

M.A.R.S on Mars

Southwestern Oklahoma State University, Weatherford, OK

Mammal Scat vs. Worm Casting Viability in Simulated Mars-regolith Soil in a Controlled
Greenhouse Environment

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INTRODUCTION

The National Aeronautics and Space Administration (NASA) is currently working on the Artemis Project, sending astronauts to the moon for the first time in decades, with the hopes of establishing a permanent settlement on the moon. Beyond this, other ventures have their sights set forward towards Mars, with the hopes of establishing a permanent settlement on the planet. With space travel and colonization, factors such as food growth must be investigated.

A group of 10 undergraduate students at Southwestern Oklahoma State University recently competed in the Plant Mars Challenge, hosted by the Institute of Competition Sciences. This team was funded by the Oklahoma Space Grant Consortium. The primary goal was to sustain crop growth in simulated Mars regolith. The international challenge was to have the highest plant yield in a 8-week growth period using simulated Mars-regolith, which is chemically similar to the composition of substrate on Mars. This regolith was produced by the University of Central Florida's CLASS Exolith Laboratory, and supplied by the competition. As part of this competition, at least 50% of the total volume of the pot must be filled with Mars-regolith or Earth soil. Beyond this requirement, all of our pots contained the following additives: activated charcoal, coconut coir, Epsom salt, and perlite.

As there were water logging issues during our participation in the previous year's competition, we included additives that are beneficial in the prevention of water logging. Coconut coir, activated charcoal, and perlite all served to do this by aerating the soil (Activated Charcoal, n.d.; Sears, 2001; Tuckeldoe *et al.*, 2023). In addition to this benefit, perlite also served as a source of the beneficial nutrient potassium (Sears, 2001). For the last of the universal soil components, we used Epsom salt. Epsom salt was used as it is a provider of magnesium which promotes high levels of chlorophyll production (Epsom, 2021). For our experimental regolith additives, we

wanted to look at how using mammalian fecal matter compared to using worm castings. We also wanted to measure differences between microbes by observing differences in pots with bacteria added and pots with mycorrhizae added. We observed how our chosen dependent variables – mass of plant, chlorophyll leaf content, seed germination, plant color, and plant height – differed within the different additives.

Our team chose two commercial microbial soil additives. Mikro-H₂O[®] rhizobacteria was chosen as it has been shown to significantly aid in increasing germination and growth rates (Gholami, 2009). This bacteria increases the length of the radicle, the part of the plant embryo that later becomes the plant's primary root, which increases its surface area and water uptake (Pérez, 2023). While also promoting plant growth, *Rhizobium leguminosarum* was chosen because it differs from our other microbial additive because it is a nitrogen-fixing bacteria (Yanni, 2010). Nitrogen-fixing bacteria take nitrogen from the environment and convert it most commonly into ammonia for plants to take in at their roots, as they cannot convert it themselves (Cocking, 2000). Unknown microbes were also present within the unsterilized rabbit droppings and the vermicompost tea.

Would these additions to our soil allow our Mars soil to become more fertile and yield better results for our crops? The interactions between plants and bacteria have been known to be beneficial to plant growth. (Palacio *et al.*, 2023). Gut bacteria from mammals have been known to be beneficial for plant growth. Additionally, rabbit compost specifically could lead to increased fruit weight (Adi *et al.*, 2020). This partially inspired our idea of rabbit tea. Vermicompost is known to have many beneficial effects on plants, like growth, yield, and quality, and also has been shown to increase germination (Vuković *et al.*, 2021). In addition to this, by introducing rhizobia bacteria into our soil, the nitrogen fixation in our legumes could lead to a higher yield and overall

more growth due to the relationship formed with the bacteria (Laysa *et al.*, 2022). These factors combined with the plant choices that were made to have faster growing plants could lead to higher yield and faster growth.

Each pot contained four crops: peas, basil, spinach, and lettuce. The species are Lizard Spinach (*Spinacia oleracea*), Red Rubin Basil (*Ocimum basilicum*), Patio Pride Snap Peas (*Pisum sativum*), and Rhone Lettuce (*Lactuca sativa*). Four plants were grown in each pot to continue our observations of diversity between crops. This was to study biodiversity within crop selections. Basil was grown with the intent of boosting morale, because of its ability to add flavor to dishes. Rubin basil is slow to bolt, so there is a longer harvest time. We chose the Lizard Spinach because it can be harvested in about 1 month. This crop also has good regrowth once leaves are cut from harvest. This would be especially useful in real-life application because we would need crops that could keep yielding food consistently as opposed to crops that could only be harvested once. Spinach carries many different minerals and vitamins that are essential and beneficial to the human body like vitamin A, C, E, and K, as well as zinc, magnesium, and phosphorus (Lasya, 2022). It is also rich in lutein, calcium, and iron (Rueda *et al.*, 2022). It is a versatile crop that can be prepared in several ways, which could keep the food choices less limited.

Patio pride snap peas were chosen as a continuation of the previous SWOSU to Mars team experiments. They grow well in the containers and vertical growth allows for more food production in small spaces. The pods add a substantial amount of mass, and the leaves could also be consumed. Peas form a symbiotic relationship with root-nodulating diazotrophic bacteria, known as rhizobia. The roots of pea plants also form mycorrhizal associations with arbuscular fungi, or the fungal microbes that colonize the area surrounding the roots of plants (Kalapchieva *et al.*, 2023).

We chose Rhone lettuce because it can be harvested in 52 days, which is within our timeframe. It also produces high yields. Head lettuce was avoided, and leaf lettuce was chosen because head lettuce takes longer to produce and can only be harvested once. Rhone is a dark leaf lettuce, which can be considered to have more nutrient content. Lettuce is generally considered low in nutritional value, but there have been studies that showed nutrient content varies on the lettuce type, and that darker colors such as red, purple, and black contain more nutrients as well. (Kim *et al.*, 2016).

We used microbes, rabbit tea, charcoal, and perlite to see if they were able to get food growth in the Mars regrowth plants, to be able to see in future plants what was able to help the plant growth and what didn't help the plant growth. We hypothesized that unsterilized rabbit tea, vermicompost, fungi, and bacteria would result in differences in plant growth and the amount of chlorophyll present.

The independent variables we measured throughout the course of this challenge are unsterilized rabbit tea, vermicompost (worm castings) tea, unsterilized rabbit droppings, rhizobia bacteria, and high fungal soil microbes. Germination of seeds, plant height, plant mass, and chlorophyll content of leaves (measured by color) are the dependent variables that were measured throughout the Plant Mars challenge.

The variables that were controlled during our experiment were the amount of water the plants receive, pot placement (within a climate-controlled greenhouse on campus), and some of the soil additives. The soil additives that remained constant throughout our 16 pots were horticultural charcoal, Epsom salt, coconut coir, and perlite.

METHODS

All of our seeds were ordered from Johnny Seeds, and they were not given any treatments prior to planting. Seeds were planted in the soil mixtures in 4 separate quadrants of the pots (Figure 1). In each pot, two Patio Pride snap pea seeds, three Rhone lettuce seeds, four Red Rubin basil seeds, and three Lizard spinach seeds were planted. The seeds were then watered gently, but thoroughly, using a pipette. They were kept on a bottom watering schedule thereafter. The pots were soaked in dechlorinated water for at least 15 minutes 2-3 times per week throughout the growth period.



Figure 1: Diagram of the pots of soil with the contents. On the left, the number of seeds are shown. On the right, the location of the plants are split into quadrants, with spinach in the top left (s), peas in the top right (p), basil in the bottom left (b), and lettuce in the bottom right (l).

Potting and planting started on February 17th. Ten pots of mixed Mars regolith were used with different additives. The same additives were added to Earth controls. The soil in each pot consisted of (by volume): 250 milliliters (mL) of Mars regolith or Earth soil, 100 mL of coconut coir, a pinch of Epsom salt (0.7 grams), 75 mL of perlite, 50mL of either sterilized or unsterilized rabbit droppings, and 40 mL of activated charcoal. Since there was a smaller amount of Epsom

salt, that was mixed in each pot separately. Other soil additives were mixed in large batches and divided between the pots. There were 5 different variable soil additives applied to 5 sets of 2 pots. After 3 weeks, commercially available products Mikro-H₂O[®], which contains *Bacillus amyloliquefaciens* and *Bacillus subtilis*, and Exceed Superior Legume Inoculant, which contains *Rhizobium leguminosarum* *bv.* *viceae*, *Bradyrhizobium* spp., and *Rhizobium leguminosarum* *bv.* *phaseoli*, were mixed in water and added to their respective pots during watering. All pots contained sterilized rabbit droppings except for two pots each of Mars and Earth treatments that contained unsterilized droppings.

Vermicompost tea was prepared by mixing 5 parts water with 3 parts worm castings, and was allowed to sit for a week. The rabbit tea was prepared by mixing 5 parts water with 1 part



Figure 2: Several members of the Mars team working on trimming Earth pot growth.

unsterilized rabbit droppings and was also allowed to sit for a week. After the mixtures had time to sit, they were stirred to homogenize and then added to pots via top watering. After three weeks, two pots with Mars soil and two Earth pots had 50 mL of unsterilized rabbit tea applied, two of each had 50 mL of unsterilized rabbit droppings added instead of sterilized rabbit droppings, two of each had 50 mL of vermicompost tea applied, two of each had various microbial mix applied, and two of each had a mycorrhizal additive applied.

RESULTS

The eight-week growth period showed a significant difference in the amount of crops grown in Earth soil compared to crops grown within the Mars soil. The overall difference in biomass between the two soil types was substantial (Figure 3). Spinach crops did sustain growth throughout the entire season within the Mars soil, however, they were much smaller and did not sprout as quickly as the spinach in the Earth treatments.

On harvest day on April 7th, we recorded the leaf color using the Munsell Plant Tissue Color Book before clipping the plants just above the soil line. These plants were placed in labeled envelopes and dehydrated for ~10 hours, until the plants were dried. The contents were then weighed and recorded to assess biomass (Table 1). Since darker color could indicate higher nutritional value, assessing the leaf color was a way to determine chlorophyll content (Kim *et al.*, 2016). The color of the leaf indicates

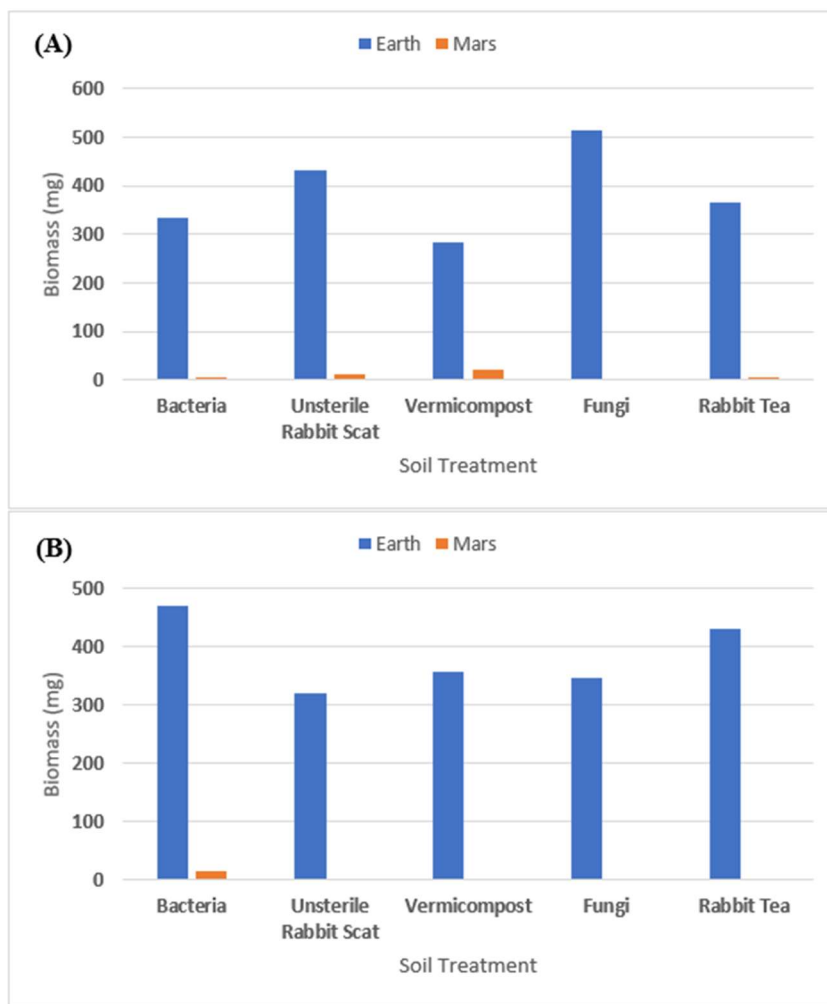


Figure 3: The difference in biomass between Earth and Mars soils containing five different treatments. Each soil type had two trials per treatment, for a total of 20 pots. Trial 1 is shown in 3A and Trial 2 is shown in 3B.

the light availability along with other factors such as chemical composition of soil and nutrient deficiencies (Munsell, 2021). The spinach leaves in the Mars soil had a slightly more yellow hue (Table 1).

Table 1: Observed leaf color on final day of growth period (April 7th, 2023). Color matched and recorded using Munsell Plant Tissue Color Book, 2021 Revised Edition.

		Peas	Spinach	Basil	Lettuce
Earth Pot #	Pot 1	5GY 6/8	7.5GY 9/4	5RP 9/8	2.5GY 6/6
	Pot 2	2.5G 7/4	5GY 4/4	5RP 3/2	2.5G 7/4
	Pot 3	2.5GY 6/8	7.5GY 3/4	5RP 3/2	7.5GY 4/4
	Pot 4	-	2.5B 7/6	5RP 3/2	2.5B 5/6
	Pot 5	7.5GY 6/8	7.5GY 3/2	5RP 4/4	7.5GY 8/6
	Pot 6	7.5GY 6/8	7.5GY 5/6	5RP 3/2	7.5GY 7/8
	Pot 7	7.5GY 6/8	7.5GY 4/6	5RP 3/2	7.5GY 6/6
	Pot 8	7.5GY 6/8	7.5GY 5/6	5RP 3/4	7.5GY 7/8
	Pot 9	5GY 7/6	7.5GY 5/4	-	-
	Pot 10	2.5GY 7/8	7.5GY 9/4	5RP 3/2	2.5GY 5/8
Mars Pot #	Pot 11	-	2.5GY 7/8	-	-
	Pot 12	-	5GY 6/8	-	-
	Pot 13	-	5GY 4/8	-	-
	Pot 14	-	-	-	-
	Pot 15	-	2.5GY 8/2	-	-
	Pot 16	-	5GY 6/8	-	-
	Pot 17	-	-	-	-
	Pot 18	-	-	-	-
	Pot 19	-	-	-	-
	Pot 20	-	2.5GY 7/8	-	-

		Spinach	Basil	Lettuce	Peas	pH
Pot 1	14-Mar	3.5	2	6.5	8.5	6
	24-Mar	4.1	3.8	7.2	7.5	6
	31-Mar	4	3.5	7.5	8.5	#
	3-Apr	5	3	5	8	#
Pot 2	14-Mar	4	2	4	8	*
	24-Mar	3.4	3.6	5.5	11.5	7
	31-Mar	3.8	4.5	5.5	10	#
	3-Apr	4.5	4.5	5.5	10	#
Pot 3	14-Mar	5	2.5	6	8	7
	24-Mar	3.2	2.7	5.8	7.5	7
	31-Mar	4	4	6	9.5	#
	3-Apr	4	4	5.5	7.5	#
Pot 4	14-Mar	3.5	3	2	-	*
	24-Mar	3.4	2.8	4.3	-	7
	31-Mar	6	5.5	8	-	#
	3-Apr	8	6	9	-	#
Pot 5	14-Mar	4.5	3	4	9	7
	24-Mar	3	4	4.3	9.1	7
	31-Mar	3.5	5	4.5	10	#
	3-Apr	4	5	4.5	9.5	#
Pot 6	14-Mar	5	1.5	2.5	6.5	*
	24-Mar	2.8	3.5	7.1	6.8	7
	31-Mar	3.5	5.5	8	10	#
	3-Apr	5	5	8	9	#
Pot 7	14-Mar	3.5	1.5	4.5	8	7
	24-Mar	3.1	2.8	3.5	9	7
	31-Mar	5.2	4.5	8	9	#
	3-Apr	4.5	4	10	9	#
Pot 8	14-Mar	5	2.5	7	8	*
	24-Mar	2.5	3	8.2	6.5	7
	31-Mar	3	5	7.5	6	#
	3-Apr	3	4.5	8	6	#
Pot 9	14-Mar	4	-	-	8	7
	24-Mar	3.4	-	-	8.8	7
	31-Mar	2.2	-	-	9.5	#
	3-Apr	4	-	-	10	#
Pot 10	14-Mar	4	3	7	8	*
	24-Mar	3	3.5	8.5	8.2	7
	31-Mar	5	4	9	9	#
	3-Apr	6.5	6	8	9	#

Table 2: Plant growth in centimeters recorded in Earth pots for each plant type at different dates. First data point (March 14) is recorded about halfway through growth period. Trimming of plants was done on March 24 and measurements taken after trimming (shown in Figure 2). Dashed line indicates no data, asterisk indicates no pH data due to similar treatment data, number symbol indicates no data due to insufficient materials.

		Spinach	Basil	Lettuce	Peas	pH
Pot 11	14-Mar	2.5	-	-	-	7
	24-Mar	1.5	-	-	-	6.5
	31-Mar	2	-	-	-	#
	3-Apr	2	-	-	-	#
Pot 12	14-Mar	1.5	-	-	-	•
	24-Mar	1.4	-	-	-	6.5
	31-Mar	1.8	-	-	-	#
	3-Apr	2	-	-	-	#
Pot 13	14-Mar	1.5	-	-	-	7
	24-Mar	2	-	-	-	6.5
	31-Mar	3	-	-	-	#
	3-Apr	2	-	-	-	#
Pot 14	14-Mar	-	-	-	-	•
	24-Mar	-	-	-	-	6
	31-Mar	-	-	-	-	#
	3-Apr	-	-	-	-	#
Pot 15	14-Mar	1.5	-	-	-	8
	24-Mar	1.2	-	-	-	7
	31-Mar	1.5	-	-	-	#
	3-Apr	1.5	-	-	-	#
Pot 16	14-Mar	1.5	-	-	0.25	•
	24-Mar	2	-	-	-	6.5
	31-Mar	2	-	-	-	#
	3-Apr	2	-	-	-	#
Pot 17	14-Mar	-	-	-	-	7
	24-Mar	-	-	-	-	6
	31-Mar	-	-	-	-	#
	3-Apr	-	-	-	-	#
Pot 18	14-Mar	-	-	-	-	•
	24-Mar	-	-	-	-	6
	31-Mar	-	-	-	-	#
	3-Apr	-	-	-	-	#
Pot 19	14-Mar	-	-	-	-	7
	24-Mar	-	-	-	-	6
	31-Mar	-	-	-	-	#
	3-Apr	-	-	-	-	#
Pot 20	14-Mar	1.5	-	-	-	•
	24-Mar	2.2	-	-	-	6.5
	31-Mar	2	-	-	-	#
	3-Apr	3	-	-	-	#

Table 3: Plant growth in centimeters recorded in Mars-regolith pots for each plant type at different dates. First data point (March 14) is recorded about halfway through growth period. Trimming of plants was done on March 24 and measurements taken after trimming (shown in Figure 2). Dashed line indicates no data, asterisk indicates no pH data due to similar treatment data, number symbol indicates no data due to insufficient materials.

DISCUSSION

To continue on last year's project, we planted multiple crops in each pot. This was to assess whether crops grew well together, since monocultures inhibit biodiversity. Growing multiple species per pot would also be a way to maximize surface area. The team took spacecraft storage into consideration, selecting soil components that could easily be packed in small spaces. We also decided against using materials like potting soil in the regolith mix, since it was assumed that there would be a shortage or problem with Earth soil if Mars was being colonized. The greenhouse we used did not account for differences in gravitational pull. We did not use artificial lighting because of the similarities in light availability between Earth and Mars.

An analysis on last year's soil was completed by Oklahoma State University's Soil, Water, and Forage Analytical Laboratory (SWFAL). This analysis showed that Mars-regolith had an average pH of 7.6, compared to the average Oklahoma soil pH of 6.3. The focus of the soil mixture was to ensure the soil would not become too alkaline throughout the growing period. The phosphorus in the Mars-regolith averaged to 1 pound per square meter (lb/m^2). Potassium results are $283 \text{ lbs}/\text{m}^2$. The recommended potassium is 85 lbs/acre (1 acre is equivalent to $4,046.86 \text{ m}^2$). The nitrate nitrogen ($\text{NO}_3\text{-N}$) concentration in the Mars soil is 0.2 per square meter. The recommended concentration of $\text{NO}_3\text{-N}$ is 92.903 per square meter.

Moving forward, the team might experiment with different crops or soil mixtures. The ratio of soil additives seemed to work well, but there may be a different combination that would allow for a larger biomass in the Mars pots. It would also be interesting to do part of the treatments with one crop vs. treatments with multiple crops.

CONCLUSIONS

Unlike last season, we managed to have sustained growth in the Mars pots for the entire 8-week growth period. We also did not experience many issues due to waterlogging, and the plants did not ever completely dry out. However, only spinach managed to grow in the Mars treatment, and it took about 3 weeks longer to germinate than the plants in Earth treatments. This might indicate that the Mars soil mixture contained inhibitory elements that severely stunted germination. The spinach in the Mars treatments had a hue that was more yellow than Earth spinach, which could indicate a nitrogen deficiency. This could be confirmed or rejected after chemical analysis of the soil.

The automatic pH indicator supplied did not give accurate readings, so we used pH strips. We ran out of the pH strips about halfway through the growth period.

It seemed odd to the team that the fungi additives were beneficial in the Earth treatment, showing the highest biomass, but did not facilitate growth in the Mars fungal treatment. While we did not get much growth in the Mars treatment, we did observe that the worm castings and bacteria had slightly more biomass. These treatments could be bioremedies for agriculturalists to consider using as opposed to commercial fertilizers and pesticides. On Earth, a mycorrhizal treatment along with soil additives may increase yield.

All of the Earth pots had growth, so the soil additives did not seem to inhibit crop production. The ratios used could provide a starting point for home gardeners or for crop production within greenhouses, where the soil could be mixed and the conditions match those used in our research.

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