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Article

Analysis of Milorganite's ability to sustain growth of *Ocimum basilicum* in simulated Martian soil

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SUMMARY

In years to come, it will be important to discover if life on Mars is a realistic option. As the population continues to grow and resources begin to dwindle, the possibility of colonizing other planets gives hope to humankind that extinction may be avoidable. Previous research suggests that some types of plants are able to survive the harsh conditions on Mars. This research aimed to discover if Milorganite could improve the growth of *Ocimum basilicum* (basil) in simulated Martian soil. Milorganite is a waste product-based fertilizer that was chosen to simulate the fecal matter of astronauts in space. We observed that basil could not grow in the simulated soil across all concentrations of Milorganite. We then shifted the study toward transplanting mature basil plants into the same soil with differing amounts of Milorganite. During the experiment, we observed that higher concentrations of Milorganite killed the basil plants. However, results were insignificant, suggesting that Milorganite has no effect on the growth of basil in simulated Martian soil. We watered some plants with a *Miracle-Gro* mixture water and some with tap water to ensure the type of water wasn't interfering with the results of the Milorganite. The *Miracle-Gro* did not significantly improve the conditions of the soil across all levels of Milorganite.

INTRODUCTION

In recent years, scientists have become increasingly interested in the terrain of Mars and whether it would be sustainable for human life (1). In fact, NASA has been working on the Mars Exploration Program to discover if Mars is, ever was, or can be a habitable planet (1). Although there are many studies on the growth of plants in simulated Martian soil, there are few that analyze the impacts of different fertilizers on a specific plant (2,3,4,5,6). Martian soil lacks the correct amount of nutrients to support plant growth and investigating the effects of different fertilizers will provide valuable data on the most efficient way to grow plants on Mars (7). As we inch closer to this possibility, finding alternative ways to improve growth will be essential for mitigating the costly process of bringing soil from Earth to Mars.

Milorganite is a waste product of living organisms made by heat-dried microbes that have digested the organic matter in wastewater (8). It is made of the microbes that eat what we flush, so it contains the organic material from human feces in the form of dried microbes (9). It also consists of 6% nitrogen, 4% phosphorus, and less than 1% potassium (10). A study conducted at the Leibniz Institute of Vegetable and Ornamental Crops in Germany showed that human urine and feces were viable and safe to use as fertilizers for plant growth (11). Milorganite was chosen due to the abundance of organic materials and similarities to human feces (8). According to a summary of waste management by NASA, the average healthy adult stool output per day is approximately 150 grams per defecation at a rate of twice a day (12). By using Milorganite, we were able to simulate the fecal matter readily available from astronauts in space. Using the fecal matter of the astronauts would possibly eliminate the need to transport fertilizer into space.

We investigated whether the amount of Milorganite added to the growing medium impacted the growth of *Ocimum basilicum* (basil), as measured by stem width. We selected this plant because according to the Red Thumbs Mars Garden Project, Guinan and his students found sweet potatoes, carrots, onions, kale, dandelions, basil, garlic, and hops to be robust crops under Martian conditions (13). The simulated Martian soil was created using the recipe from Chicago Botanic Garden's soil scientist Louise Egerton-Warburton (14). The ingredients of this mixture used Earth-sourced ingredients to approximate the mineral particles present from weathering of rocks on Mars (14).

We hypothesized that the addition of Milorganite would increase the growth of basil plants in a dose-dependent manner, as indicated by greater stem width and better overall health of the plant. We also hypothesized that the *Miracle-Gro* water would improve the growth of the basil plants in all treatments of Milorganite.

In this study, the independent variable was the amount of Milorganite added to the simulated Martian soil. The dependent variable was the stem width of each basil plant. Using the stem width to measure the growth of the plant is not as common as measuring the height, but due to the overall lack of growth, stem width was the most measurable factor. Measuring these variables allowed us to determine if there is a correlation between plant growth and the amount of Milorganite added to the soil. Variables controlled were soil bacteria, nutrients, and the growing environment, thus ensuring that changes in growth were in response to the independent variable. Another variable that we measured throughout the experiment was the difference in water types (tap water with added *Miracle-Gro* or tap water alone). We wanted to ensure an optimal water source that would provide the best plant growth and not impact the results of the Milorganite.

After the experiment provided insufficient data due to the repeated death of all of the basil plants, we conducted a final experiment to determine if one element of the simulated Martian soil (volcanic rock, basalt dust, and feldspar) was more

JOURNAL OF
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toxic than the others. This would be shown by a significant difference in stem widths grown in separate elements of the simulated Martian soil.

Insignificant results were produced from all three experiments. The concentration of Milorganite did not have a significant impact on the growth of basil, *Miracle-Gro* did not significantly improve the growing conditions, and none of the individual elements appeared more toxic than the rest.

RESULTS

To test the effect of various concentrations of Milorganite on basil plant growth, mature basil plants were transplanted into simulated Martian soil containing 0% (T_1), 1% (T_2), 5% (T_3), or 15% (T_4) Milorganite. After conducting this experiment, we found that the Milorganite had no significant impact on the viability of basil planted in simulated Martian soil. With an *f*-ratio value of 2.3437 and *p*-value of 0.085978, the results were not significant ($p > 0.05$). Pairwise comparisons using the post hoc Tukey's Honest Significant Difference (HSD) test revealed no significant differences in growth between the control and any concentration of Milorganite ($p > 0.05$) (**Table 1**).

To determine if stem widths decreased faster over time as concentration of Milorganite increased, basil stem widths in the four different treatments were measured each day. It was found that the slope values for each treatment increased as the concentration increased. Increased slope indicates a faster death rate of the basil plant over time. For 15% Milorganite, linear trends showed that it took around five days for the basil plant to die, whereas it took around eight and a half days for basil plants to die in the control of 0% concentration (**Figure 1**).

Daily measurements were also used to determine if there was a significant difference in overall mean stem width. It was found that the mean stem width decreased across all four treatments. For basil grown in 0% Milorganite concentration, the mean stem width decrease was -1.175 mm (SD = 0.7238) compared to a mean decrease of -1.93 (SD = 0.7670) mm for basil grown in 15% Milorganite concentration (**Figure 2**). Although the results showed an insignificant p-value of 0.07753, it is worth noting that the higher concentration of Milorganite had a decreasing trend in mean stem width than the other concentrations. It was also observed that the more concentrated levels of Milorganite caused blackened stems, indicating toxicity (**Figure 3**).

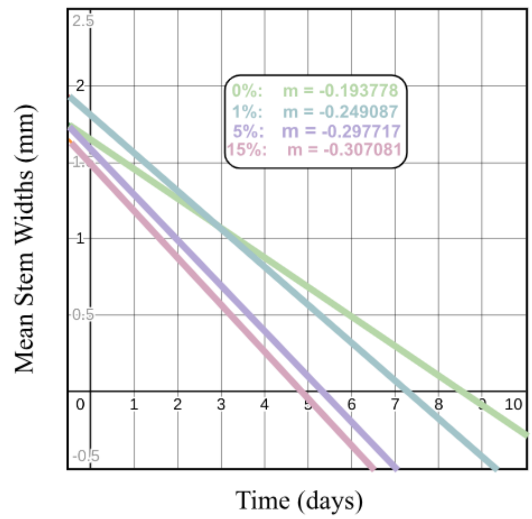

Figure 2: Linear trends of stem widths across all four treatments. Each line represents the decrease in stem width over time for each treatment. Slope values are displayed above the lines. As the Milorganite concentration increases, the slope gets steeper which represents a faster death rate.

Then, to determine if Miracle-Gro improved soil conditions across all levels of Milorganite, stem widths of basil grown in a

		Honestly Significant Difference (difference in means)	Q-Distribution / P-Values
Pairwise Comparisons		$HSD_{.05} = 0.8135$ $HSD_{.01} = 1.0055$	$Q_{.05} = 3.7760$ $Q_{.01} = 4.6673$
T_1:T_2	$M_1 = -1.18$ $M_2 = -1.73$	0.55	Q = 2.59 (p = .27219)
T_1:T_3	$M_1 = -1.18$ $M_3 = -1.77$	0.59	Q = 2.78 (p = .21612)
T_1:T_4	$M_1 = -1.18$ $M_4 = -1.93$	0.75	Q = 3.50 (p = 0.07753)

Table 1: Pairwise comparisons of means from each treatment. Mean decreases in stem widths from each treatment are displayed in the first column, with their differences in the second column, and the p-values/Q-values in the third. T_1 represents 0% concentration, T_2 represents 1% concentration, T_3 represents 5% concentration, and T_4 represents 15% concentration. Post Hoc Tukey HSD test. M_1, M_2, M_3, and M_4 represent the mean stem width decrease for each treatment (T_1, T_2, T_3, T_4). HSD is the honestly significant difference that shows the difference between the two means in each treatment. The q-value is the minimum false discovery rate.

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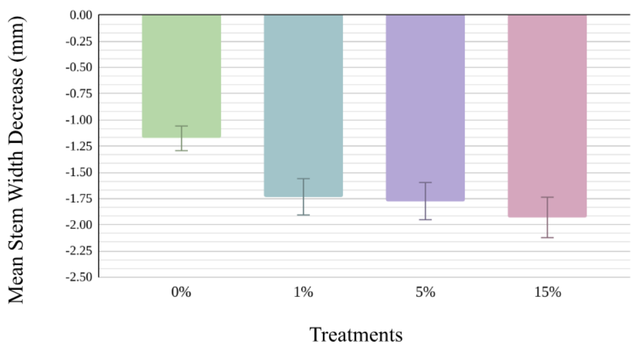

Figure 2: Mean stem width decreases across all four treatments. Bar graph displays the mean stem width decrease from each treatment. The decrease in width was observed as compared to the measurement of the stem when planted. Basil plants were grown in either 0%, 1%, 5%, or 15% concentrations of Milorganite. The sample size for each trial was 12 basil plants. The error bars represent the variation of the stem width measurements in each treatment. The error bars get larger as the concentration increases because there was more variation in the results. A One-Way ANOVA hypothesis test through the Social Science Statistics calculator was used to determine significance.

mixture of tap water and *Miracle-Gro* or basil grown in tap water alone were measured daily and compared. The *Miracle-Gro* across all levels of Milorganite did not significantly improve the conditions of the soil compared to tap water. In fact, the basil grown in 1% Milorganite had a p-value of 1.0, suggesting that there was no difference between the plants grown with tap water versus the plants grown with the *Miracle-Gro* mixture. The remaining treatments showed p-values of 0.794786 (T_1: 0% concentration), 0.969083 (T_3: 5% concentration), and 0.955339 (T_4: 15% concentration) which were all insignificant at the 0.05 cut-off.

Finally, to eliminate the possibility that an individual element of the simulated Martian soil was toxic, basil was grown in each element (with the addition of sand) and stem widths were measured each day. This experiment did not include the addition of Milorganite. It was found that there was no significant difference between the net decrease of stem widths between the three individual elements of the simulated Martian soil. The mean stem width decrease for basil grown in volcanic rock was approximately -0.82 (p-value = 0.74324) mm compared to -0.72 mm for basalt dust and -0.775 (p-value = 0.89331) mm for feldspar (p-value = 0.858909). These results suggest there was no difference in toxicity between any of the individual components of the simulated Martian soil.

DISCUSSION

Without the addition of organic material and elements like nitrogen, phosphorus and potassium, Martian soil lacks the nutrients to support plant life (13). If humans are ever in need of using Mars as a planet to sustain life, a method to make the Martian environment suitable for plant life will be necessary. This study attempted to look at how the Martian soil could be improved through the use of Milorganite as a way to add the proper organic nutrients and elements of fertile soil. As this method of improving the conditions of simulated Martian soil was investigated, it was revealed that Milorganite did not have a significant positive effect on the growth of basil plants.

Throughout the experiment it was observed that an increase in Milorganite correlated with a greater decrease in stem width than the control soil. However, the statistical analysis did not show that difference to be significant. This could be due to the lack of a sufficient amount of results, as indicated by the p-value reaching closer to the significance level ($p<0.05$) as sample sizes increased. If there had been more time to collect more data, there could have been sufficient results to show that an increase in Milorganite was killing the basil plants more quickly. Although the decrease in stem widths was not statistically significant among the different treatments of Milorganite, it was observed that the more concentrated levels of Milorganite caused blackened stems, indicating toxicity. The word toxic was chosen because after only one or two days, plants grown in the 5% and 15% Milorganite appeared to have blackened stems. In contrast, the basil grown in the 0% and 1% Milorganite still retained a green stem and had fewer wilted leaves after the same period of time. This observed correlation could indicate that the Milorganite concentration was too intense for the basil to survive. It was unexpected that more stem width degradation was observed with basil grown in higher concentrations of Milorganite, as the use of Milorganite was intended to combat the toxic conditions of simulated Martian soil. In a study where undergraduate students grew various vegetables in Martian-like soil, the fine clay like qualities of the soil led to many difficulties with dryness and the issue was combated with more frequent watering and a controlled humidity level in the greenhouse. While this study indicated the need for adding fertilizer and biologically rich materials like compost waste, they did not indicate the exact material that was used to supplement their soil. It would be interesting to know what supplements were used in this experiment, as they had successful growth (7).

When trying to determine an optimal water source for best growth of the basil plants, two types of water were tested. *Miracle-Gro* mixed with tap water and regular tap water were tested across all treatments of Milorganite with simulated Martian soil. Statistical analysis did not show a significant difference between the two water sources. After finding the difference in stem widths to be insignificant, the sample size was increased to include both the basil watered with the *Miracle-Gro* mixture and the regular tap water. This increase in sample size caused the p-value of the stem widths to be closer to the significance level ($p<0.05$), indicating that an even larger sample size may lead to significant results as to how Milorganite affects basil grown in simulated Martian soil.

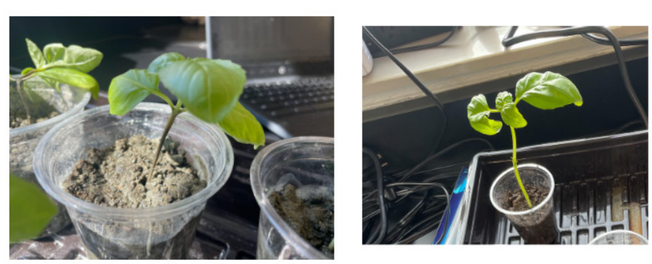

Figure 3: Basil grown in 15% Milorganite vs. 0% Milorganite. Basil grown in 15% Milorganite after 1 day is displayed on the left. Stem appears to be blackened and hardened. Basil grown in 0% Milorganite after 1 day is displayed on the right. Stem retained its green color and was not blackened or hardened.

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Finally, after many difficulties with the initial experiment, individual elements of the simulated Martian soil were isolated with sand (no Milorganite added) to see if a particular element was toxic to the basil plants. Results showed no significant difference between the stem widths of basil grown in the different elements of the simulated Martian soil. Future studies could test individual ingredients of the simulated Martian soil with the addition of Milorganite to see if it was the reaction between the Milorganite fertilizer and a particular element that was causing the toxicity. A constraining factor with the simulated Martian soil was that it may not have accurately represented real Martian soil. In other studies, Martian soil has been purchased from the Martian Garden. Unfortunately, this growing medium was costly to buy in large quantities. Instead, a simulated Martian soil from a set of steps published by the Chicago Botanic Garden was crafted. Because this is a different mixture of materials, results may have differed from what would have occurred if the Martian soil from the Martian Garden was purchased.

A limitation of this study is that it did not account for the atmospheric conditions of Mars. The atmosphere on Mars is too cold and full of carbon dioxide to support human or plant life (15). This experiment looked at manipulating Martian soil in the Earth's atmosphere and it is important to note that the Martian atmosphere might interact with the soil and affect the growth of the basil plants. This study explored a possible solution to only one of the factors that would need to be changed to survive on Mars. If the Martian soil could be successfully manipulated with the use of human feces, it would eliminate the need for the costly transport of Earth's soil.

If this study were to be conducted again, germination of the basil seeds in a wet paper towel overnight before planting them might improve growth. Also, it is recommended that the basil is grown and measured in a more controlled environment. It may be beneficial to grow the basil during the summer months when there is an appropriate amount of sunlight and no overheating issues at night. If the experiment was to be repeated indoors, the plastic covers from the kits should be removed. The amount of water used for each plant should be optimized and adequate drainage should be ensured. In a follow up experiment, it may be advantageous to lower the Milorganite concentration even more. It is possible that the concentration of Milorganite was too harsh for the basil plants to survive in. Procedurally, width measurements should have been taken at a set time point, as this may have created biased results. Also, after the original plants died and new plants were used, baseline measurements may have varied and time became another variable in the experiment. These are all setbacks and limitations from this research that can be countered and improved if this were to be replicated.

MATERIALS AND METHODS

In this study, the impact of Milorganite on the growth of basil plants in simulated Martian soil was investigated. The simulated soil was created using a recipe from Chicago Botanic Garden and basil plants were used to measure the effects of the Milorganite (8).

Soil Preparation

To begin, a growing medium to simulate Martian soil was created. To do this, 16 pounds of crushed volcanic rock (American Fireglass Small Lava Rock, American Fireglass, CA, USA) 16 pounds of sand, 8 pounds of basalt dust (Basalt Rock Dust, Redbud Soil Company, OK, USA) and 1.6 pounds of feldspar (Feldspar Custer, The Ceramic Shop, PA, USA) were mixed following the recipe on Chicago Botanic Garden (8). Three cups of Earthen soil collected from a forested area near Williamston, Michigan were added to the entire batch of simulated Martian soil to provide bacteria to promote soil viability. A 10-10-10 NPK fertilizer (All Purpose 10-10-10, Arizona's Best, AZ, USA) was added to the soil at 1 pound per 1000 square feet according to the manufacturer's directions.

Once all parts of the simulated Martian soil were mixed, they were separated into three equal amounts (approximately 13 ⅓ lbs). One batch was the control with no Milorganite (Milorganite, Milorganite, WI, USA), while the other two contained varying amounts of Milorganite (5% and 15%). These two values were recommended by Dr. Kurt Guter at Michigan State University as an appropriate range of levels of Milorganite. After plant decline was observed under the 5 and 15% Milorganite conditions, an additional experimental group containing 1% Milorganite was also added.

Plant Selection and Other Materials

Basil seeds were purchased online (Basil Genovese Non-GMO Heirloom Seeds, Gardeners Basics, UT, USA) and then from Christian's Greenhouse in Williamston, MI. The basil seeds were planted into growth kits (Super Sprouter Deluxe Propagation Kit, Hawthorne Hydroponics LLC, OH, USA) with individual growth lights placed on top of heating pads (BN-LINK Heat Pads, BN-LINK, CA, USA) set to 70°F. After failed germination of basil seeds in all conditions during the first ten weeks of experimentation, mature basil plants were used instead of trying to grow basil from the seed. Mature basil plants were purchased from Meijer (Edible Garden 4" Fresh Organic Potted Basil, Edible Garden Corp, NJ, USA).

Collection Methods

The stem widths (mm) of the mature basil plants were measured daily at the same point on the stem to determine how much degradation had occurred. The decrease in width was observed as compared to the measurement of the stem width when planted. As the basil plants were dying quickly in these conditions, measurements were taken for anywhere from 3-8 days, as determined by the death of the plant. Each time a plant died, the process was repeated with new mature basil plants. Data was collected for a total of 27 days. A digital caliper was used for the measuring (Electronic Digital Caliper, Entatial, USA). A total of 72 plants were used in this study, 12 for each of the six trials. Two basil plants were planted in each treatment condition and the remaining plants were grown in topsoil, collected from the forest areas nearby, until used for experimentation.

Testing Different Water Types

The two plants grown in each medium were given different types of water. Both water types were tried to ensure that the basil was getting an optimal water source and the plants' response to Milorganite was best measured. One was watered with tap water while the other with a *Miracle-Gro* mixture (Miracle-Gro Water Soluble All Purpose Plant Food, Miracle-Gro, USA). Each day every plant was watered with one tablespoon of the correct water and stem widths

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(mm) were measured. Once the majority of the plants died, new basil plants were replanted in the same cups. Since the difference in stem widths (mm) between tap water and water with *Miracle-Gro* was not significant, all data collected from basil grown in each treatment was used to determine if Milorganite had a significant effect on the growth of basil. Thus, data from both plants grown in the same soil each trial were used.

Testing Individual Elements of the Simulated Martian Soil

Because the plants were still having difficulty lasting any significant length of time, 12 basil plants in each individual element of the simulated Martian soil were planted in an attempt to determine if the individual elements were toxic to the basil. This consisted of putting the correct amount of each element in combination with sand. There were four cups of feldspar and sand, four cups of basalt dust and sand, and four cups of volcanic rock and sand. Each group had two plants watered with tap water, and two watered with the *Miracle-Gro* solution. Stem widths were measured daily to determine toxicity.

Statistical Analysis

Significance was determined by a One-Way ANOVA hypothesis test through the Social Science Statistics calculator (16). In addition, the post hoc Tukey HSD test was performed to produce comparisons between treatments. All research was conducted at Williamston High School in the Math and Science Academy lab.

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