Ozarks on Mars: The Effects of Azotobacter and Mycorrhizal Fungi on Red Globe Radishes

PTMC Fall 23

Team # 16126

Roberca Chery

Patrice Dames

Casey Gomez

Dylan Herring

Jessie Berth

Alexandra Kasper

Benjamin Stormes

Coach: Dr. Christie Sampson

Introduction

Space, a place long thought to be out of reach, grows ever closer as humankind continues to innovate and reach for the stars. Looking to continue the mission starting back on July 20, 1969, with first man landing on moon and the work of the scientist who on July 4th, 1997, landed the first rover on the moon. Nasa is looking too further these momentous feats and establish settlements on these astral bodies. The Artemis program currently under development at the National Aeronautics and Space Administration (NASA) seeks to create long-term living conditions first on the moon and then on Mars. A critical aspect of living on these celestial bodies is a food source cultivated on the land.

The Plant Mars challenge seeks the help of competitors to find innovative ways to cultivate this otherwise desolate land. A group of seven diverse students from the University of the Ozarks in Clarksville, Arkansas put their minds to work solving these issues. The group had to decide on two things with one goal in mind, plant mars. The first thing to consider what to plant: the scarlet globe radish was chosen for its fast growth period, high soil and drought tolerability, and health benefits. The second thing to consider was the additives for the soil. The Martian soil and environment presented several issues for plants to thrive with water and nutrient retention being the focus of our additives. The two additives we focused on were azotobacter and mycorrhizal fungi. Azotobacter is a nitrogen fixer used commonly as a biofertilizer. Mycorrhizal fungi are a symbiotic fungus with the purpose of improving water intake as well as nutrient intake. The goal of this project is to see what additive mixture produces the healthiest plants with the most water retention.

Theory

Martian regolith is a very dry sandy soil with a variety of nutrients spread across the planet. The soil provided by the plant mars competition matched this composition well, being extremely dry and dusty. The soil needed a few things to be used as proper farming soil organic matter, nutrients, and water. To fix these the group theorized that the azotobacter and mycorrhizal fungi would be efficient and as well as light enough to include in space travel. Both azotobacter and mycorrhizal fungi improve water uptake and nutrient fixation. Studies have shown that azotobacter makes plants drought resistant and very adept at fixing nitrogen to the soil (1). Mycorrhizal fungi are also shown through study to benefit the plants in nutrient fixing of most minerals having more effect in water poor environments though they do say this may be because water rich environments wouldn't need to rely on this symbiotic relationship as much (2&3). The organic matter needed for these plants was provided by a potting soil mix containing moss, bark, and other organic particulates.

Measurement Methods

Once the soil was received, it was prepped with its initial mix of potting soil and water. Following this it was split into two batches, one group containing the mycorrhizal fungi and the other with no additives, both were mixed at a 50/50 mix. This soil was then spilt up into 23 small cups. The cups were split into 4 quadrants with each quadrant of the cup getting 1 scarlet globe radish seed. The cups were then split up into groups based on their additives as well as control groups for the soil and water see *Table 1*. Looking at *Table 1* it is noticeable that there is one more factor than tested this was due to time and money constraints on the project. The not tested group was black soldier fly larvae (B) compost made from biodegradable containers. These were not used due to premature death of the larvae and lack of time too reset new larvae in a compost container. The cups included a control water cup with no plants and a control with plants but no additives aside from potting soil. The azotobacter was added once a week every week throughout the experiment for consistency. We did replant.

Sampling Design

Groups	# of Individuals
AZ+M+P	**3**
AZ+M+P +B	**3**
M+P Control	**4**
MY+**M+P**	**3**
AZ + MY+ **M+P**	**3**
AZ + MY+**M+P+**B	**3**
Water Control	**1**
MY M+P	**3**
Total	**23**

Table 1 Distribution of additives Black soldier fly (B) Azotobacter (AZ) Mars Soil (M) Potting Soil (P) Mycorrhizal Fungi (MY)

The cups themselves had a small hole put in the bottom that a cotton water wick was ran through to keep the soil moisture up and to reduce water waste *Figure 1*. These cups were placed into larger cups that we filled with 200ml of distilled water. The cups were then placed in an incubator set to 29.44 degrees Celsius and 95% humidity, with a UV light cycling off and on with a 12-hour timer. The plants were checked twice a week

Figure 1 Diagram of wick cup

for moisture, growth, and water intake. The moisture is measured with the provided moisture meter, growth using a ruler (cm), and water intake using a graduated cylinder (ml). We used a Govee thermometer to keep track of the temperature and humidity throughout the experiment.

Analysis and Results

Our plants had growth in almost all groups aside from the water control and one of the mycorrhizal +B group. To measure the success of each group we took the averages for each measurement day then we took those averages and averaged all of them together to get our overall total. We measure plant growth to how much the additives improved the growth rate and height of the greens. Looking at *Figure 2* we can see a comparison of the growth of each soil type in cm. The results of our growth from all the additives found that the control soil group had the most growth overall. The next closest by only .02 centimeters is the combination of azotobacter and mycorrhizal fungi mix. With the next three following all being spin offs of the azotobacter. This helps support the idea that the azotobacter additives were effective in increasing plant growth in the regolith. This is however not to overlook the growth we see in the control group which has managed to keep pace exceedingly well with the azotobacter, suggesting its effects might not be as important as they may seem. The Mycorrhizal fungi on the other hand saw the least amount of growth between its groups, only managing to grow at its best with azotobacter present in the soil.

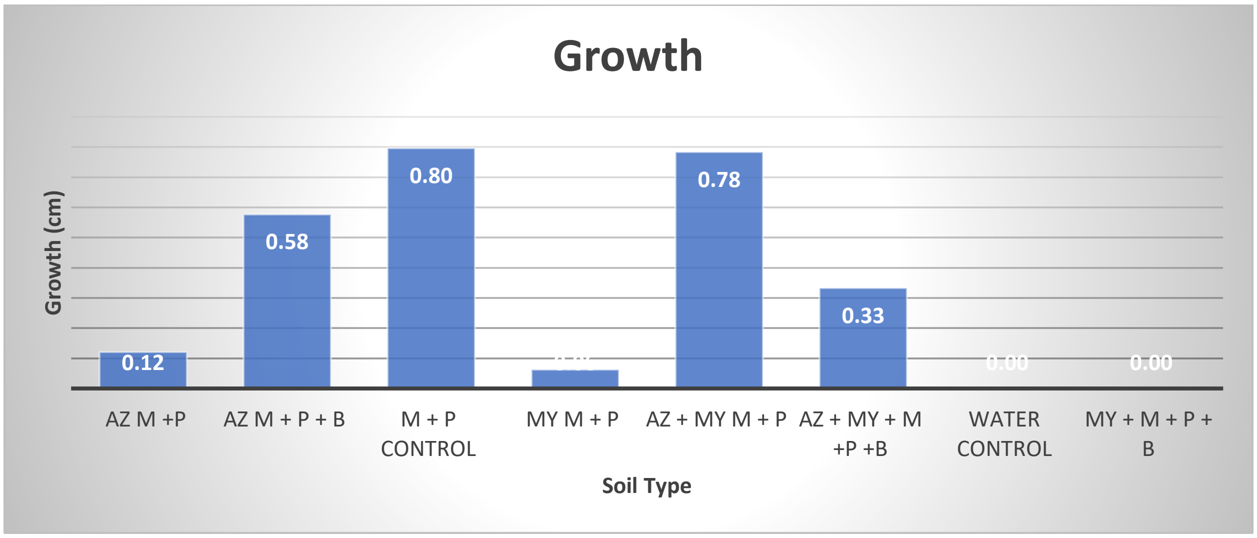


Figure 2 Growth by soil type

Looking at the moisture we can see how well the different additives retained moisture within the soil. *Figure 3* shows the average moisture of each soil type tested. All the soils stayed relatively moist with or without the additives still comparing them nonetheless we can see that highest moisture levels were found in the azotobacter and the controls all having 9.0 and above. These results do support the idea that the azotobacter is helping the soil keep moisture if only by a slim margin compared to the other additives and the control. We can also see here that the mycorrhizal fungi are not helping retain the water to the soil this could lend to the idea discussed earlier in the paper without the plant being in a more stressful environment the symbiotic relationship is having a harder time forming between the two specimens.

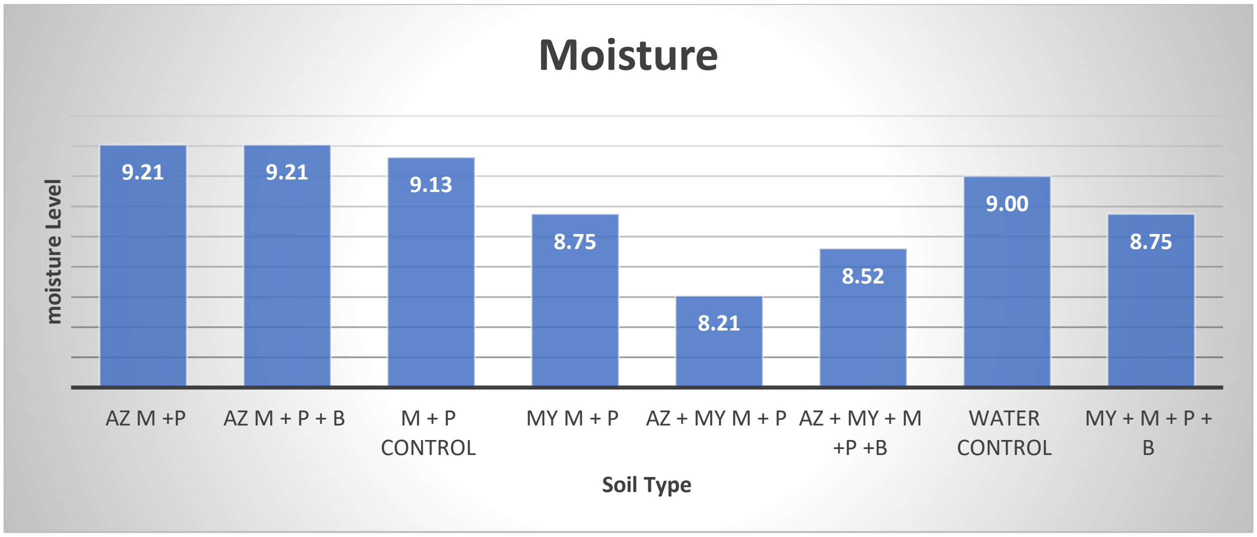


Figure 3 Moisture Levels by soil type

The water intake of each plant was also measured and averaged to see how well the additives increased water consumption of the plants. Looking to *Figure 4* we can observe the highest water consumers to be the azotobacter all of which are below 130 aside from the azotobacter and mycorrhizal mix which is 133.33. The lowest intake was seen in the controls which took in roughly 10ml less in water than all the azotobacter did. We can see her that azotobacter does use more water than the rest of the additives and control this could be due to the better utilization of it keeping the soil moister or possibly an inefficiency of the azotobacter this could warrant further study.

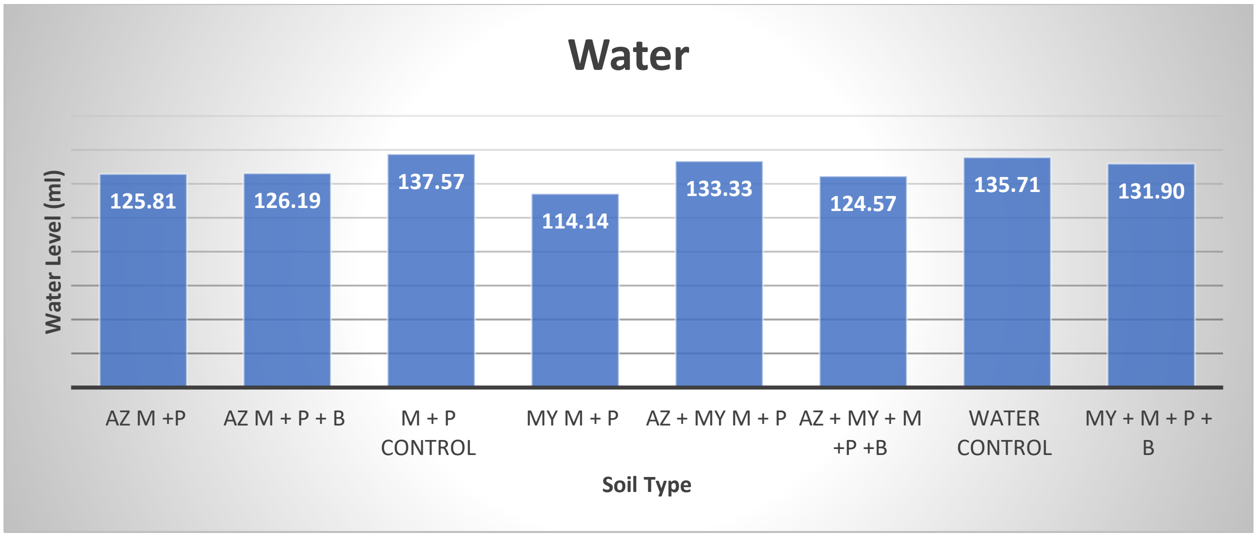


Figure 4 Water intake by soil type

Discussion and Conclusions

The point of this experiment was to improve soil quality of Martian regolith to allow for future colonization of the red planet. The way we sought to improve the land had very clear results. The most advantages way to increase water intake while fixing more nutrients to the soil and plant is to use azotobacter. Azotobacter groups in our study had the best results out of all the groups aside from maybe the control who slightly outgrew it but didn't take as much advantage of its water supply. The azotobacter also managed to hold onto the most moisture in its soil managing out compete but not by a large amount. The best result of the azotobacter came when it was used alone and not mixed with other additives. The ones mixed with additives had more varied results though the mixture could use more study on a longer scale looking at more parameters than just water consumption. The results did not come without their own problems and setbacks.

We had a low germination success rate causing us to replant most of the seedlings with a few successful seedlings still managing to push through. Other issues faced included consistency in the humidity and temperature due to malfunctions in our growth chamber. We also had some incomplete data as one of the data collections days no data was recorded for water level this week was left out to avoid anomalies in the data. To fix these issues we changed the power supply to the system and monitored the chamber more regularly. The humidifier was also automated to reduce human error. The soil itself stayed moist but as for the plant itself it seems to have shriveled up.

The Martian soil proved to be relatively tolerable for the plants with the added potting soil with no additives performed better than expected. This could lead to the idea that less is more for growing these plants. The additives could be consuming more resources such as a precious water supply on a dry rock than what they are making up for in growth and moisture retention. This is not to say that azotobacter didn’t perform well but does this small increase warrant its inclusion. In future studies we would like to see if azotobacter had any more positive effects specifically in nutrient uptake as well as the weight of the plant post growing season compared to its control counterpart. These additives could be the next step closer needed to prepare the Artemis mission for a sustainable life on other worlds.

References

1. Frew, A. (2023). Water availability alters the community structure of arbuscular mycorrhizal fungi and determines plant mycorrhizal benefit. *PLANTS, PEOPLE, PLANET*, *5*(5), 683–689. https://doi.org/10.1002/ppp3.10372
2. Jain, D., Sharma, J., Kaur, G., Bhojiya, A. A., Chauhan, S., Sharma, V., Suman, A., Mohanty, S. R., & Maharjan, E. (2021). Phenetic and Molecular Diversity of Nitrogen Fixating Plant Growth Promoting Azotobacter Isolated from Semiarid Regions of India. *BioMed Research International*, *2021*, 1–9. https://doi.org/10.1155/2021/6686283
3. Shafiq, M., Casas-Solís, J., Neri-Luna, C., Kiran, M., Yasin, S., González-Eguiarte, D. R., & Muñoz-Urias, A. (2023). Arbuscular Mycorrhizal Fungi as a Plant Growth Stimulant in a Tomato and Onion Intercropping System. *Agronomy*, *13*(8), 2003. https://doi.org/10.3390/agronomy13082003

Appendixes

	10/6/2023			10/13/2023		
fertilization type	moisture	growth(cm)	water (mL)	moisture	growth(cm)	water (mL)
AZ M +P (1)	8.00	0.00	170.00	10.00	0.00	115.00
AZ M +P (2)	7.00	0.00	152.00	10.00	0.00	110.00
AZ M +P (3)	10.00	0.00	160.00	10.00	0.00	118.00
Average	8.33	0.00	160.67	10.00	0.00	114.33
AZ M + P + B	10.00	1.80	150.00	10.00	0.00	112.00
AZ M + P + B	10.00	2.00	155.00	10.00	0.00	116.00
AZ M + P + B	10.00	3.50	250.00	10.00	2.00	112.00
					1.00	
Average	10.00	2.43	185.00	10.00	0.75	113.33
M + P Control (1)	10.00	5.00	160.00	10.00	3.50	128.00
M + P Control (2)	8.00	0.00	145.00	10.00	4.50	110.00
M + P Control (3)	8.00	1.40	209.00	10.00	1.50	115.00
		0.50			0.50	
M + P Control (4)	10.00	0.00	160.00	10.00	1.50	100.00
					1.00	
Average	9.00	1.38	168.50	10.00	2.08	113.25
MY M + P (1)	5.00	0.00	140.00	8.00	0.00	100.00
MY M + P (2)	8.00	0.00	119.00	10.00	0.00	127.00
MY M + P (3)	10.00	1.50	152.00	10.00	0.00	115.00
Average	7.67	0.50	137.00	9.33	0.00	114.00
AZ + MY M + P (1)	5.50	0.00	170.00	10.00	0.00	123.00
AZ + MY M + P (2)	6.00	0.00	164.00	10.00	6.00	120.00
					6.00	
AZ + MY M + P (3)	8.00	0.00	156.00	10.00	0.00	110.00
Average	6.50	0.00	163.33	10.00	3.00	117.67
AZ + MY + M +P +B (1)	6.50	0.00	180.00	10.00	0.00	129.00
AZ + MY + M +P +B (2)	10.00	0.00	117.00	10.00	0.00	100.00
AZ + MY + M +P +B (3)	8.00	0.00	154.00	10.00	0.00	120.00
Average	8.17	0.00	150.33	10.00	0.00	116.33
Water Control	7.00	0.00	180.00	10.00	0.00	124.00
MY + M + P + B (1)	7.00	0.00	162.00	10.00	0.00	128.00
MY + M + P + B (2)	7.00	0.00	153.00	10.00	0.00	110.00

MY + M + P + B (3)	10.00	0.00	157.00	10.00	0.00	120.00
Average	8.00	0.00	157.33	10.00	0.00	119.33

	10/16/2023			**10/20/2023**		
fertilization type	moisture	growth(cm)	water (mL)	moisture	growth(cm)	water (mL)
AZ M +P (1)	8.00	0.00	135.00	9.00	0.00	
AZ M +P (2)	9.00	0.00	142.00	10.00	0.00	
AZ M +P (3)	9.00	0.00	132.00	10.00	0.00	
Average	8.67	0.00	136.33	9.67	0.00	
AZ M + P + B	7.00	0.00	136.00	10.00	0.00	
AZ M + P + B	7.00	0.00	142.00	10.00	0.00	
AZ M + P + B	8.00	2.50	146.00	10.00	1.80	
Average	7.33	0.83	141.33	10.00	0.60	
M + P Control (1)	8.00	4.00	138.00	10.00	3.50	
M + P Control (2)	7.00	0.00	202.00	10.00	0.00	
M + P Control (3)	7.00	0.00	138.00	10.00	0.00	
M + P Control (4)	7.00	2.00	202.00	10.00	0.00	
Average	7.25	1.50	170.00	10.00	0.88	
MY M + P (1)	6.00	0.00	133.00	10.00	0.00	
MY M + P (2)	6.00	0.00	107.00	9.00	0.00	
MY M + P (3)	7.00	0.00	105.00	10.00	0.00	
Average	6.33	0.00	115.00	9.67	0.00	
AZ + MY M + P (1)	8.00	0.00	143.00	10.00	4.00	
AZ + MY M + P (2)	6.00	5.00	148.00	7.50	0.00	
AZ + MY M + P (3)	7.00	0.00	157.00	10.00	0.00	
Average	7.00	1.67	149.33	9.17	1.33	
AZ + MY + M +P +B (1)	5.00	0.00	143.00	10.00	0.00	
AZ + MY + M +P +B (2)	4.00	0.00	158.00	10.00	0.00	
AZ + MY + M +P +B (3)	8.00	0.00	110.00	10.00	0.00	
Average	5.67	0.00	137.00	10.00	0.00	
Water Control	7.00	0.00	151.00	10.00	0.00	
MY + M + P + B (1)	7.00	0.00	156.00	10.00	0.00	
MY + M + P + B (2)	5.00	0.00	145.00	10.00	0.00	

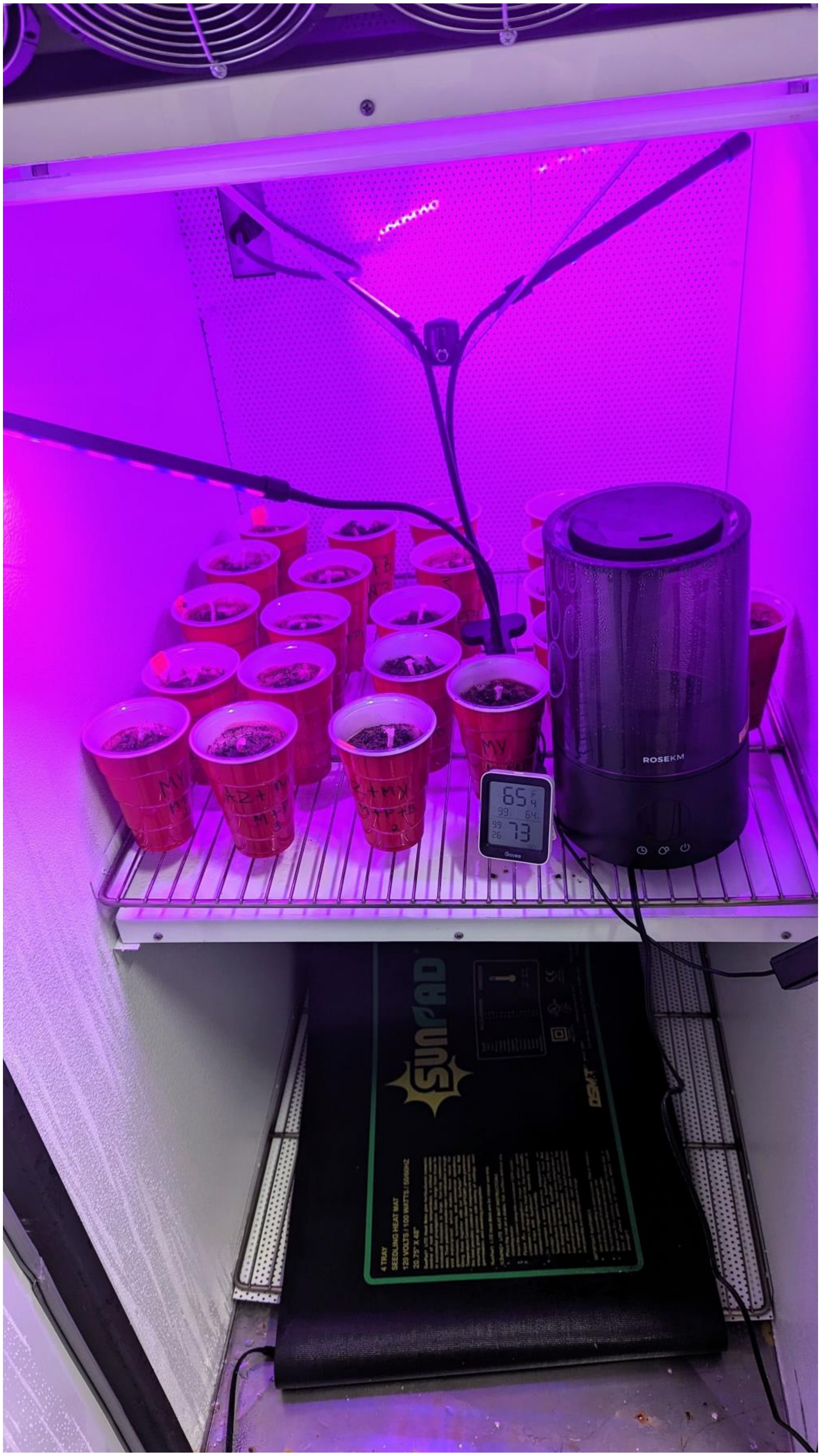
MY + M + P + B (3)	6.00	0.00	156.00	10.00	0.00	
Average	6.00	0.00	152.33	10.00	0.00	

	11/3/2023			**11/10/2023**		
fertilization type	moisture	growth(cm)	water (mL)	moisture	growth(cm)	water (mL)
AZ M +P (1)	10.00	0.00	113.00	10.00	0.00	150.00
AZ M +P (2)	10.00	0.00	110.00	9.00	0.00	107.00
AZ M +P (3)	10.00	0.70	100.00	10.00	1.00	110.00
					0.20	
Average	10.00	0.23	107.67	9.67	0.30	122.33
AZ M + P + B	10.00	0.00	112.00	9.00	0.00	110.00
AZ M + P + B	10.00	0.00	100.00	10.00	0.00	100.00
AZ M + P + B	10.00	0.00	110.00	10.00	0.00	113.00
Average	10.00	0.00	107.33	9.67	0.00	107.67
M + P Control (1)	10.00	0.50	118.00	9.00	0.00	110.00
M + P Control (2)	10.00	0.00	115.00	10.00	0.30	120.00
M + P Control (3)	10.00	0.90	135.00	10.00	0.00	135.00
M + P Control (4)	10.00	0.00	127.00	10.00	0.00	120.00
Average	10.00	0.35	123.75	9.75	0.08	121.25
MY M + P (1)	10.00	0.00	100.00	9.00	0.00	100.00
MY M + P (2)	9.00	0.00	110.00	9.00	0.00	100.00
MY M + P (3)	10.00	0.00	122.00	10.00	0.00	123.00
Average	9.67	0.00	110.67	9.33	0.00	107.67
AZ + MY M + P (1)	8.00	0.00	115.00	8.00	0.00	115.00
AZ + MY M + P (2)	10.00	0.80	115.00	9.00	0.00	120.00
AZ + MY M + P (3)	8.00	0.00	117.00	8.00	0.00	127.00
Average	8.67	0.27	115.67	8.33	0.00	120.67
AZ + MY + M +P +B (1)	10.00	1.20	120.00	9.00	2.00	120.00
AZ + MY + M +P +B (2)	8.00	0.00	125.00	9.00	0.40	100.00
AZ + MY + M +P +B (3)	10.00	0.00	100.00	8.00	0.00	117.00
Average	9.33	0.40	115.00	8.67	0.80	112.33
Water Control	10.00	0.00	120.00	10.00	0.00	115.00
MY + M + P + B (1)	10.00	0.00	118.00	10.00	0.00	120.00
MY + M + P + B (2)	8.00	0.00	125.00	8.00	0.00	110.00
MY + M + P + B (3)	10.00	0.00	127.00	10.00	0.00	120.00

Average	9.33	0.00	123.33	9.33	0.00	116.67
	11/14/2023			11/17/2023		
fertilization type	moisture	growth(cm)	water (mL)	moisture	growth(cm)	water (mL)
AZ M +P (1)	8.00	0.00	141.00	10.00	0.00	110.00
AZ M +P (2)	7.00	0.00	133.00	10.00	0.00	100.00
AZ M +P (3)	8.00	0.50	124.00	9.00	0.80	110.00
Average	7.67	0.17	132.67	9.67	0.27	106.67
AZ M + P + B	8.00	0.00	133.00	10.00	0.00	100.00
AZ M + P + B	8.00	0.00	102.00	9.00	0.00	100.00
AZ M + P + B	5.00	0.00	141.00	10.00	0.00	110.00
Average	7.00	0.00	125.33	9.67	0.00	103.33
M + P Control (1)	7.00	0.00	129.00	9.00	0.00	100.00
M + P Control (2)	7.00	0.00	131.00	10.00		110.00
M + P Control (3)	8.00	0.00	195.00	10.00	0.00	100.00
M + P Control (4)	8.00	0.40	175.00	9.00	0.03	125.00
Average	7.50	0.10	157.50	9.50	0.01	108.75
MY M + P (1)	8.00	0.00	100.00	10.00	0.00	100.00
MY M + P (2)	8.00	0.00	100.00	10.00	0.00	100.00
MY M + P (3)	8.00	0.00	134.00	10.00	0.00	110.00
Average	8.00	0.00	111.33	10.00	0.00	103.33
AZ + MY M + P (1)	8.00	0.00	143.00	9.00	0.00	120.00
AZ + MY M + P (2)	7.00	0.00	150.00	8.00	0.00	110.00
AZ + MY M + P (3)	6.00	0.00	157.00	10.00	0.00	120.00
Average	7.00	0.00	150.00	9.00	0.00	116.67
AZ + MY + M +P +B (1)	7.00	2.00	150.00	9.00	2.00	115.00
AZ + MY + M +P +B (2)	7.00	0.40	104.00	8.00	0.00	100.00
AZ + MY + M +P +B (3)	8.00	0.00	144.00	10.00	0.00	110.00
Average	7.33	0.80	132.67	9.00	0.67	108.33
Water Control	8.00	0.00	140.00	10.00	0.00	120.00
MY + M + P + B (1)	7.00	0.00	147.00	10.00	0.00	115.00
MY + M + P + B (2)	8.00	0.00	139.00	10.00	0.00	100.00
MY + M + P + B (3)	7.00	0.00	147.00	10.00	0.00	115.00
Average	7.33	0.00	144.33	10.00	0.00	110.00

Photos

Final growth of plants in environmental chamber.


ROSEKM
SUNPAD