Extracted from Each Group Utilized to Measure Growth (Leaves, Length, Roots)



Figure 7: Summary of Each Groups' Root Formation Compared Inside the Pots

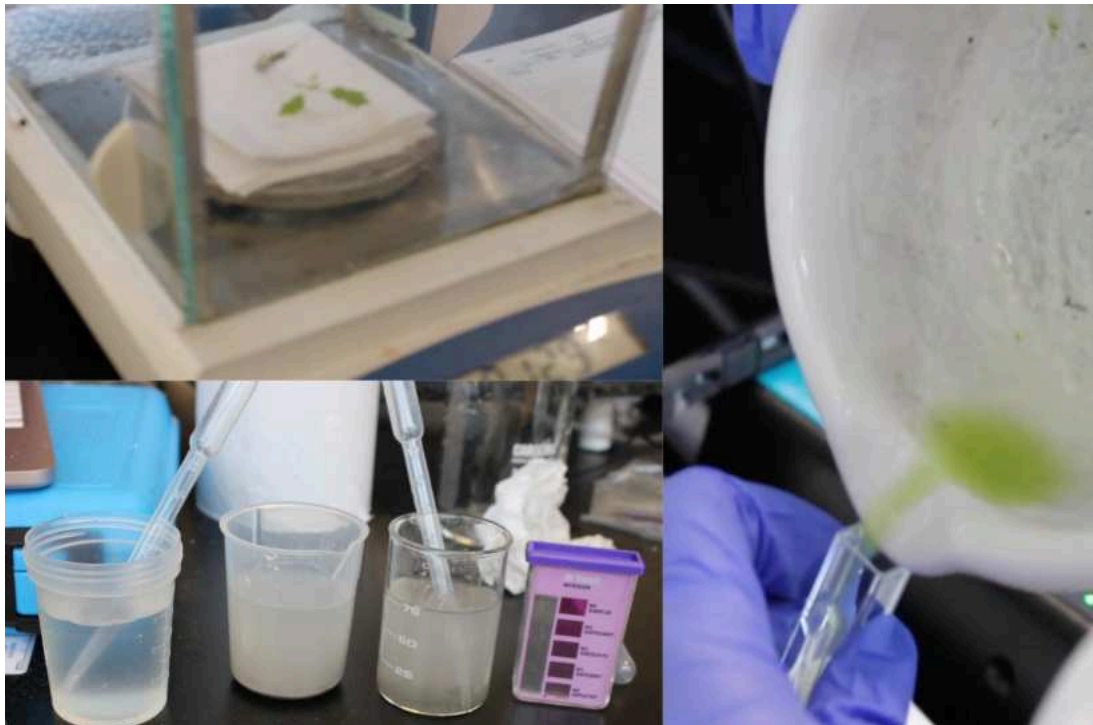


Figure 8: Pictures Taken When Collecting Data for Biomass, Chlorophyll, and Nitrogen Levels



Figure 9: Final Overhead Shot of End of the Experiment

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