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Growth of Four different Crop Species in Supplemented Loamy Lunar Regolith

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ABSTRACT

The ability to sustain crop growth in lunar regolith is imperative for long-term lunar missions. Due to the difficulty of transporting materials, using available lunar regolith for food growth is vital; however, past research has shown that dependence on crude lunar regolith is not feasible due to its absence of necessary nutrients and poor structural composition. Our experiment analyzed the possibility of growing crops in lunar regolith that was modified to have a loamy texture and to imitate the properties of Earth soil. Four crop species were grown in a 52:48 lunar regolith to additive concentration for seven weeks. Fagopyrum esculentum plants had significantly greater biomass in lunar regolith than in the control, which was earth soil. Triticum aestivum L., Solanum lycopersicum, and Pisum sativum crops showed no significant difference in overall biomass. Both lunar regolith and soil-grown crops produced flowers and fruits. We then determined the ideal lunar regolith to additive ratio by growing Fagopyrum esculentum in 52:48, 60:40, 70:30, 80:20, 90:10, and 100:0 concentrations over a seven-day period. Biomass results indicate that the 90:10 concentration of lunar regolith to additive is the most effective ($p<0.01$ when compared to all concentrations excluding 80:20). Such results are promising because they suggest a potential for effective crop development in lunar regolith supported by a relatively small quantity of additives.

Introduction

NASA's Artemis missions aim to establish the first long-term human settlement on the moon in the coming years [1]. Significant progress has been made toward this goal; however, food accessibility remains an obstacle [2]. Current astronauts rely heavily on dried and pre-made foods [2]. These foods are not sustainable for long-term missions due to nutritional deficits and the expense of transporting them [2]. For humans to live on extraterrestrial bodies, such as the moon, the capability to grow fresh food crops using readily available materials is vital. The moon does not contain soil, the usual substrate for growing crops. Instead, it is covered by a layer of fine dust called lunar regolith [3]. Lunar regolith is infertile and lacks many qualities essential for healthy plant development [3].

Previous research has compared plant growth in simulated lunar regolith to plant growth in both fertile and infertile earth soil. It was found that the lunar regolith simulant was less capable of growing plants than infertile earth soil [4]. Such findings show that pure lunar regolith cannot support high-yield crop growth. Further research explored how the addition of organic matter affected biomass production in lunar regolith. Lunar regolith supplemented with organic matter produced a greater biomass than unsupplemented regolith; however, the yield was significantly less than the quantity of biomass produced by earth soil modified in the same way [5].

To effectively grow crops in lunar regolith, more research needs to be done to reduce the biomass production discrepancy between lunar regolith and earth soil. Loamy earth soil is composed of a relatively equal ratio of sand, silt, and clay, and organic matter [6]. It is very fertile due to the balanced variety of particle sizes that provide ideal moisture and nutrient retention [6]. A soil additive composed of many materials was designed to introduce the beneficial attributes present in loamy soil to lunar regolith, increasing its overall growth productivity. Peat moss increased the total porosity of the soil, allowing for better aeration and drainage. Vermiculite served a similar purpose, improving drainage, aeration, and nutrient retention. Compost added organic matter and nutrients to the lunar regolith. Red clay was added due to its small particle size, allowing it to retain large amounts of moisture and nutrients. Lastly, GardenTone Vegetable fertilizer was used to increase the availability of usable nutrients like Nitrogen and Phosphorus.

In contrast to the research method used by Wamelink et al. in 2014, a fertile soil control was used to gauge how modified lunar regolith compares to soil capable of ample crop production. Using this control would more adequately determine lunar regolith's ability to support human life. The crops tested in this research were *Fagopyrum esculentum* (common buckwheat), *Triticum aestivum L.* (hard red spring wheat), *Pisum sativum* (iona pea), and *Solanum lycopersicum* (glacier tomato). These species were selected due to their yield ability, preferred climate, and hardiness. They have the potential to produce a good quantity of viable crops in a relatively short period, which is pertinent to extraterrestrial farming. It was hypothesized that when designed to imitate properties of loamy earth soil, modified lunar regolith with a 48% additive concentration would sustain seed and plant development. If the findings support this hypothesis, effective crop production using lunar regolith would be conceivable.

To investigate the applicability of lunar regolith additive to long-term lunar missions, the soil additive was dried and compressed to make it more cost and space efficient. It was speculated that the dehydration of the soil additive might impact plant growth negatively due to potential heat sterilization caused by the dehydration process. An experiment was designed to test this concern. It was hypothesized that the dried and condensed soil additive would produce statistically similar results to the standard soil additive. To further address lunar regolith additive efficiency, different concentrations of lunar regolith to additive were tested for crop growth productivity. It was hypothesized that additive concentration and crop growth levels would increase proportionally.

Materials and Methods

Soils

LHS-1 Lunar Highlands Regolith simulant was used in this experiment. It was purchased from Exolith Labs. This simulant contains specific minerals that can also be found in magmatic rocks on earth. The primary minerals within the lunar regolith simulant are anthorosite and glass-rich basalt [7]. The loamy earth soil was gathered at 36.12432*N, 86.82381*W, which has a soil type classified as maury silt loam [8]. The soil was collected at a 0–3-inch depth and had a high organic matter content. No additives were combined with the earth soil at the time of planting.

The lunar regolith was combined with many additives, each contributing to a trait found in loamy earth soil. The following materials were added to the lunar regolith in order of concentration. Specific percentages of each material within the additive can be found in Table 1. Organic Sphagnum Peat Moss was sourced from Espoma. Organic fine Grade horticultural Vermiculite was sourced from Burpee. Compost was sourced from a residential anaerobic compost pile in planting zone 7a. The Red Clay was sourced from 36.12386*N, 86.82377*W at a depth of seven feet. GardenTone Vegetable fertilizer was sourced from Espoma. The earth soil, compost, and clay additives were not sterile or free from seeds and insects. At planting, the pH for the earth soil control and the lunar regolith mixture was 6.4. The pH of the lunar regolith mixture was lowered using white vinegar.

Table 1. The percentage of each component of the regolith mixture

Percentage	Additive
52	Regolith
2	GardenTone
11	Clay
11	Compost
12	Vermiculite
12	Peat Moss

Each additive in the regolith mixture is listed above next to its corresponding concentration percentage.

Species Selection

Four species were chosen for this experiment, each from different taxonomic families. From the Solanaceae family, Solanum lycopersicum (glacier tomato) was selected. Pisum sativum (iona pea) was selected from the Fabaceae family, Triticum aestivum L. (hard red spring wheat) was selected from the Poaceae family, and Fagopyrum esculentum (common buckwheat) was selected from the Polygonaceae family. The hard red spring wheat seeds were sourced from All Good Things Organic Seeds, the common buckwheat seeds were sourced from Baker Creek Heirloom Seeds, and the two remaining seeds were sourced from Botanical Interests. External bacteria may have been present on the seeds, as it was not eradicated.

Experimental Design

The growth portion of the experiment was conducted in an indoor environment, as such conditions seemed the most likely to be present in an extraterrestrial mission. The location was kept at a temperature of around 70°F. Plants were placed in front of an east-facing window, from which they received sunlight each day. Additionally, light was supplemented by four 100w, full spectrum, led grow lights for six hours daily.

Four plastic growing chambers were used to house the plants in this experiment. The chambers included ventilating lids; however, the crops quickly outgrew them and were removed after the first week. Each chamber had twelve individual units. Two chambers were filled with the lunar regolith mixture, and the remaining two were filled with our control earth soil. One seed was planted per unit, and six were planted per species in each soil type. Figure 1 shows the experimental setup, including where each seed was planted. The species and soil type locations were grouped, and the chambers were randomly rotated weekly to prevent location-related bias. The seeds were planted at the depths recommended on their packages. Specific depths are detailed in table 2.

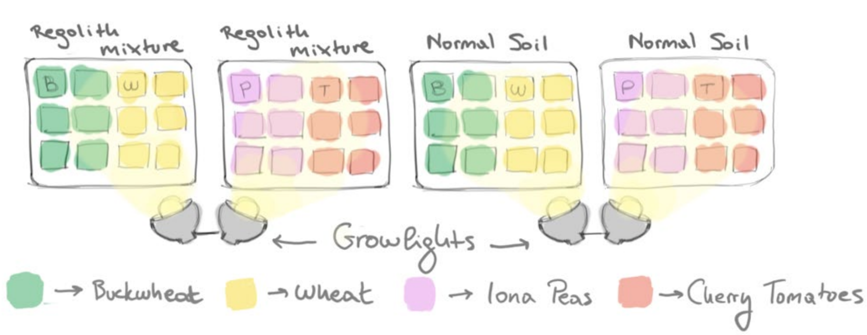

Figure 1. Crop growth in modified lunar regolith experimental set up. Buckwheat was planted in the chamber units labeled green, wheat was grown in those labeled yellow, iona peas were grown in those labeled pink, and cherry tomatoes were grown in those labeled red. The two chambers on the left contain modified regolith and the chambers

on the right contain earth soil. The locations of the grow lights are represented at the bottom of the image. Figure 1 provides a visual representation of the growing setup containing the lunar regolith and earth soil mixtures as well as the four different plant species.

The growing period lasted for seven weeks. During this time, the plants were watered regularly with reverse osmosis water. One chamber unit from each species section was tested for moisture level using a moisture meter every weekday. The species section was watered if the meter indicated that the soil was moist or dry. If the meter indicated soil wetness, no water would be added for that day. On week two of the growing period, all plants were fertilized with Monty's PlantVantage Premium Growth water-soluble organic fertilizer.

Table 2. Seed depth in inches

Species	Depth in Inches
White Buckwheat	1/4
Hard Red Spring Wheat	1
Glacier Tomato	1/4
Iona Pea	1

This table shows the depth the seeds of each species were planted in inches.

Measurements and Harvest

During the growing period, several measurements were taken each week. Plant height data was gathered for each plant using a ruler. Leaf count was taken each week, and it accounted for living leaves that had fully unfurled. The wheat plants' leaf count was not taken due to their structural difference. Starting on week four, observations regarding the development of flowers were recorded for all plants. Flowering was quantified by the presence of reproductive organs on a plant. Fruiting was recorded on week seven of growth, and it was quantified by the presence of seeds or fruit. Each week photos were taken of all plants to document development. At the end of the growth period, each specimen was harvested from its growing chamber. Excess soil was removed from the roots, and each plant was dried, first in an oven for approximately 1 hour at 60 °C and then left in an open area for a week to ensure total dryness. From the dried specimens, both above and below-ground weight was collected.

Pellet Experimental Design

For the second experiment, the additive detailed in Section 2.1 was compacted and dried. A mold was created using TinkerCad to achieve uniform and compact pellet shapes. This mold was printed on an Ultimaker 3-D Printer with PLA filament. Each produced pellet was cylindrical in shape with a diameter of 33 mm and a height of 15 mm and contained 21g of additive. After being shaped, the moist pellets were put into an isotemp oven to dry for 3.5 hours at approximately 80 °C. Subsequently, the additive was hydrated and combined with 20g of lunar regolith, creating approximately a 52:48 concentration. The pH was adjusted to 6.6. Buckwheat was selected to test the effectiveness of pellet soil due to its rapid growth rate. It was grown in the environmental conditions previously stated in Section 2.3; however, the growing period was adjusted to a week. After the growing period, the buckwheat plant heights were measured with a ruler. This data was compared to the height data of buckwheat growing in non-pellet regolith-additive mixture on week 1 of the first experiment.

Concentration Testing

For this experiment, the previously described lunar regolith additive was used in varying concentrations. The previously tested 52:48 concentration of lunar regolith to additive was used as a control. To see which additive concentration produced the most effective crop growth, six different concentrations were mixed and tested. Concentrations are referenced in Table 3. Buckwheat was the species used to test soil fertility due to its fast-growing nature. Experimental conditions were the same as mentioned in Section 2.3; however, the growth duration was only a week in length. The growing chamber layout is detailed in Figure 2. Germination and height were measured during each of the final five days of the growing period. Germination was quantified as the above ground sprouting of a seed, and height was measured using a centimeter ruler. Biomass was measured at the end of the growing period in the same way as referenced in Section 2.4.

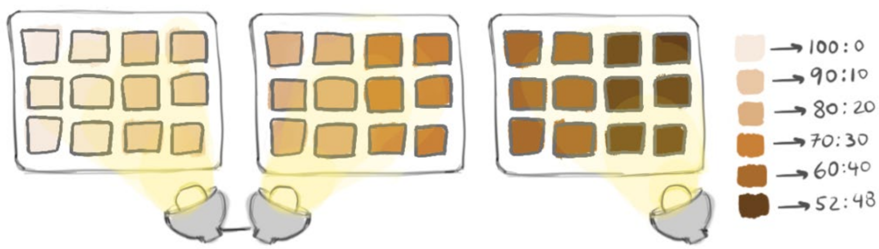

Figure 2. Crop growth in different concentrations of regolith experimental design. The concentrations of regolith to additive are labeled according to color, with the lightest color representing the concentration with the most regolith and the darkest color representing the concentration with the most regolith additive. Buckwheat was grown in all chambers for a one-week period. The locations of the grow lights are represented at the bottom of the image. Figure 2 is a visual representation of the growing setup of concentration testing containing six concentrations of regolith to additive and one crop type.

Table 3. The ratio of lunar regolith to additive

Number	Regolith	Additive
1	58	48
2	60	40
3	70	30
4	80	20
5	90	10
6	100	0

Table 3 summarizes the ratios of regolith to additive used in each concentration mixture tested.

Data Analysis

To analyze the data collected from all three experiments, Excel was used. The correlation was represented by P-Values found via two-tailed, type one T-Tests.

Results

Crop Growth in Modified Lunar Regolith

Plant performance was measured in terms of germination rates, plant height, leaf count, flowering rates, fruiting rates, and biomass. By the end of the growing period, the modified lunar regolith outperformed the earth soil in terms of total germination rate (Table 3). There was no significant difference between the average heights of the plants during most weeks of the growing period (Figure 3). Leaf count was significantly greater for the buckwheat plants growing in lunar regolith compared to those growing in earth soil during the last two weeks (Figure 4). All species of plants except for tomatoes flowered. This included 11 buckwheat plants, 5 pea plants, and 6 wheat plants. Two peas grown in lunar regolith produced fruits, as did three peas grown in earth soil. One buckwheat plant and all wheat plants were able to fruit in the earth soil, while none fruited in the lunar regolith. No tomatoes developed to the point of fruiting in either soil. In terms of total biomass, buckwheat grown in lunar regolith had a significantly higher biomass than the buckwheat grown in the earth soil ($p<0.01$). There was no significant difference between the total biomasses of other plants (Figure 5).

Table 4. Germination rates of plants growing in lunar regolith and earth soil

Plant Type	Germination Rates in Lunar Regolith (germinated/total)	Germination Rates in Earth Soil (germinated/total)
Fagopyrum esculentum (White Buckwheat)	6/6	5/6
Triticum aestivum (Hard Red Spring Wheat)	6/6	6/6
Pisum sativum (Iona Peas)	4/6	5/6
Solanum lycopersicum (Glacier Tomatoes)	4/6	3/6
Total Germination Rates	20/24	19/24

Each soil type housed 24 total plants with 6 chamber units for each type of plant. Overall, the lunar regolith had a higher germination rate than the earth soil.

Table 5. Flowering and fruiting ratios for plants grown in lunar regolith and earth soil.

Plant Type	Flowering Rates in Lunar Regolith (flowered/total)	Flowering Rates in Earth Soil (flowered/total)	Fruiting Rates in Lunar Regolith (fruited/total)	Fruiting Rates in Earth Soil (fruited/total)
Fagopyrum esculentum (White Buckwheat)	6/6	5/6	0	1/6
Triticum aestivum (Hard Red Spring Wheat)	0	6/6	0	6/6
Pisum sativum (Iona Peas)	2/6	3/6	2/6	3/6
Solanum lycopersicum (Glacier Tomatoes)	0	0	0	0
Total Germination Rates	8/24	14/24	2/24	10/24

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Each soil type housed 24 total plants with 6 chamber units for each type of plant. All wheat plants were able to flower and fruit in the earth soil, while wheat plants growing in lunar regolith were unable to do so.

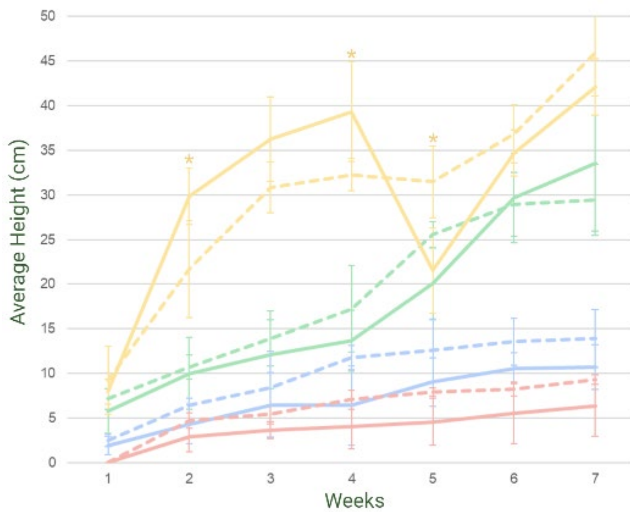

Figure 3. Average weekly plant height. Yellow represents wheat, green represents buckwheat, blue represents peas, and red represents tomatoes. The dotted lines represent plants that were grown in earth soil. The solid lines represent plants that were grown in the lunar regolith mixture. Error bars show standard deviation. There was no significant difference between crops grown in modified regolith and earth soil at the end of the growing period (week seven). (* $p < 0.05$).

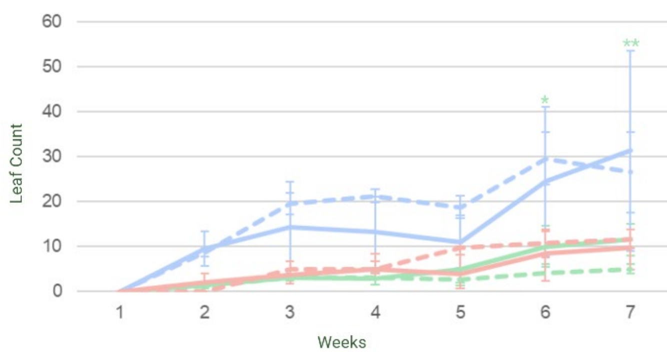

Figure 4. Average weekly leaf count. Blue represents peas, green represents buckwheat, and red represents tomatoes. The dotted lines represent plants that were grown in earth soil. The solid lines represent plants that were grown in the lunar regolith mixture. Error bars show standard deviation (* $p < 0.05$, ** $p < 0.01$. Buckwheat grown in modified lunar regolith had a significantly higher leaf count at the end of the growing period compared to buckwheat grown in earth soil. None of the other crop types showed significant differences between soil types. Wheat was excluded from this measure due to difference in plant structure.

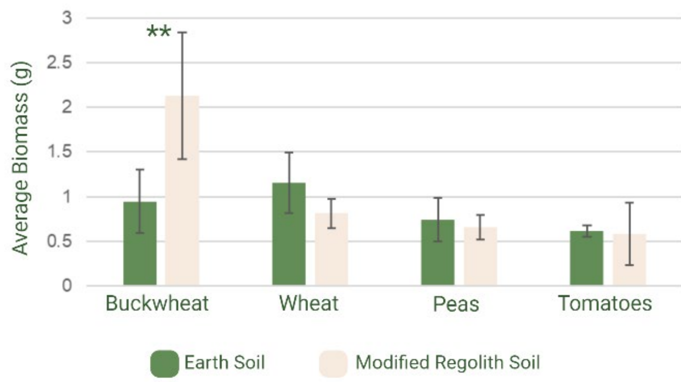

Figure 5. Average total biomass per plant type. Green represents the plants grown in earth soil, while tan represents plants grown in the modified lunar regolith. Error bars show standard deviation. The buckwheat crops grown in modified lunar regolith had a significantly greater biomass than that grown in earth soil. The remaining crops showed no significant difference in biomass. (**$p < 0.01$).

Comparison of Pellet Additive

The effectiveness of dried, compacted pellet additive was tested in comparison to the original additive solution. There was no significant difference between the height of crops produced in the pellet treatment compared to the mixture treatment.

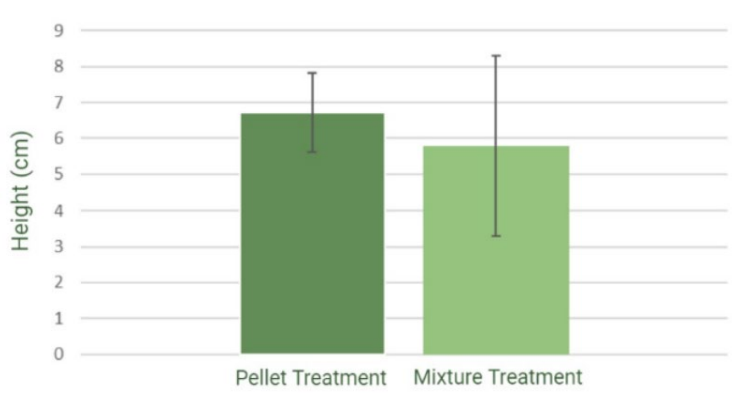

Figure 6. Buckwheat height in different lunar regolith treatments. Plants grown in the pellet treatment are represented by dark green and the ones grown in the mixture treatment are represented in light green. Error bars show standard deviation. There is no significant difference between buckwheat health in the two treatments.

Crop Growth in Different Concentrations of Regolith

The different lunar regolith concentrations were tested to discover the ideal concentration for plant growth. After a week, the average total biomass of the 90:10 concentration was significantly greater when compared to 52:48, 60:40, 70:30, and 100:0 concentrations ($p<0.01$). 80:20 had a significantly greater height when compared to 53:48 and 100:0 ($p<0.001$). 52:48, 60:40, 70:30, and 90:10 had a significantly greater height than 100:0 ($p<0.01$).

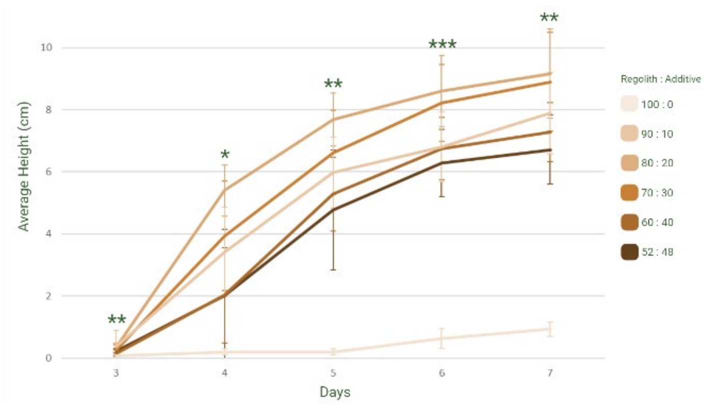

Figure 7. Average buckwheat height in different soil concentrations. The concentrations of regolith to additive are labeled according to color, with the lightest color representing the concentration with the most regolith and the darkest color representing the concentration with the most regolith additive. The height was recorded over seven days. Error bars show standard deviation. There was significance between 80:20 and 60:40 concentrations (Day 3), 80:20 and 52:48 concentrations (Day 4, 5, and 6), and 90:10 and 100:0 concentrations (Day 7). {*p<0.05, **p<0.01, ***<0.001).

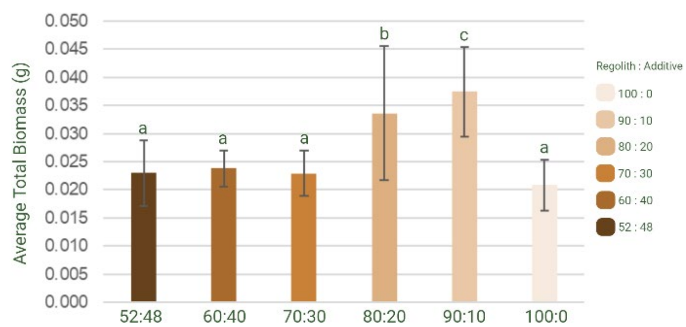

Figure 8. Average total buckwheat biomass of different soil concentrations. The concentrations of regolith to additive are labeled according to color, with the lightest color representing the concentration with the most regolith and the darkest color representing the concentration with the most regolith additive. Error bars show standard deviation. There was significance between the data of the different concentrations. The average total biomass of the 'c' value is significantly larger than those of 'a' values, (p<0.01), whereas the 'b' value is not significantly different from the rest of the values.

Discussion

The goal of this research was to modify lunar regolith to imitate the properties of loamy earth soil. Overall, statistical analyses show that plants grown in modified lunar regolith performed similarly to, if not better than, plants grown in fertile, loamy earth soil (Figures 3, 4, 5). With more testing, specific lunar regolith modification has the potential to allow astronauts to grow their own food on the moon. Additionally, drying the additive is an effective method to reduce volume and weight without significantly altering performance (Figure 6). Such a finding could be promising, as it would make transport of this additive more affordable and feasible. It was concluded that an 80:20 concentration of lunar regolith to additive performed the best in terms of plant height (Figure 7) while a 90:10 concentration performed the best in terms of biomass (Figure 8). Evidence suggests that that additive is effective in smaller concentrations, which is promising because it allows for efficient crop growth without the necessity for large amounts of imported materials.

The results found in previous research contrast our findings regarding crop growth ability in lunar regolith. Wamelink et al. 2014 and Wamelink et al. 2019 reported significantly less plant development in lunar regolith than in an infertile earth soil control. It is believed that our results differ from past findings due to the specific additives combined with lunar regolith. Such additives increased overall nutrient availability and improved soil texture which aided in plant growth.

There were several limitations in this study, the most significant of which was water. Due to the varying drainage rates of the soils, some periods of dryness occurred. Another important limitation was container size. The plants quickly outgrew the growing chambers, which may have altered growth patterns. Additionally, external seeds were not eradicated from the native soil before planting, and because of this, plants grew that were not intended as part of the experiment. No data was collected on these plants; however, they may have affected the growth of the plants in the same chamber unit. In the tests of pellet effectiveness and concentration, only buckwheat was used for plant growth analysis. It would be beneficial to test a variety of plant species in these conditions to formulate more comprehensive claims about additive effectiveness. Future research addressing the aforementioned limitations would portray the efficiency of the lunar regolith additive with more accuracy.

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References

[1] “NASA: Artemis,” *NASA*. https://www.nasa.gov/specials/artemis/index.html [Accessed 27-Apr-2022].

[2] M. Cooper, G. Douglas, and M. Perchonok, “Developing the NASA Food System for Long-Duration Missions,” *Journal of Food Science*, vol. 76, no. 2, pp. R40–R48, 2011, doi: 10.1111/j.1750-3841.2010.01982.x

[3] D. W. Ming and D. L. Henninger, “Use of lunar regolith as a substrate for plant growth,” *Advances in Space Research*, vol. 14, no. 11, pp. 435–443, Nov. 1994, doi: 10.1016/0273-1177(94)90333-6

[4] G. W. W. Wamelink, J. Y. Frissel, W. H. J. Krijnen, M. R. Verwoert, and P. W. Goedhart, “Can Plants Grow on Mars and the Moon: A Growth Experiment on Mars and Moon Soil Simulants,” *PLOS ONE*, vol. 9, no. 8, p. e103138, Aug. 2014, doi: 10.1371/journal.pone.0103138

[5] Wamelink, G.W.W., Frissel, J.Y., Krijnen, W.H.J., and Verwoert, M.R., “Crop growth and viability of seeds on Mars and Moon soil simulants,” *Open Agriculture*, vol. 4, no. 1, pp. 509–516, 2019, doi: 10.1515/opag-2019-0051

[6] N. Bumgarner and H. Savoy, “Soil Science and Plant Nutrition,” in *Tennessee Master Gardener Handbook*, The University of Tennessee Institute of Agriculture, Ed. Knoxville, TN: The University of Tennessee, 2018.

[7] “LHS-1 lunar highlands simulant,” *Exolith Lab*. [Online]. Available: https://exolithsimulants.com/collections/regolith-simulants/products/lhs-1-lunar-highlands-simulant. [Accessed: 27-Apr-2022].

[8] Nrcs, *Web soil survey - home*. [Online]. Available: https://websoilsurvey.nrcs.usda.gov/app/. [Accessed: 27-Apr-2022].