Final Report: Can Wool Pellets Promote Plant Growth in Lunar Regolith?

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Objective:

The aim of this PTMC project is to find out whether wool pellets can stimulate growth in plants in lunar regolith.

Background, Experimental Parameters

Raw wool is an environmentally friendly, sustainable organic material. It easily breaks down and releases nutrients back into the soil. Wool pellets are made from 100% raw wool from sheep. The main fleece of the wool is sold to make clothes, but the belly wool and the "tags" (wool from around the back end of the sheep) are compressed and formed into pellets. Using wool pellets to fertilize plants on the moon has many benefits:

- Wool pellets are sources of animal protein. Animal protein is made up of amino acids, which release nitrogen when they break down Wood pellets thus form a nitrogen-rich fertilizer with NPK (Nitrogen-Phosphorous-Potassium) ratio of 9:0:2. That means, they can be very valuable to grow leafy vegetables such as spinach, cabbage, bok choy, and lettuce. These vegetables are nutrient-rich for astronauts.
- Wool pellets absorb water helping to reduce watering by 25%, which is an asset when growing food on the lunar landscape. This also prevents the plants from overwatering.
- Wool pellets expand when they break down, and during the process, they leave space in the soil. This increases the porosity and levels of oxygen in the soil. Lunar regolith has higher density than the soil on Earth, and some loosening may also be helpful to growing root vegetables such as radishes, because the roots can spread better in loose, porous soil.
- A very small quantity of wool pellets is needed (1/2 cup per gallon of soil or growing medium), so the payload transported from the Earth may be minimal.
- Wool pellets break down slowly, and after applying once, they can feed nutrients to the plants for six months, further reducing the payload to be carried from the Earth.
- Wool pellets keep away pests because they naturally repel slugs, snails, and weeds.
- They also have calcium, magnesium, iron, sulfur, and other micronutrients necessary for plant growth.
- They are all-natural, and free of chemicals. Thus, they will not burn plants and are also safe for the lunar environment.
- Using wool pellets can help reduce waste and promote sustainability, because they are made from waste and undervalued wool that was never going to be useful for fabric.

We read some studies which reported good success with using wool pellets as fertilizers. This motivated us to experiment with using these pellets to stimulate plant growth in lunar regolith. Initially, we chose two vegetables for our experiment, spinach and radish. Both spinach and

radish are on the list of vegetables recommended by the PTMC for their short harvest and germination periods. In our experiment, each of these two vegetables can benefit from different characteristics of wool pellets. Spinach can benefit from its high nitrogen content, radish greens can benefit from its nitrogen richness, and also radish roots/bulbs can benefit from the increased porosity and loose structure of the soil.

Independent Variables

Control – 50% lunar regolith and 50% topsoil
Experimental/Treatment – 50% lunar regolith and 33.33% topsoil, 16.66%wool pellets

Dependent Variables

Radish – Plant height, Number of leaves, Biomass, Width of leaves, Number of Cotyledons, Width of Cotyledons, pH of soil.
Spinach -- Plant height, Number of leaves, Width of leaves, Number of Cotyledons, Width of Cotyledons, pH of soil.
Qualitative variables will be the general health and vigor of plants.

Control Variables

Environment – Amount of light, Humidity, Temperature. Type of seeds, Type of containers, Amount of growing medium in each container, Moisture.

Hypothesis:

Plants in the growing medium treated with wool pellets (consisting of 50% lunar regolith, 33.33% topsoil, and 16.66% wool pellets) will show better growth (taller, more leaves, higher root mass) and will be healthier compared to plants in the medium without the wood pellets.

Materials:

20 4-inch planting pots, Topsoil, Lunar regolith, Cherry Belle radish seeds (1 packet), Spinach seeds (1 packet), Wild Valley farm wool pellets – 1 packet, pH, moisture and light meter meter, 1 cup, ½ cup, ¼ cup, 1 tablespoon, 1 teaspoon measures, Measuring tape, Yardstick, Electronic weighing scale, Distilled water, Lemon, Sulfur pellets

Initial Experimental Design & Procedures

1. Obtain 20 4-inch pots, label 5 pots as Control-Radish-1, Control-Radish-2, Control-Radish-3, Control-Radish-4, Control-Radish-5. Label 5 pots as Control-Spinach-1, Control-Spinach-2, Control-Spinach-3, Control-Spinach-4, Control-Spinach-5. Label 5 pots as Treatment-Radish-1, Treatment-Radish-2, Treatment-Radish-3, Treatment-Radish-4, Treatment-Radish-5. Label 5 pots as Treatment-Spinach-1, Treatment-Spinach-2, Treatment-Spinach-3, Treatment-Spinach-4, Treatment-Spinach-5.
2. Prepare the control growing medium by mixing equal parts of lunar regolith and topsoil. This ratio is according to the PTMC constraints that 50% of the material must be lunar regolith to minimize any payload transported from the Earth. We used topsoil because it does not contain any added nutrients.
3. Fill each of the 10 control pots with the control growing medium.

4. Prepare the treatment growing medium by mixing 3 parts of lunar regolith, 2 parts of topsoil, and 1 part of wool pellets.
5. Fill each treatment pot with the treatment growing medium.
6. Soak the seeds for 6-8 hours to speed up the germination. This is seed priming.
7. Sow 2 radish seeds in each pot labeled Control-Radish and Treatment-Radish, one inch apart.
8. Sow 2 spinach seeds in each pot labeled Control-Spinach and Treatment-Spinach, one inch apart.
9. Water all the pots with 2 tablespoons of water.
10. Wrap the pots in saran wrap to seal the moisture. Poke holes in the saran wrap for free air flow.
11. Place the pots by an east facing window, but with shades closed. This is because seeds need a warm, dark place to germinate.
12. Measure the moisture level in each pot every day using a moisture meter. Water the plants to maintain an optimal level of moisture in each pot.
13. Once the seeds start to germinate and seedlings emerge from the soil, remove the saran wrap and expose the plants to sunlight by opening the shades.
14. Measure the height of the plants, the number and width of leaves, number and width of cotyledons, every Sunday. The height of a plant will be measured from the point where the hypocotyl (stem) emerges from the soil to the top of the tallest leaf when it is held upward. The width of the cotyledon and leaves is measured at its widest point. The average width across all the leaves and cotyledons will be calculated for each plant.
15. Measure the pH of each pot every Sunday and Wednesday using the pH meter.
16. Measure the ambient light every Sunday and Wednesday.
17. Measure the biomass for the radish on the last day. To measure biomass, remove the plants from the pots, rinse away the soil, and measure the weight using an electronic scale.
18. Run t-tests on each metric of growth to understand if the difference between the control and treatment groups is statistically significant.

Pots	**Soil Composition**	**Plant**
Control-radish-1, Control-radish-2, Control-radish-3, Control-radish-4, Control-radish-5	50% Regloith, 50% Topsoil	Radish
Treatment-radish-1, Treatment-radish-2, Treatment-Radish-3, Treatment-radish-4, Treatment-radish-5	50% Regolith 33.33% Topsoil 16.66% Wool Pellets	Radish
Control-spinach-1, Control-spinach-2, Control-spinach-3, Control-spinach-4, Control-spinach-5	50% Regolith, 50% Topsoil	Spinach
Treatment-spinach-1, Treatment-spinach-2, Treatment-spinach-3, Treatment-spinach-4, Treatment-spinach-5	50% Regolith 33.33% Topsoil 16.66% Wool Pellets	Spinach

Updated Experimental Design (Oct. 13, 2023)

We assessed the growth in all the pots after 3 weeks. None of the spinach seeds had grown in both control and treatment pots. Radish seeds grew in four of the five control pots. In one control pot, and all five pots treated with wool pellets, the radish seeds also did not grow. Our conjecture is that due to the excessive humidity in Massachusetts, the wool pellets expanded quite a bit by absorbing the moisture in the air. Combined with the regolith and potting soil, this created a densely packed soil, which prevented any kind of growth. We did use the moisture meter to measure the moisture in the pots and watered every day with 2 teaspoons for control pots and 1 tsp for treatment pots to maintain the moisture at the level of 7. Despite using the moisture meter to figure out the best watering frequency and amount, the soil in both control and treatment pots remained very damp and tightly packed.

Therefore, after 3 weeks, we loosened the soil, and re-planted radish seeds in the one control pot which had not shown any growth, and also all the five treatment pots. To overcome the problem that many radish seeds may have been defective, and hence did not grow, we planted 4 seeds per pot instead of the 2 seeds in the initial experimental design. Because spinach seeds did not grow at all, we planted fava beans in all the five control and treatment pots in which spinach was initially planted. We chose fava beans for re-planting because they are easy to grow, prefer mild to cooler weather conditions which is what we would expect in Massachusetts in October and November. They are low maintenance, which will be desirable for growing on the moon. They also do not attract pests and fruit flies easily. Even though their size is relatively small, fava beans contain an incredible amount of nutrients. They are rich in plant protein, folate, and several other vitamins and minerals. They are also loaded with soluble fiber that can aid digestion and also lower cholesterol levels. Also, they are not very particular about the soil quality or soil structure, making them ideal for experimentation with lunar regolith. We planted four fava beans in each pot for the same reasons as planting four radish seeds.

Another challenge was that the pH in both the control and treatment pots consistently remained high around 8. This was the case in both control and treatment pots. This was an additional concern as wool pellets have a high pH between 8.3 and 8.6. So, we added crushed/powdered sulfur pellets following a suggestion from the PTMC guide after one week. However, even the addition of sulfur did not lower the pH. Therefore, after a week, we started watering the plants with lemon water. We prepared the lemon water by squeezing half a lemon in 8 oz of water and then used the lemon water to water the plants every other day. Measuring the pH, we still consistently found that it is around 7.5, and not near the desired 4.5 to 5.5. In our updated experimental design we reduced the watering frequency to every other day, and watered the control pots with 2 teaspoons lemon water and treatment pots with 1 teaspoon lemon water. As suggested by the PTMC webinar, we did not measure the pH beyond three weeks.

Data, Observations & Analysis

We measured the height of the plants and the number of leaves every week, and recorded these measurements in a template similar to Table 1. All heights are measured in centimeters. Table 1 shows the last measurements taken on November 17. On this day, we also harvested the plants to check the development of roots. The overall growth (stems and leaves) is shown in Figure 1, and the roots are shown in Figure 2. Because the plants in the treatment group did not grow well, we

measured only the heights of the stem and the numbers of leaves, and not the width and area of the leaves as outlined in the initial experimental design. Also, because the radish plants did not grow any bulbs we did not weigh them to measure biomass.

Radish								Fava Beans							
		Hgt	Lvs			Hgt	Lvs			Hgt	Lvs			Hgt	Lvs
C-1	P-1	10	3	T-1	P-1	0	0	C-1	P-1	15	5	T-1	P-1	0	0
	P-2	0	0		P-2	0	0		P-2	8	5		P-2	0	0
	P-3	0	0		P-3	0	0		P-3	0	0		P-3	0	0
	P-4	0	0		P-4	0	0		P-4	7.5	2		P-4	2.5	0
C-2	P-1	9	3	T-2	P-1	0	0	C-2	P-1	23	5	T-2	P-1	0	2
	P-2	7	4		P-2	0	0		P-2	0	0		P-2	0	0
	P-3	7	3		P-3	0	0		P-3	0	0		P-3	0	0
	P-4	5	4		P-4	0	0		P-4	0	0		P-4	0	0
C-3	P-1	3	3	T-3	P-1	0	0	C-3	P-1	7	5	T-3	P-1	0	0
	P-2	9	3		P-2	0	0		P-2	4	2		P-2	0	0
	P-3	0	0		P-3	2	0		P-3	13	2		P-3	0	0
	P-4	0	0		P-4	0	0		P-4	14	5		P-4	0	0
C-4	P-1	6.5	2	T-4	P-1	0	0	C-4	P-1	16	5	T-4	P-1	9	1
	P-2	7	2		P-2	0	0		P-2	0	0		P-2	1.5	1
	P-3	0	0		P-3	0	0		P-3	0	0		P-3	0	0
	P-4	0	0		P-4	0	0		P-4	0	0		P-4	0	0
C-5	P-1	9	2	T-5	P-1	5	4	C-5	P-1	4	2	T-5	P-1	0	0
	P-2	7	2		P-2	5	4		P-2	17	5		P-2	0	0
	P-3	0	0		P-3	4	2		P-3	8	5		P-3	8	2
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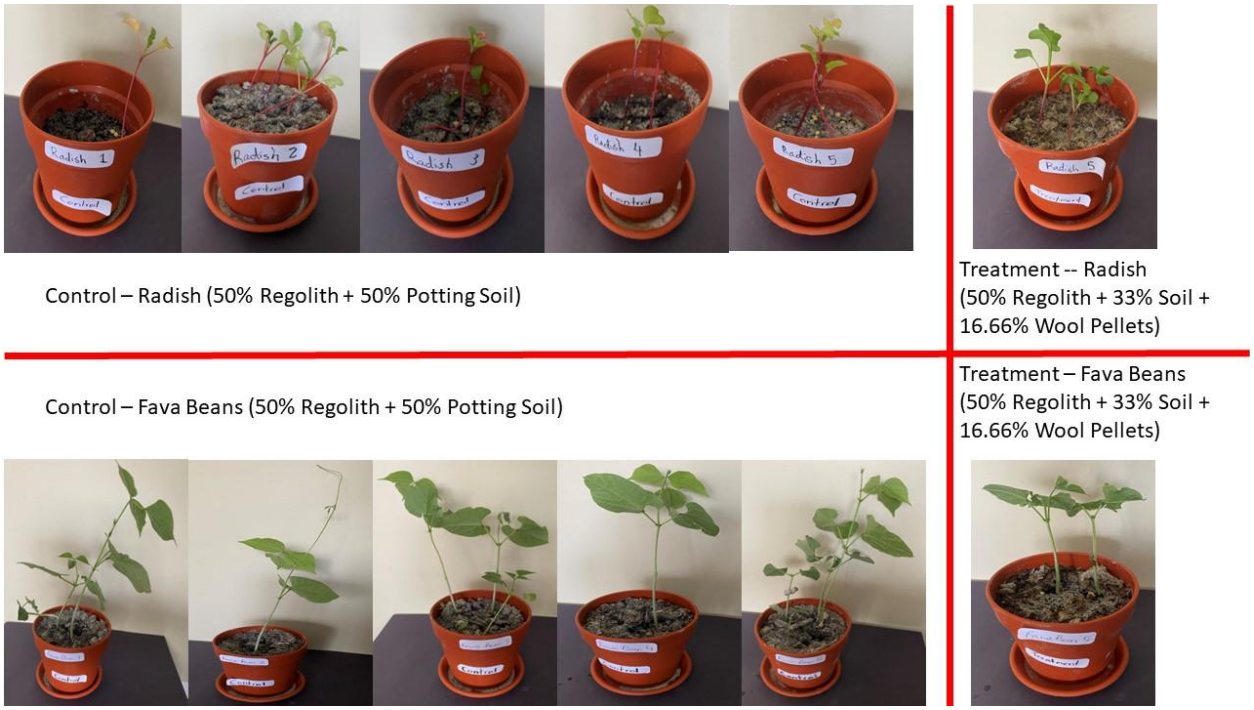

Figure 1: Stem & Leaves Growth

Figure 2: Development of Roots

For both radish and fava beans, at least one seed in each of the five control pots showed growth. However, only very few seeds across the treatment pots showed growth for both radish and fava beans. In Table 1, seeds that did not sprout are recorded as having zero height and zero leaves.

Statistical analysis could not be conducted (as in initial experimental design) because very few seeds in the treatment pots grew. Therefore, we compared the growth between control and treatment groups by calculating the average plant heights and number of leaves per plant. The health of the plants, and how intricate the root structure was assessed only by eyeballing.

Table 2 shows the average height and number of leaves per plant for both the control and treatment groups. Only those plants which showed stem and leaf growth are included in the average calculations. The average heights of the radish and fava bean plants in the control group was almost twice the average heights of the respective plants in treatment groups (Table 2). Radish plants in the treatment group were healthier (greener, bigger leaves, and more intricate and evolved root structure – Figures 1 and 2) compared to the plants in the control group. Radish plants in the treatment group also showed a slightly greater average number of leaves. For fava beans, however, plants in the control group were healthier (greener, bigger leaves, higher average number of leaves per plant, and a significantly more evolved root structure – Figures 1 and 2) compared to the plants in the treatment group. Figures 1 and 2 only include plants that showed healthy robust growth with leaves.

Table 2: Average Height and Leaves

	Radish		Fava Beans	
	Height	Leaves	Height	Leaves
Control	7.23 cm	3.00	11.04 cm	3.85
Treatment	4.00 cm	3.33	5.80 cm	2.00

We also noted other incidental observations:

1. None of the radish plants grew bulbs. We believe this may be because of overcrowding, or pots not being deep enough (radish plants need a depth of 6 inches to grow bulbs).
2. Because wool pellets absorb and hold moisture, watering needs of the treatment group were consistently lower than the control group to maintain the same level of moisture.

Conclusions & Future Suggestions

There was not enough data to conduct any statistical analysis. However, based on the observations we can conclude that in our experiment using wool pellets as soil amendment did not promote the growth of plants in lunar regolith. We do not know why many seeds in the treatment group did not grow for both types of plants. However, our plants in the combination of lunar regolith and topsoil without wool pellets grew very well. Our conjecture is that because of the excessive humidity wool pellets expanded and created a densely packed medium along with the lunar regolith. Because the size of the pots were very small, it compounded the problem.

We feel that based on this single experiment it is premature to rule out wool pellets outright as a soil amendment. We say that for several reasons. First, there was growth in at least some of the plants in the treatment group. Second, at least for radish, the plants treated with wool pellets were healthier, and grew more intricate root structures with slightly greater average

number of leaves. Finally, we also ran a parallel experiment growing radish and spinach to compare whether wool pellets can promote growth in just topsoil **without** the lunar regolith. In this experiment, the control variable was just topsoil and the treatment was topsoil treated with wool pellets. Our radish and spinach plants, treated with wool pellets, thrived in this experiment, which did not include lunar regolith. Since using wool pellets as a fertilizer is relatively recent, additional experimentation may be needed to figure out whether it works with lunar regolith. Therefore, in a future experiment with wool pellets, we would like to try these changes.

1. Use bigger pots, maybe with the capacity of one gallon.
2. Set up a growing environment with tighter control over the environmental parameters – lower humidity, possibly by running a dehumidifier, and/or growing the plants under a grow light.
3. It may take anywhere between 2 weeks to 6 months to a year for the wool pellets to decompose and release nutrients into the soil. Therefore, to really take in the benefits of the wool pellets, it may be necessary to experiment over a longer period of time. Another approach would be to prepare the mixture and let it just sit for a period of time, giving a chance for the wool pellets to start decomposing.
4. Experiment with different percentages of regolith to topsoil to wool pellets.

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