The Influence of Endophytic Fungi on Plant Growth in a Martian Soil Simulant

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Introduction

There has been a major increase in both public and private interest regarding Martian colonization, however, the path to sustainable living on Mars remains hindered by logistics [1]. Participation within a NASA sponsored program through the Institute of Competition Sciences guided this project to conduct unique experimentation on crops that utilize a Martian regolith simulant. The independent variable of this experiment involves endophytic fungi, which is a microorganism that proliferates within plant cells [2]. A goal of NASA's Artemis II mission is to plant crops with native regolith, in addition to necessary foreign amendments; this is known as *in situ* resource utilization. Foreign amendments like organic compost are required in addition to native regolith due to the lack of a microbiome (life) and high soil salinity present in Martian regolith [3]. These features of Martian regolith are detrimental to plant health in the absence of organic amendments. The endophytic variable in this experiment was chosen for two main reasons: 1) Endophytic granules are light weight, which leads to decreased fuel requirements; 2) There is minimal research in the *in situ* field regarding how endophytic inoculations may affect plant growth in a Martian regolith simulant [4]. The experimental methods used two sets of growing pots to vary the recommended dosage of microorganisms. This research hypothesizes that while endophytic inoculations will increase overall prosperity, the minimum volume thereof will produce equal prosperity to that of the maximum volume.

Materials

- NASA's PTMC Project Guide
- Concord's Tap Water
- 7 planting pots
- LED grow lamps
- 1 kg Exolith's Martian Regolith
- 1 kg Timberline's Organic Compost
- 2 oz Mikro-root granules
- Organic seeds: *S. oleracea*, *B. oleracea*, & *R. sativus*
- 5--375 mL sized growth pots
- 2—1000 mL sized growth pots

Pot Name	Regolith Amendment	Compost Amendment	Endophytic Dose
Compost control	0 mL	1000 mL	NO DOSE
Regolith	850 mL	150 mL	
50/50 control	188 mL	188 mL	
Maximum #1 & #2	188 mL	188 mL	TWO DOSES
Minimum #1 & #2	188 mL	188 mL	ONE DOSE

Table 1. Composition of each experimental pot

Methods

The growth period began on February 5th, with seeding of *S. oleracea* within all pots; here, a dry application of endophytic granules took place on the soil mixture surface. Watering was completed in accordance with soil moisture and LED lights were cycled at 12- hour intervals. Weeks 3 & 4 experienced re-seeding of *B. oleracea* and *R.* sativus, respectively, due to a lack of germination. Upon germination at week 5, the independent variable was incorporated: dates of inoculations are recorded in Table 2. Using a water suspension of 2.5 g microbes dissolved in 1 gallon of water, the maximum pots were dosed twice, at 14-day intervals, and the minimum pots were dosed once.

Pot Name	Dry Application Weight, Date	Water Suspension Volume, Date
Max. #1	2.5 g, February 5th, 2024	25 mL, March 4th & 18th, 2024
Max. #2	2.5 g, February 5th, 2024	25 mL, March 4th & 18th, 2024
Min. #1	2.5 g, February 5th, 2024	25 mL, March 4th, 2024
Min. #2	2.5 g, February 5th, 2024	25 mL ,March 4th, 2024

Table 2. Timeline of endophytic inoculations

Results

Quantitative data is summarized in Table 3, while qualitative data is summarized in Figures 1 & 2. The 2nd minimum pot produced two, small sprouts from week 4-6, however, leading up to the termination of the growth period, both sprouts within this pot had died. The regolith pot had produced no sprouts during the period.

Pot Name	Cumulative Crop Weight	# of Sprouts
Compost control	.752 grams	9 sprouts
50/50 control	.079 grams	1 sprout
Regolith	NO GROWTH	
Maximum #1	.084 grams	2 sprouts
Maximum #2	.048 grams	1 sprout
Minimum #1	.050 grams	1 sprout
Minimum #2	DEATH UPON TERMINATION PERIOD	

Table 3. Results upon termination of the growth period: Week 9

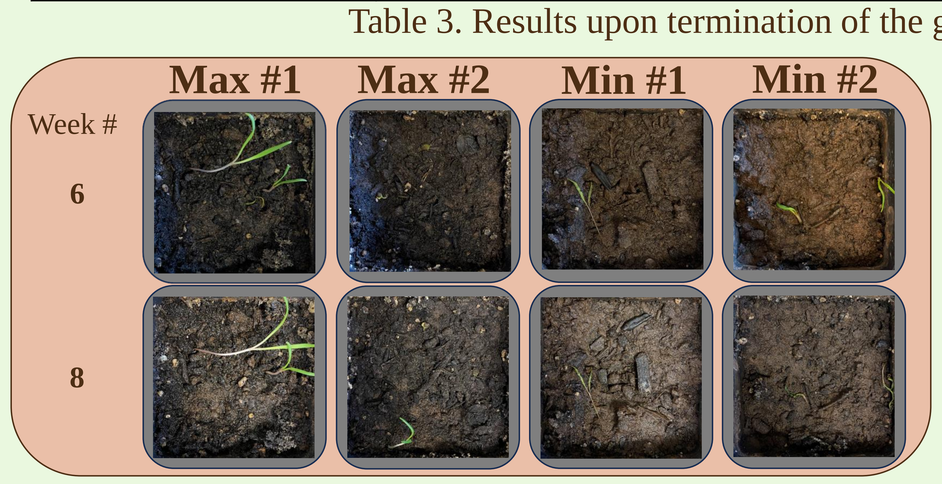

Figure 1. Experimental Pot Results

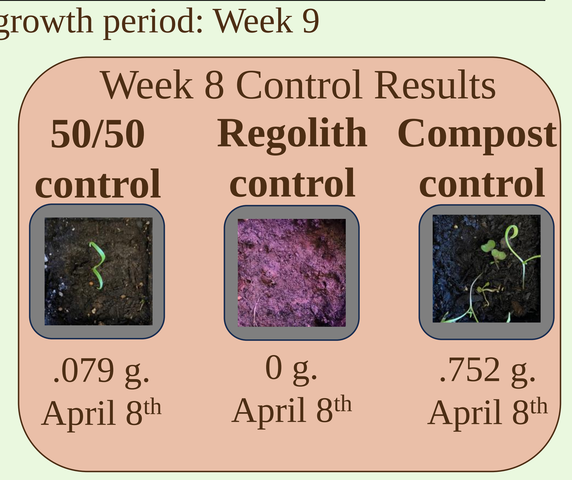

Figure 2. Control Pot Results

Conclusions

Mars Global Simulant-1, MGS-1, produced by the University of Central Florida's Exolith lab, is a comprehensive standard for Martian soils based off mineralogy samples sourced from NASA's Curiosity rover [5]. While this simulant does not contain many toxic components of native Martian regolith like calcium perchlorate, this simulant maintains an absence of a microbiome, as well as increased salinity due to the high levels of sodium, Na, and potassium, K, added to the product.

The factors mentioned would hinder crop proliferation within a pure simulant growth mixture, however, the experimental pots utilized in this research were composed only of 50% regolith, therefore, the lack of crop viability that resulted from this experiment may have been due to reasons other than the regolith simulant composition.

While the relative ratio of regolith to organic compost stayed consistent across the experimental pots, the size of the growth pots did vary. The compost control pot and the regolith pot were situated within 1000 mL containers whereas the experimental pots and 50/50 control pot were situated within 375 mL containers. This variability within the container sizes may have had a negative effect on the experimental results.

The inferences regarding varied volumes of endophytic inoculations on plant prosperity in a Martian soil simulant produced by this experiment are inconclusive. While the data suggested that the pots dosed with the maximum level of endophytic inoculant produced the greatest mass, the small sample size, as well as the initial challenges pertaining to germination, may have caused inaccurate representations of the independent variable. Extensive samples, as well as a more controlled seeding method are suggested for future research involved with endophytic fungi on plant prosperity in a Martian soil simulant.

References & Acknowledgments

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