

OVERCOMING SEED GERMINATION CHALLENGES IN MARTIAN REGOLITH

Abstract

As we work on long-term space exploration sending astronauts back to the Moon and even to Mars, we need to think about ways to provide food. One potential is to bring the food with them, but the spaceship can only hold so much. Because of this, we have joined the Plant Mars Challenge organized by the Institute of Competition Sciences (ICS) to help NASA and a team of scientists work on cultivating plants in native soil. We are using Martian soil simulant for this experiment and focusing on some of the challenges experienced while attempting to germinate radish and pole bean seeds in the Martian regolith along with identifying a potential solution to increasing the rates of seed germination and growth. The results show that plants can germinate in Martian soil simulant but quickly shrivel and die. During the study we had initial concerns about the pH levels and their impact on seed germination. We added coffee grounds to all variables to reduce pH. We attempted to grow the pole beans with soil acidifiers and apple cider vinegar to all groups. Between the coffee grounds and the soil acidifiers / apple cider vinegar added, they had little impact on the pH levels and germination rates. The 100% Martian soil simulant control performed the best getting Radishes in the first part and Pole Beans in the second part of the experiment to germinate along with supporting the Pole Bean past its first true leaves. We also determined that there is a salt that accumulates at the top of the simulant that may be hindering seed germination and once we leached the salt off, we were able to see increased germination rates in all variables. These results show that although we can germinate seeds in Martian soil simulant, further questions remain to sustain the plant past germination and the first true leaf stage and whether the soil simulant is indicative of the soil that astronauts would encounter on Mars.

Introduction

It was made clear when the president signed the White House Space Policy Directive 1 on December 11, 2017 that human expansion across the solar system will be the intention [1]. This included long-term efforts for returning humans back to the moon for the first time since 1972 and building upon that experience for human exploration of Mars through NASA's Artemis program [2].

With that being said, we need to begin studying methods and processes for growing sustainable food sources on the Moon, Mars, and beyond. There is a level of impracticality with the cost of continued resupply of food resources from Earth to other celestial bodies such as the Moon or Mars. Cargo space on space craft is preciously limited and would be better served with specialized instruments and equipment rather than for the continuous resupply of food. Therefore, it is important to start considering ways in which we can be more self-sufficient on places we intend to colonize such as the Moon, Mars and beyond. And this colonization begins with ability to grow plants. It is not so much a question of 'what' plants should be grown on Mars, but how we can amend the soils in the most optimal way so that the plants themselves can be successfully started and grown in them. Based on data returned from the Martian lander

missions, scientists have been able to determine a general mineral / chemical composition of the soils (regolith) on Mars [3]. Labs can create regolith 'simulants' that enable us to simulate the soil for researchers to study [4].

As part of the Plant the Mars Challenge, this study will explore some of the challenges experienced while attempting to germinate radish and pole bean seeds in the Martian regolith along with identifying a potential solution to increasing the rates of seed germination and growth.

Materials and Methods

Regolith

Martian regolith simulant was provided for the challenge by The Exolith Lab (<https://exolithsimulants.com/>). We received the MGS-1 Mars Global Simulant [5]. We can compare Martian soil to what we have available on Earth to create comparable Martian soil simulants [4].

As a control, we used Sta Green Potting Mix that is commercially available. This potting soil was chosen because it represents the average soil in any home garden typically used to start seeds.

Species Selection

We initially selected radish seeds because we know that the entire Radish plant is edible and nutrient rich [6]. Radishes have been successfully grown by astronauts in space [7], [8]. Radishes have a relatively short germination period and shorter amount of time needed before harvest can occur. Radishes also can grow in various types of soils ranging from thick, dense clay to a sandy loam. We also considered that the Radish (the root part of the plant) would not be the part harvested by astronauts for consumption because the edible biomass consisting of the Radish leaves and seed pods far outweighs that of the root. The Radish can continue growing as long as one harvests the leaves or cuts off the seed pods; lasting 6 months or more. The Radish variety chosen was Champion because they take less time between germination and harvest. This Radish variety is commercially available.

For the second plant species, we decided to use Blue Lake Pole Bean seeds. We chose Pole Beans because they germinate quickly, can continue producing for a long time and add nitrogen in the soil through their roots. This Pole Bean variety is commercially available.

Experimental Design and Observations

Kirkland brand disposable drinking cups were used for this experiment. The cups had 2 holes poked in the bottom and a paper towel filter cut to fit the inside bottom to prevent the soil from leaking. The cup was then filled and placed inside a small garden pot to contain any water overflow. For this experiment, we used 2 controls- 100% potting soil and 100% Martian regolith simulant. We also tested 5 variables measured by volume and then measured the mass of each cup. The variable cups were filled with a 2-1 mixture in order to maintain 66% volume of Martian regolith in each container so mass varied between control and variables (Fig. 1). Each

control and variable had 2 cups. This created a total of 14 cups, 2 for each control and 2 for each variable tested. (Fig. 2)

ID	Test Group	Martian Composition (%)	Variable Composition (%)	weight
TGM-1	Martian Soil (control)	100%	0%	502g
TGM-2	Martian Soil (control)	100%	0%	496g
TGM-3	Earth Soil (control)	-NA-	-NA-	98g
TGM-4	Earth Soil (control)	-NA-	-NA-	94g
TGM-5	Worm Castings	66.6%	33.3%	396g
TGM-6	Worm Castings	66.6%	33.3%	394g
TGM-7	Perlite	66.6%	33.3%	340g
TGM-8	Perlite	66.6%	33.3%	366g
TGM-9	Radish Leaves	66.6%	33.3%	408g
TGM-10	Radish Leaves	66.6%	33.3%	424g
TGM-11	Egg (baked)	66.6%	33.3%	348g
TGM-12	Egg (baked)	66.6%	33.3%	342g
TGM-13	Paper Wick	100%	0%	502g
TGM-14	Paper Wick	100%	0%	510g

Figure 1

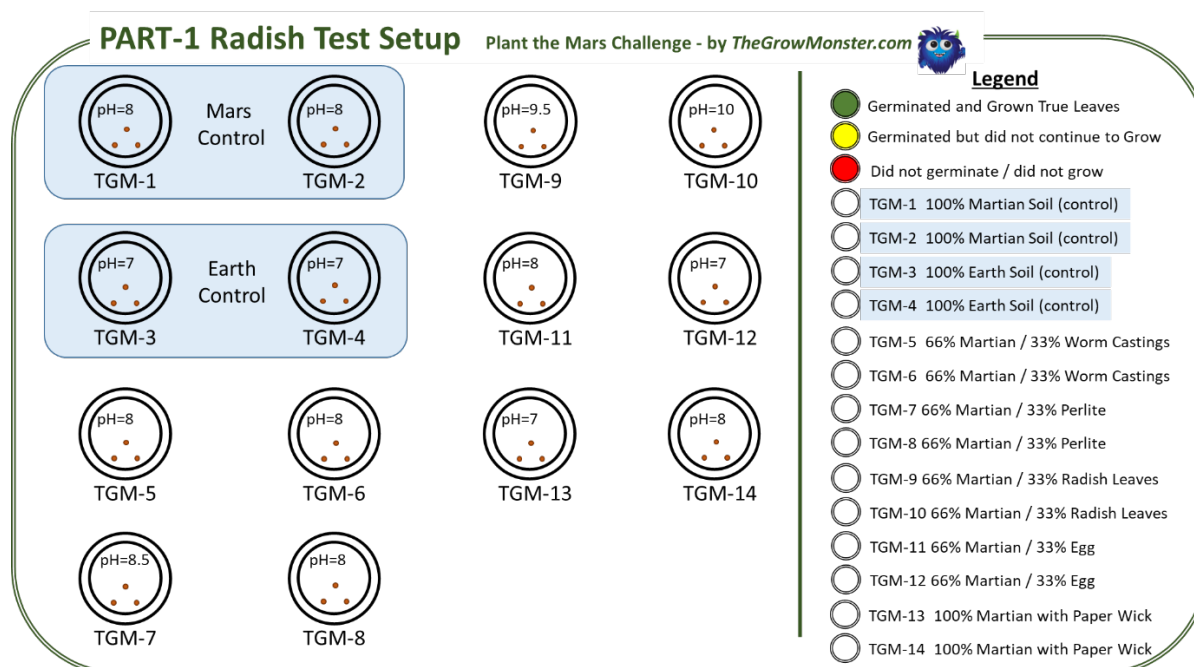


Figure 2

We tested 5 variables- worm castings, perlite, shredded radish leaves, baked Martian soil simulant with egg, and paper towel wick. (Fig. 3)

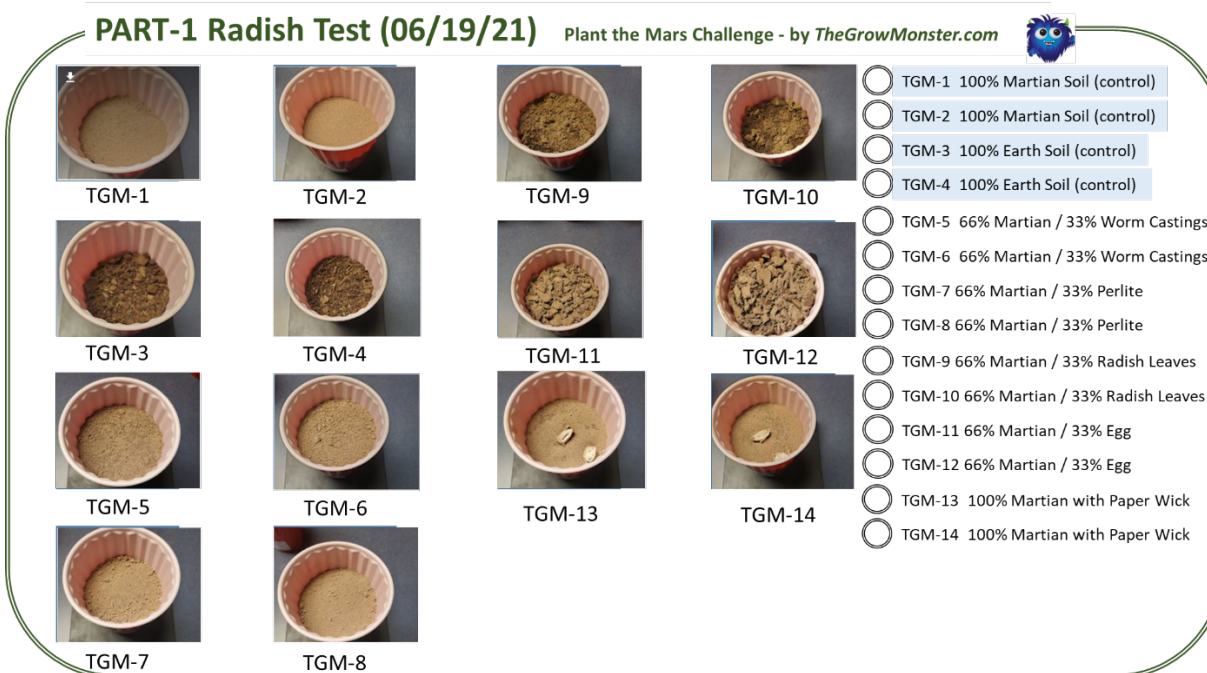


Figure 3

Worm Castings

We chose worm castings to add to the Martian soil simulant to introduce organic matter that might also aid in aeration and nutrition of the soil. We added 2 parts Martian soil simulant to 1 part worm castings in 2 separate cups.

Perlite

We chose Perlite to add to the Martian soil simulant since it is a lighter and porous material that helps provide aeration and drainage that will counteract the regolith simulant's tendency to become compacted. We added 2 parts Martian soil simulant to 1 part Perlite in 2 separate cups.

Shredded Radish Leaves

We chose shredded Radish leaves to add to the Martian soil simulant to introduce organic matter that might be readily available on the spaceship. If the astronauts are already growing Radishes, it can be used directly in the Martian soil to counter the soil compaction, provide nutrients when decomposed, and help in aeration of the soil as the leaves decompose. We added 2 parts Martian soil simulant to 1 part Perlite in 2 separate cups.

Baked Martian Regolith with Egg

We knew that soil compaction was a problem with the Martian soil simulant so we decided to try to make the particles larger that would be used in the container. We first tested 3 additions to the simulant- water, egg, and cornstarch, to determine which would be best to use. We added 2 tbsp

water to 1 tbsp Martian soil simulant, 2 tbsp egg to 1 tbsp Martian soil simulant, 2 tbsp cornstarch to 1 tbsp Martian soil simulant. We then spread each mixture out on aluminum foil on a baking pan and baked in the oven at 350 degrees for 30 minutes and then allowed it to cool (Fig. 4). After the mixtures were cool, we cut the larger pieces up and took a sample and added a drop of water to see which mixture would retain its shape best (Fig. 5). The egg was chosen because it retained particle size while also soaking in the water like a sponge. We thought this may reduce compaction and alleviate aeration issues in the Martian soil simulant. We added 2 parts Martian soil simulant to 1 part egg to create the larger particles and did this for 2 separate cups.

Recipe: baked at 350 degrees for 30 minutes, then let cool			
Variable Group Bake Candidate	Martian Composition	Variable Composition	Water Required for Baking
Water (H ₂ O)	2 tbsp	1 tbsp	-NA-
Egg	2 tbsp	1 tbsp	-NA-
Corn Starch	2 tbsp	1 tbsp	1 tbsp

Method task: Identify a variable bake candidate to address anticipated aeration/drainage issues with regolith			
Variable Group Bake Candidate	Observation	Chosen for Variable Group?	Justification
Water (H ₂ O)	When adding water to candidate, it immediately dissolved to prior state	No	Will not provide the particle size needed to test aeration/drainage
Egg	When adding water to candidate, retained it's form	Yes	This candidate should provide the aeration/drainage along with particle size we suspect we need
Corn Starch	When adding water to candidate, it dissolved (but slower) to prior state	No	Will not provide the particle size need to test aeration/drainage

Figure 4



Figure 5

Paper Towel Wick

We chose to add a paper towel wick to the Martian soil simulant to help the soil retain moisture around the site where we would place the seeds. We took a rolled-up half of a paper towel and added it in the middle of the Martian soil in 2 separate cups.

The experiment consisted of 3 parts in order to try to increase the germination rate.

Part 1

The experiment started June 13th, 2021. The Martian simulant was measured and added to the cups outside but once added to the cups the entire experiment took place in our basement under Surpsun Full Spectrum 600W Grow Lights set to Veg (Fig. 6) with mean day time lasting approximately 13 hours each day (7am-8pm). During the experimental period, the temperature range was 66 F- 81 F and humidity level ranged 34 to 57% measured on a Govee Bluetooth Hygrometer Thermometer. The pots for the first part of the experiment were watered with 20mL water. The pots were then switched to 10mL water once we noticed the soil wasn't drying between watering. We used distilled water to prevent contaminants from tap water like fluoride.



Figure 6

We took photos of all pots and noted any germination, pH of soil, and any other observations. (Fig. 7). We took the biomass produced from the germination and measured the entire plant from bottom of roots to top of leaves (Fig. 20). We couldn't get a weight measurement because the biomass was not enough to create a measurement. There were 8 total plants to be measured (6 from the Earth soil control and 2 from the Martian soil control).

ID	Test Group	Part1 initial pH	Part1 final pH	Part2 initial pH	Part2 final pH	Part3 initial pH	Part3 final pH
TGM-1	Martian Soil (control)	8	7	9	7.5	8.5	8
TGM-2	Martian Soil (control)	8	7	9	7	7.5	7
TGM-3	Earth Soil (control)	7	7	7	7	-NA-	-NA-
TGM-4	Earth Soil (control)	7	7	7	7	-NA-	-NA-
TGM-5	Worm Castings	8	7	7	8.5	8	8
TGM-6	Worm Castings	8	7	7	8.5	8.5	9
TGM-7	Perlite	8.5	7	7	9	8	8
TGM-8	Perlite	8	7	7	9.5	9.5	8.5
TGM-9	Radish Leaves	9.5	9	9	9.5	9.5	8.5
TGM-10	Radish Leaves	10	9	7	9.5	9.5	7
TGM-11	Egg (baked)	8	9	8	9	9	8.5
TGM-12	Egg (baked)	7	9	8	9	9.5	8.5
TGM-13	Paper Wick	7	7	8	9	8.5	7
TGM-14	Paper Wick	8	7	8	9	9	7.5

Figure 7

The week starting July 9, the plants in the Martian soils started showing signs of distress and shriveling. We decided to conduct a further experiment on July 21 with the Radish seeds after taking soil pH levels and noticing they were more alkaline than Radish seeds prefer when germinating. We added 1 tbsp unused coffee grounds to the soil in all 14 containers and placed 3 more Radish seeds to germinate. We followed the same process in measuring any growth, but there was only growth in the Earth potting soil control containers.

Part 2

The second part of the experiment was started July 30 in the existing 14 containers. We took the soil pH levels (Fig. 7) and decided to put 1 tbsp apple cider vinegar in half of the containers to reduce pH and 1 tbsp soil acidifier in the other half to reduce the pH level to be more hospitable to seed germination. We placed 2 Blue Lake pole bean seeds in each container and 10mL water daily (Fig. 8). The pH levels did not change much (Fig. 7) but one of the bean seeds did germinate and we were able to get a measurement of the plant.

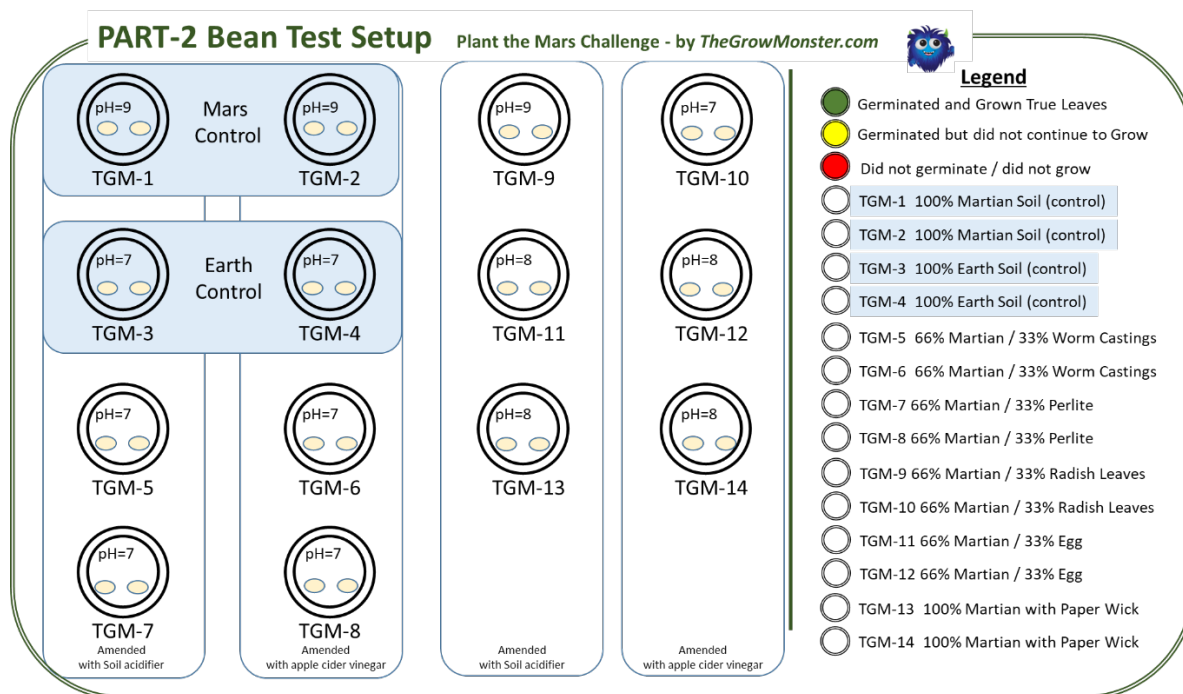


Figure 8

Part 3

We then did a third part to the experiment to try to leach the salt that would form on the top of the soil off to see if that would aid in germination. We added 2.5 tbsp from each Martian soil container and 5mL water for the first run-off followed by 5mL of water for a second run-off to a container to dissolve and dispose of the salts that may have hindered seed germination (9). 4 Radish seeds were then added to the soil to observe if there would be higher germination rates (Fig. 9).

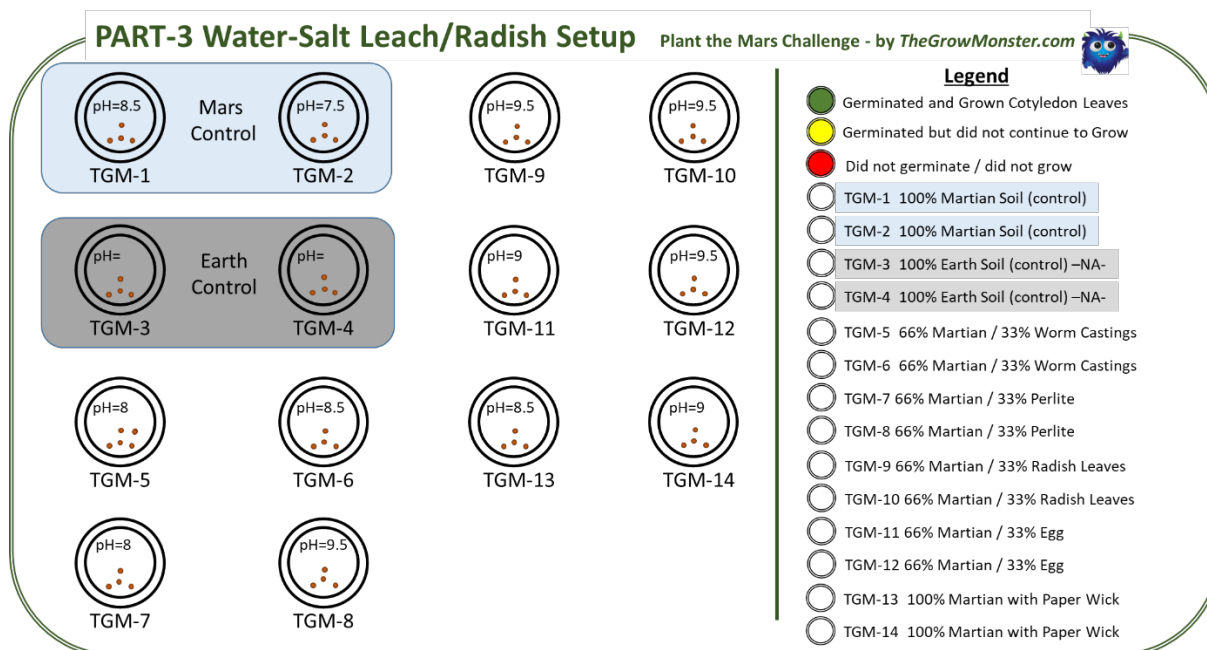


Figure 9

Results

While both Radishes and Beans did germinate in both the earth soil and Martian soil controls, they did not germinate in some of the variables (Fig 10-16). Even though germination was observed in multiple variables, the radish seeds were slow growing and began dying quickly. The radish plants did not get to the true leaf stage in either part of the experiment. Only 1 bean seed germinated, and it did get past the true leaf stage (Fig. 17-19). The germination rates for the radish seeds in part 1 was much higher than the bean seeds in part 2 (Fig. 24-27). Overall, of the seeds that germinated only the 1 bean seed went on to develop true leaves, the others (except in the earth soil control in part 1) stopped growing and shriveled. Seed germination occurred mostly in the control (both 100% Earth soil and 100% Martian soil). The pH levels were more alkaline in the variables (range 7-10) and dropped slightly after we tried amending the soil to lower the pH (range 7-9.5) (Fig. 7). After leaching the salts from the soil, we were able to increase the germination rate in all variables (Fig. 22, 23, 27).

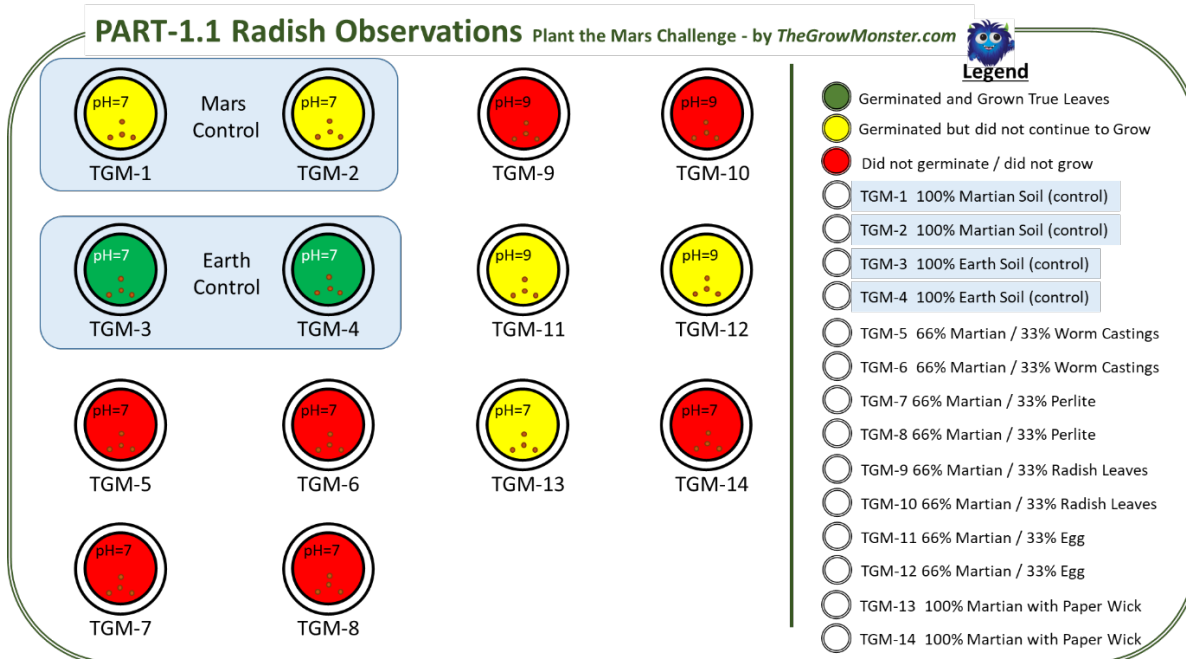


Figure 10

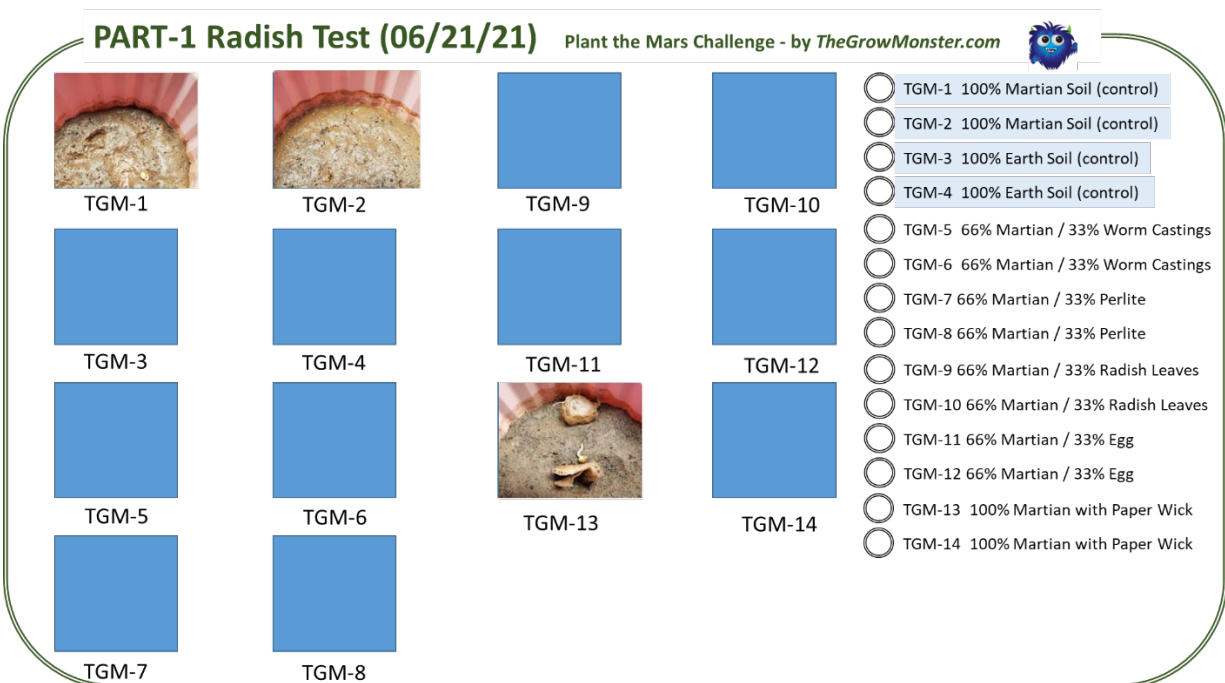


Figure 11

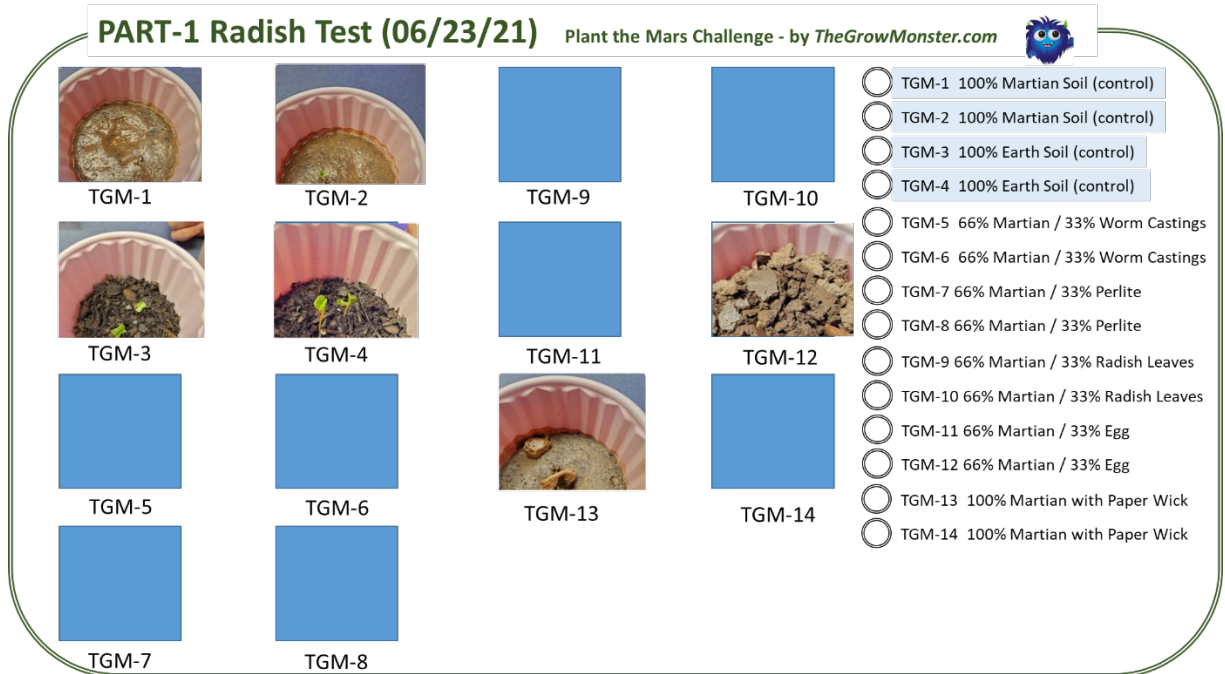


Figure 12

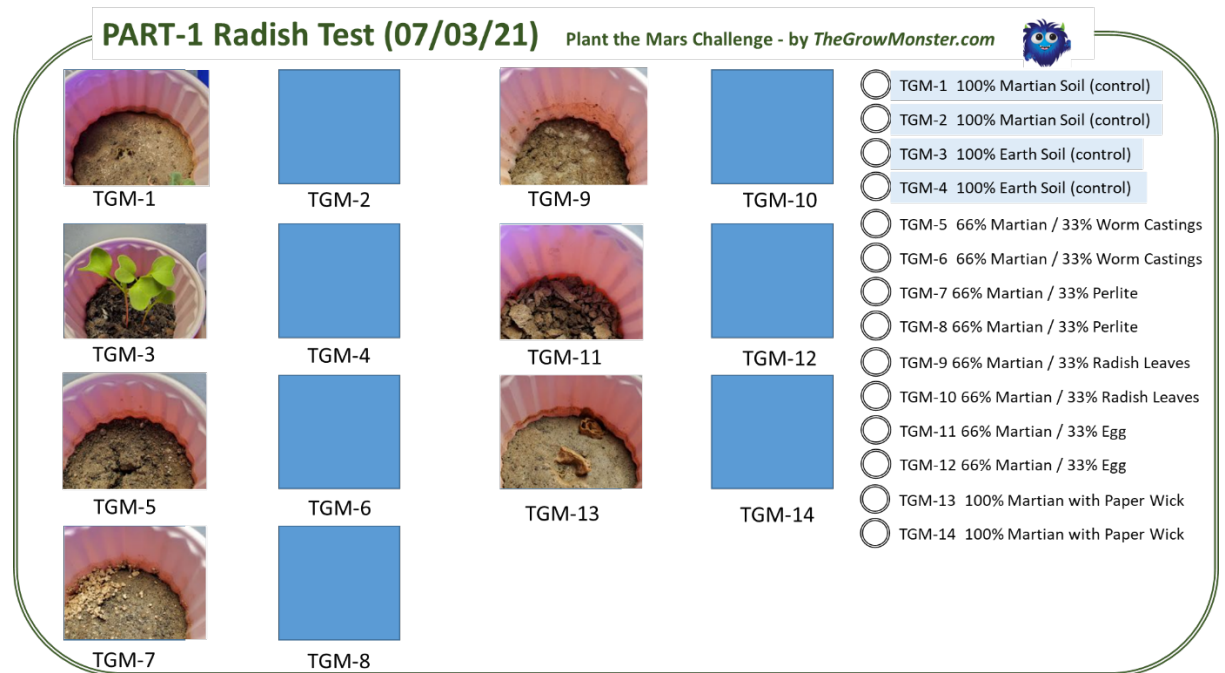


Figure 13

PART-1 Radish Test (07/21/21)

Plant the Mars Challenge - by TheGrowMonster.com

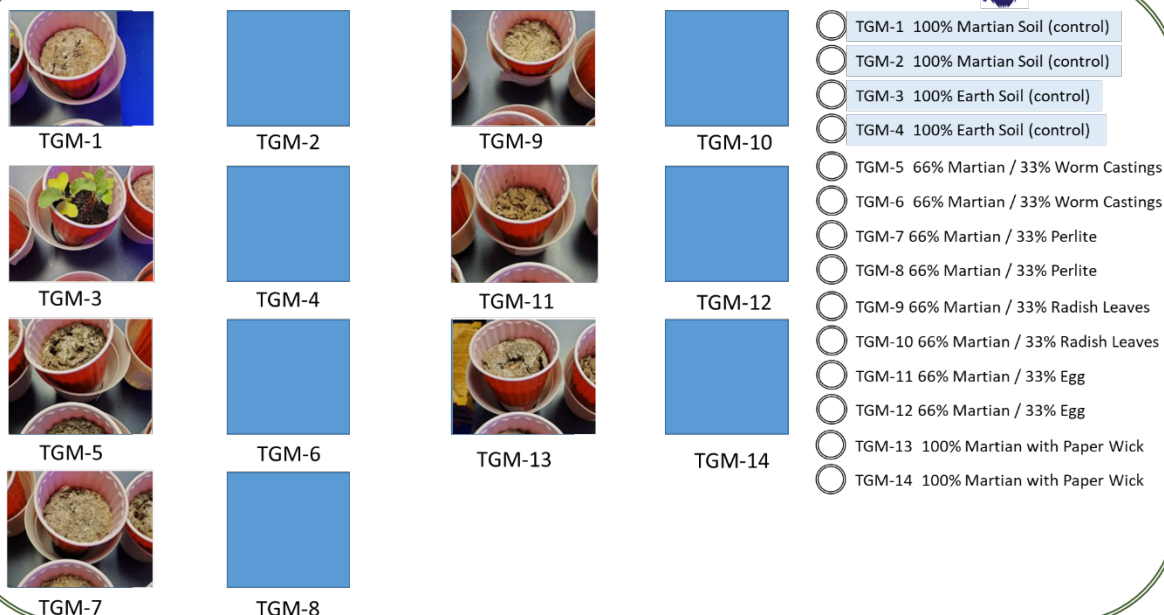


Figure 14

PART-1.2 Radish Observations

(with 1 tbsp of coffee grounds in each cup)

Plant the Mars Challenge - by TheGrowMonster.com

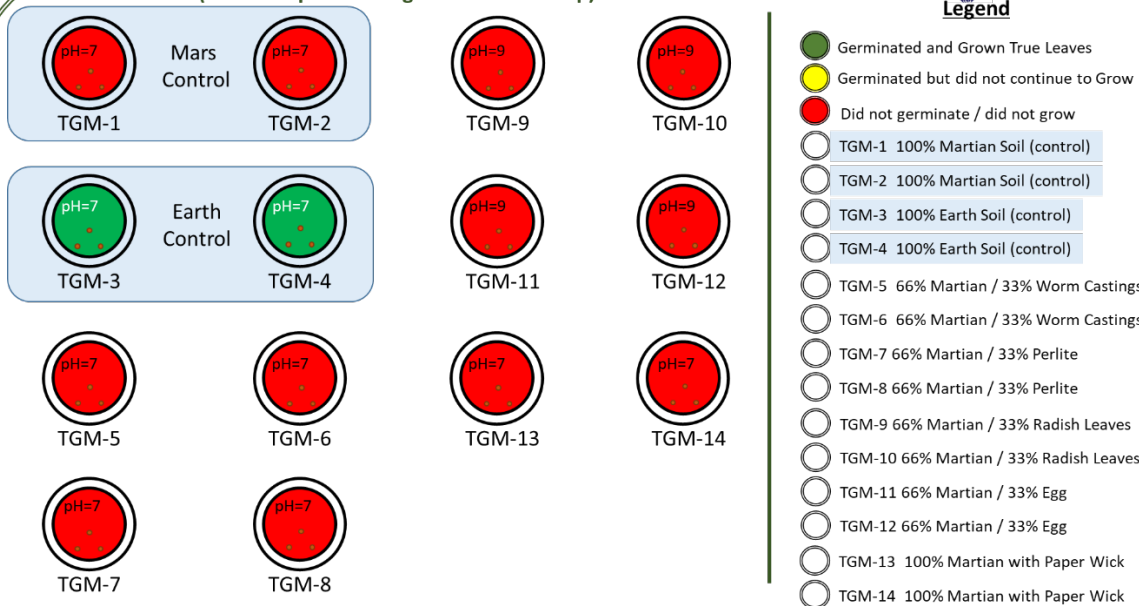
**Legend**

Figure 15

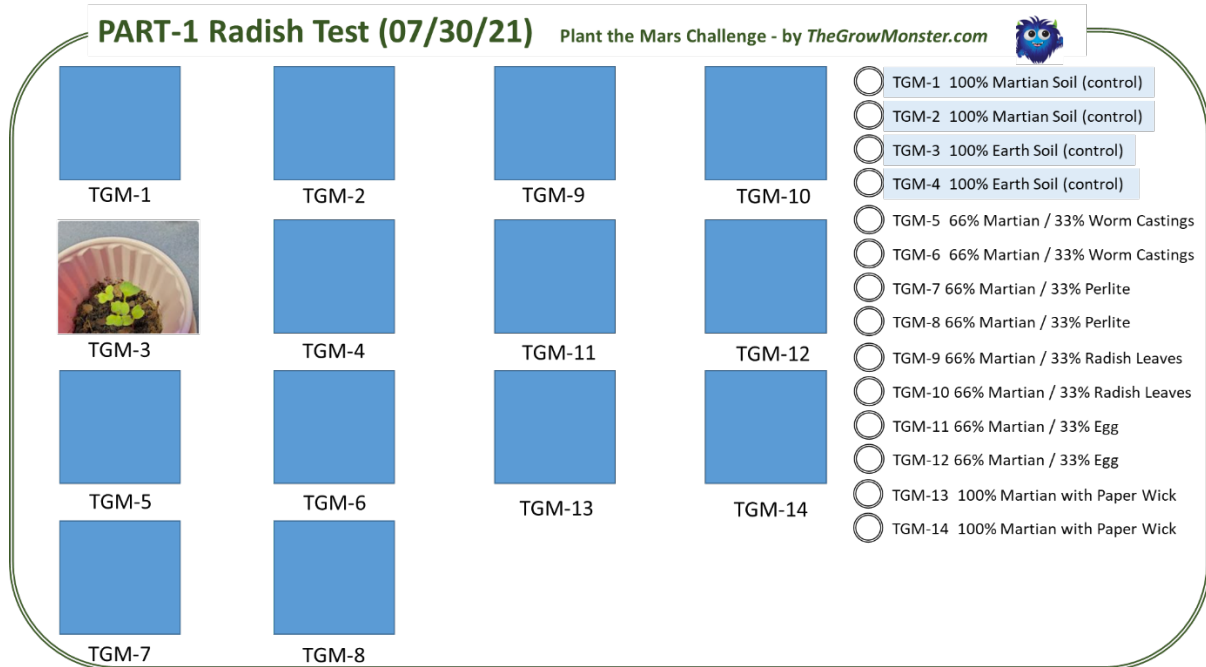


Figure 16

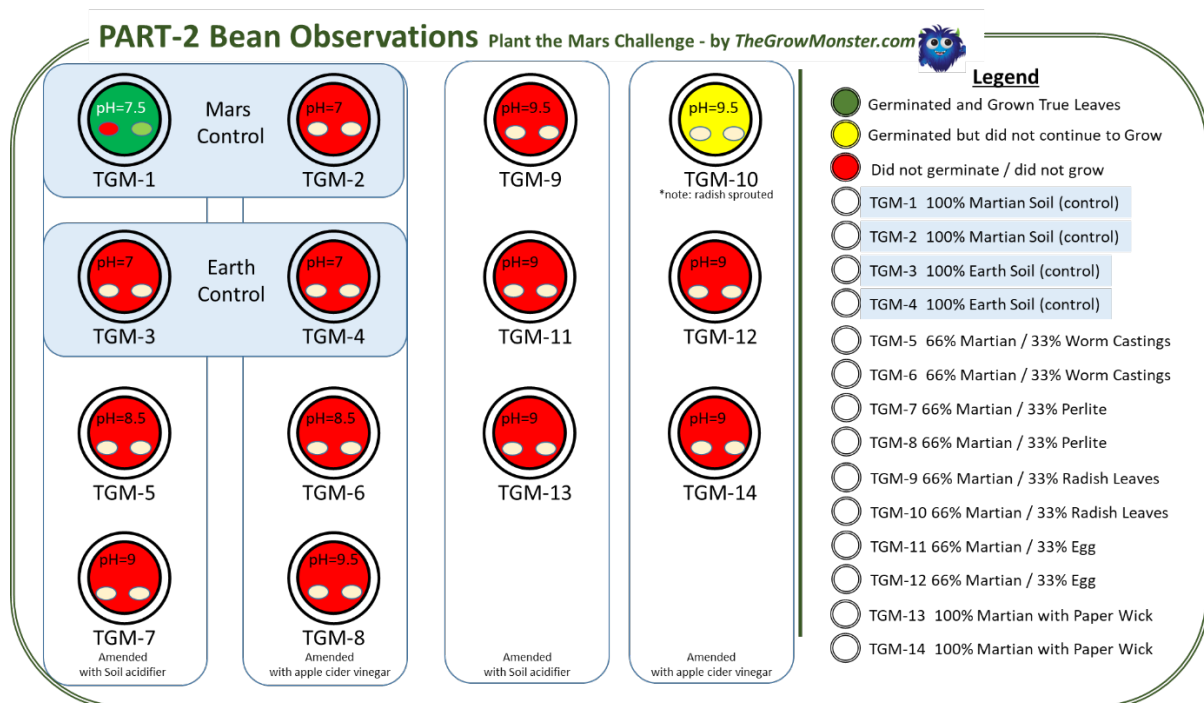


Figure 17

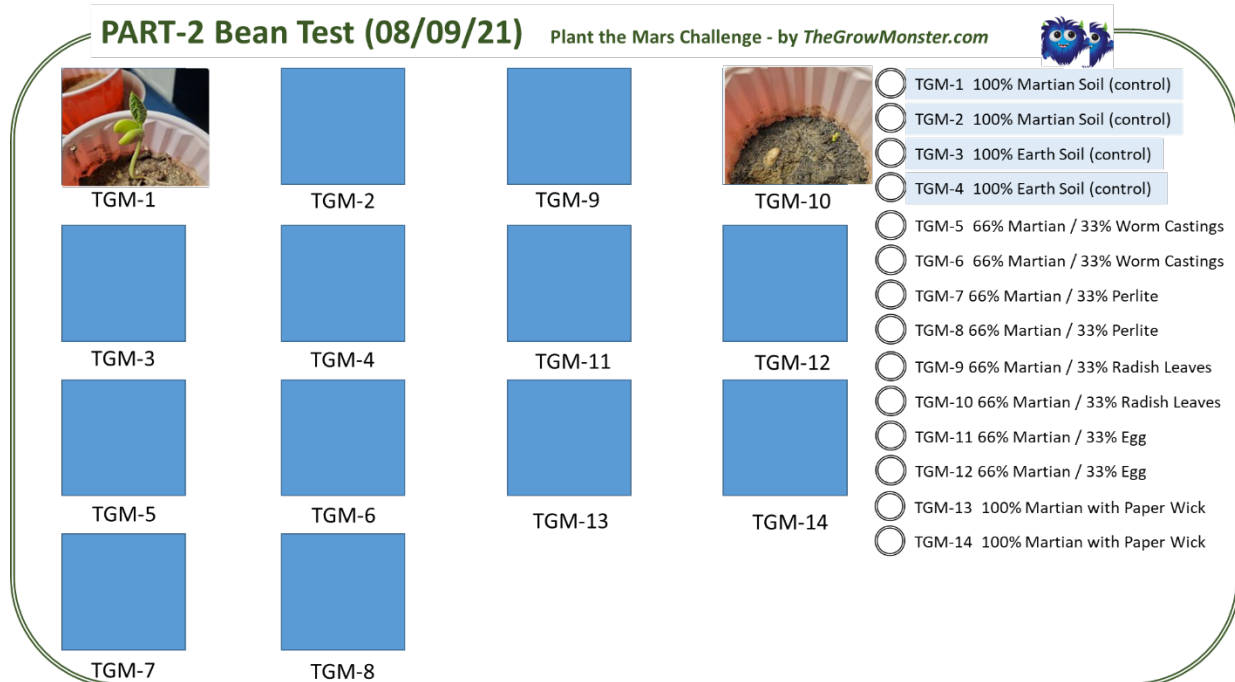


Figure 18

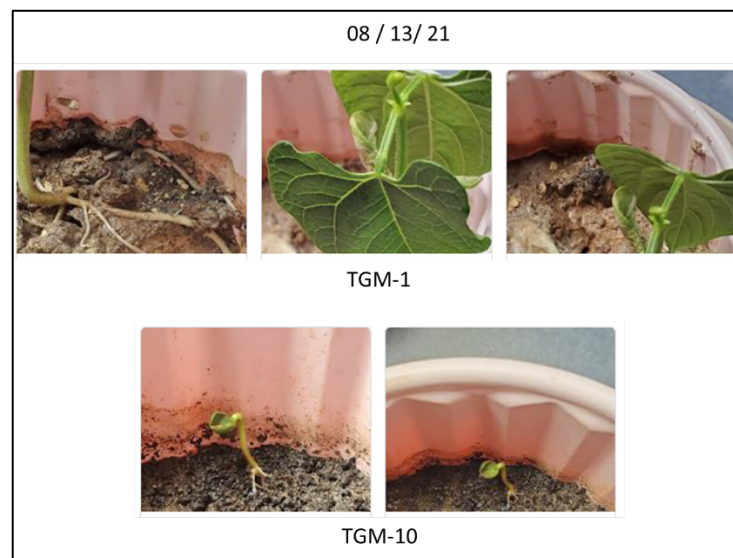


Figure 19

The biomass at the end of the first part of the experiment was higher in the Earth control soil compared to the Martian control and any of the other variables (Fig. 20). At the end of the 3rd part of the experiment, there was 1 bean that germinated and grew true leaves producing the largest biomass compared to all other variables in the first part of the experiment (Fig. 21)

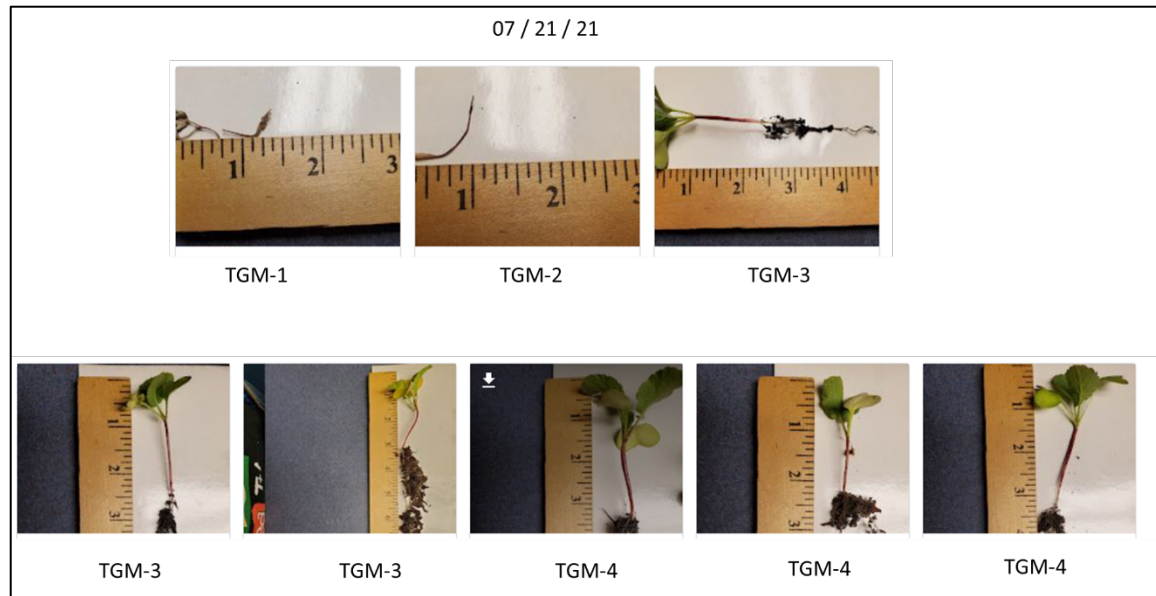


Figure 20

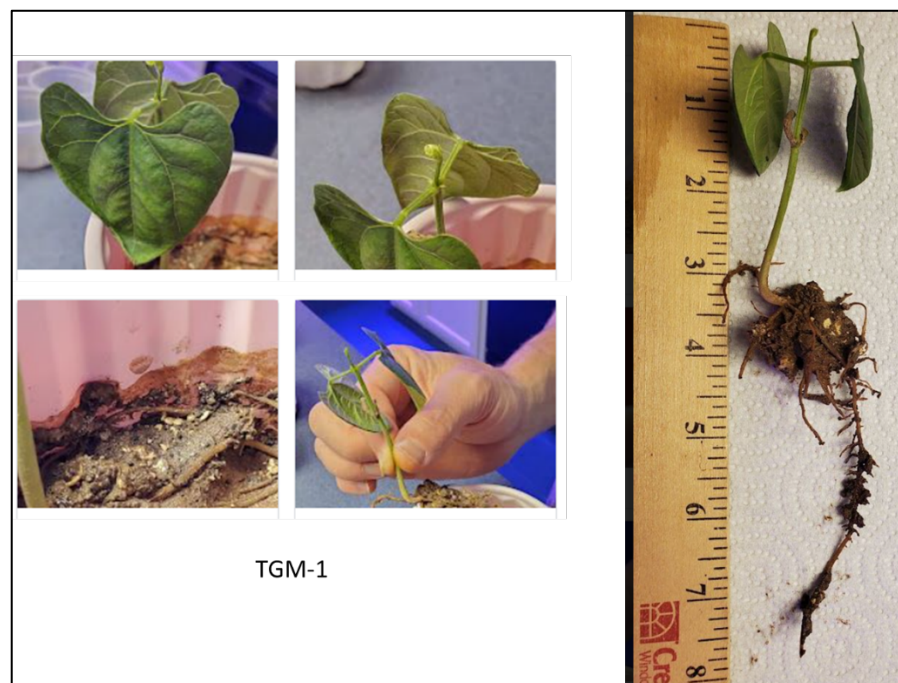


Figure 21

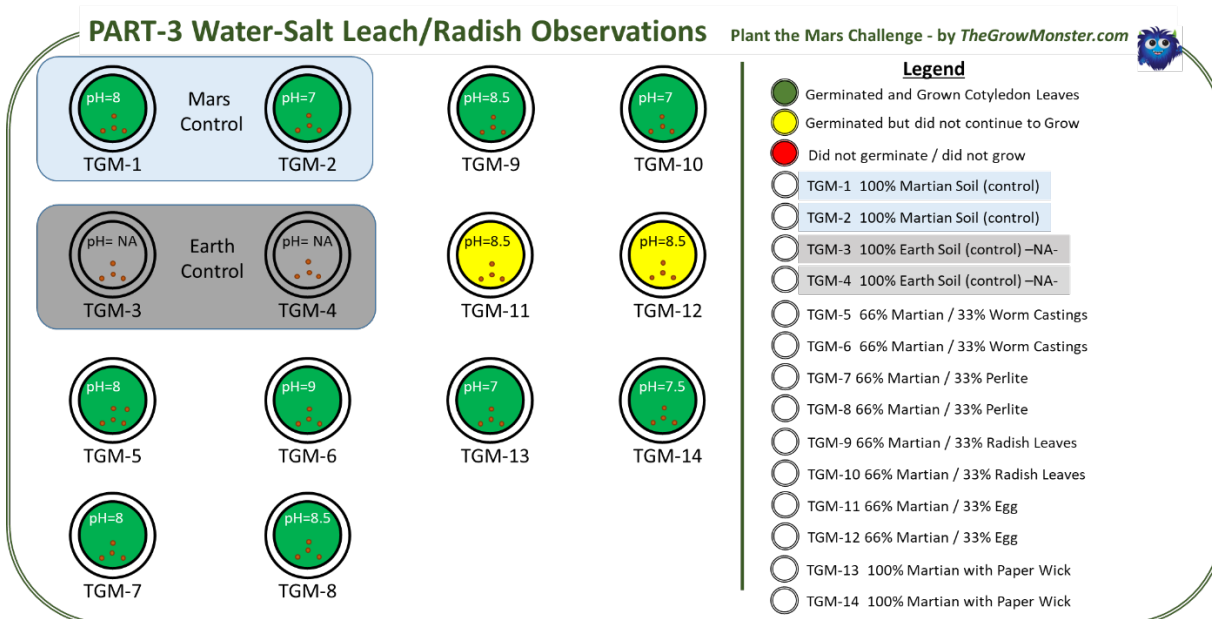


Figure 22

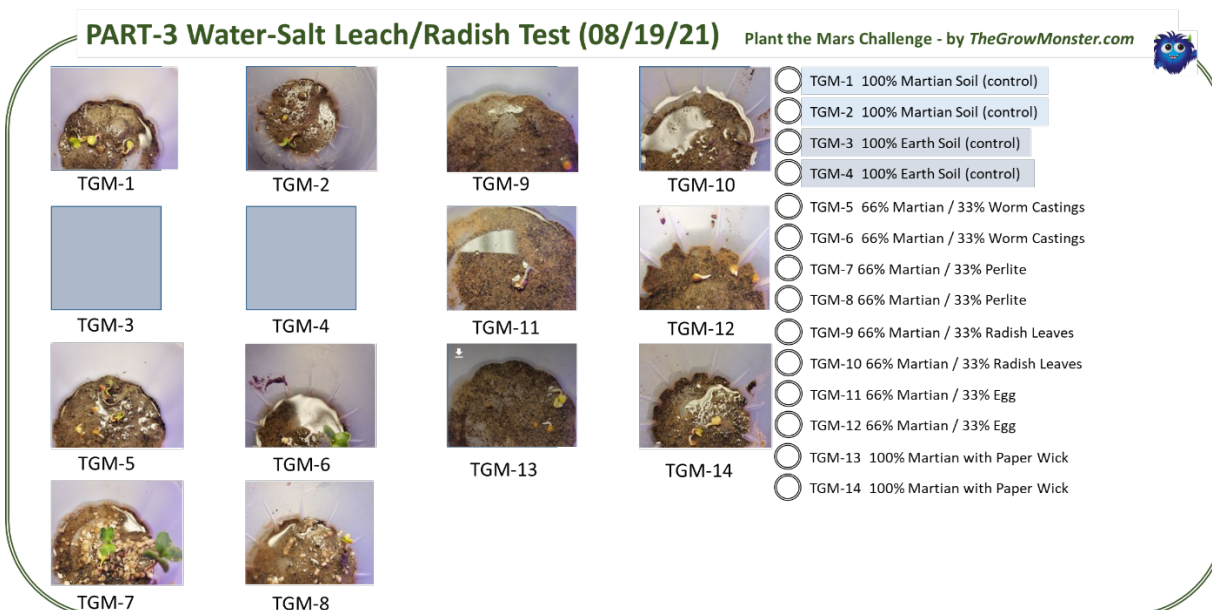


Figure 23

Discussion

We had germination and plant growth for both Radish Seeds and a Bean seed in the Martian regolith, which is consistent with prior research. However, we were not able to get any Radish seeds in Martian soil to grow true leaves and we had very low overall germination rates, especially in the Bean seeds. But we did have higher germination rates once we leached some of the salt from the Martian soil.

The biomass produced in the 100% Earth soil was much higher for the Radish seeds compared to the Martian soil and any of the other seeds that germinated shriveled before we could measure them. There was only biomass from 1 bean seed and that was the only one that germinated in the second part of the experiment. The Martian soil is very dense and compacted with very little to no nutrients for plant germination. Martian soil is also very good at retaining water, we noticed the pots were still wet and the water would sit on the surface for a while after watering. Because of this, we adjusted the amount we were watering down from 20mL a day to 10mL. After a few days of the 10mL watering, we started noticing a white crusty coating on the top of the Martian soil that is most likely a salt. This salt may be what is causing such a low germination rate in the seeds no matter what we added to the soil.

Another hurdle we encountered is the pH level being more alkaline in the Martian soil and variable containers. This higher pH level is not ideal for seed germination. We first tried to lower the pH with 1tbsp of unused coffee grounds, but this did not lower the pH. We then added 1 tbsp Sulphur Hydrangea food to half of the containers and 1 tbsp apple cider vinegar to the other half of the containers to lower the pH. This had mixed results with only 1 bean seed germinating. We then noticed a radish seed had germinated in a corner of TGM10 with the Radish leave variable group. The bean seed that had germinated and was still growing was also on the edge of the container TGM1 with 100% Martian soil. This led us to believe that maybe watering the soil was somehow washing the salt away from that side of the container and this could be why those seeds were able to germinate.

In order to see if the salt could be affecting germination rate, we leached some of the salt from the soil. The germination rate was much higher increasing from avg of about 17% for part 1 and 4% for part 2 to 80%. Further testing would be needed to see if those plants are able to grow longer and past the first true leaf stage.

PART-1.1 Radish Germination Results

21.4% germination rate

ID	Test Group	# planted	# germinated	germination rate
TGM-1	Martian Soil (control)	3	1	33.3%
TGM-2	Martian Soil (control)	3	1	33.3%
TGM-3	Earth Soil (control)	3	2	66.6%
TGM-4	Earth Soil (control)	3	2	66.6%
TGM-5	Worm Castings	3	0	0%
TGM-6	Worm Castings	3	0	0%
TGM-7	Perlite	3	0	0%
TGM-8	Perlite	3	0	0%
TGM-9	Radish Leaves	3	0	0%
TGM-10	Radish Leaves	3	0	0%
TGM-11	Egg (baked)	3	1	33.3%
TGM-12	Egg (baked)	3	1	33.3%
TGM-13	Paper Wick	3	1	33.3%
TGM-14	Paper Wick	3	0	0%

Figure 24

PART-1.2 Radish Germination Results (with 1 tbsp coffee grounds)

14.3% germination rate

ID	Test Group	# planted	# germinated	germination rate
TGM-1	Martian Soil (control)	4	0	0
TGM-2	Martian Soil (control)	4	0	0
TGM-3	Earth Soil (control)	4	4	100%
TGM-4	Earth Soil (control)	4	4	100%
TGM-5	Worm Castings	4	0	0
TGM-6	Worm Castings	4	0	0
TGM-7	Perlite	4	0	0
TGM-8	Perlite	4	0	0
TGM-9	Radish Leaves	4	0	0
TGM-10	Radish Leaves	4	0	0
TGM-11	Egg (baked)	4	0	0
TGM-12	Egg (baked)	4	0	0
TGM-13	Paper Wick	4	0	0
TGM-14	Paper Wick	4	0	0

Figure 25

PART-2 Bean Germination Results

4% germination rate

ID	Test Group	# planted	# germinated	germination rate
TGM-1	Martian Soil (control)	2	1	50%
TGM-2	Martian Soil (control)	2	0	0
TGM-3	Earth Soil (control)	2	0	0
TGM-4	Earth Soil (control)	2	0	0
TGM-5	Worm Castings	2	0	0
TGM-6	Worm Castings	2	0	0
TGM-7	Perlite	2	0	0
TGM-8	Perlite	2	0	0
TGM-9	Radish Leaves	2	0	0
TGM-10	Radish Leaves	2+1(radish)	1 (radish)	33%
TGM-11	Egg (baked)	2	0	0
TGM-12	Egg (baked)	2	0	0
TGM-13	Paper Wick	2	0	0
TGM-14	Paper Wick	2	0	0

Figure 26

PART-3 Water-Salt Leach/Radish Germination Results

80% germination rate

ID	Test Group	# planted	# germinated	germination rate
TGM-1	Martian Soil (control)	4	4	100%
TGM-2	Martian Soil (control)	4	4	100%
TGM-3	Earth Soil (control)	-NA-	-NA-	-NA-
TGM-4	Earth Soil (control)	-NA-	-NA-	-NA-
TGM-5	Worm Castings	5	5	100%
TGM-6	Worm Castings	4	3	75%
TGM-7	Perlite	4	3	75%
TGM-8	Perlite	4	3	75%
TGM-9	Radish Leaves	4	3	75%
TGM-10	Radish Leaves	4	1	25%
TGM-11	Egg (baked)	4	2	50%
TGM-12	Egg (baked)	4	4	100%
TGM-13	Paper Wick	4	3	75%
TGM-14	Paper Wick	4	4	100%

Figure 27

Germination is just one of the challenges faced growing plants in Martian soil simulant. We also encountered the issues of no aeration, no nutrition, and compaction which we tried to solve with the variables chosen. Adding organic matter to the soil from plants already produced, like Radish leaves or Bean leaves, could help solve those problems. We did notice that the pots with the Radish leaves and Martian soil simulant baked with egg had much better drainage and ability to retain moisture than the other pots. This is something to explore further- maybe adding a mixture of Martian soil baked with egg to Radish or Bean leaves could help provide nutrients to the soil through the organic matter while also providing the aeration so the Martian soil simulant isn't as compacted.

Conclusions

While seeds can germinate and grow in the Martian regolith simulant, plants will not be able to grow in the Martian regolith on their own. In addition to light, water, and climate requirements for plant growth that are not addressed by this study, the regolith itself will need to be amended.

There will need to be continued study on the effects of Martian regolith simulants with higher pH on plants along with how to reduce the salts that we suspect are preventing seed germination and stunting plant growth. Eventually scientists need to address the perchlorates that are found in actual Martian soil and toxic to humans, perhaps they can also be removed through water leaching. With salts and perchlorates removed via water leaching and/or other methods, we can begin to include in the discussion the addition of microbes to the regolith soil to make it truly come alive with nutrients for the plants to grow.

Therefore, we participate in events like Plant the Mars Challenge to find ways and methods to make the dream of colonizing Mars a reality. Can plants grow on Mars? Yes, and if we continue this research, we can make it happen. As the character Mark Watney from *The Martian* movie said, "They say once you grow crops somewhere, you have officially colonized it..."

Acknowledgments

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Author Contributions

Joseph and Therese Vogel from TheGrowMonster.com team conceived and designed the experiments, performed the experiments, analyzed the data, and wrote the paper. Joey Vogel (age 7) helped test pH in part 3 of the experiment. Michael Vogel (age 5), David Vogel (age 3), and Thomas Vogel (age 1) – patience testers.

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