PLANT THE MARS AND MOON CHALLENGE

TEAM#: 10726

Coriander in martial regolith: Promoting growth with optimal amendments

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INTRODUCTION:

A fundamental challenge in human missions to Mars is producing consumable foods efficiently with the in-situ resources such as soil, water, nutrients and solar radiation available on Mars (Kasiviswanathan P et al., 2022). For long-term missions on Mars, it is crucial to develop techniques to increase the nutrient content of the Martian soil and to desalinate. It is therefore essential to develop strategies to enhance nutrient content in Mars soil and to desalinate for long-term missions on Mars. To tackle this issue, we have undertaken various amendments to improve the nutrient supplying capability of the soil and salinity reducing bacteria to deter the salinity levels.

Coriander (*Coriandrum sativum*) is a flavorful, high-antioxidant herb with a wide range of culinary use and has vitamins, minerals, and antioxidants in coriander that provides significant health benefits. It may aid in lowering blood sugar, preventing infections, and enhancing the health of your heart, brain, skin, and digestive system. Coriander seeds or leaves, often known as cilantro, are simple to incorporate into our diet. Vitamin K, which is essential for assisting with blood clotting, is abundant in the leaves and seeds of coriander. Additionally, vitamin K aids in bone healing, assisting in the prevention of conditions like osteoporosis.

Simulated Martian regolith was tested with alfalfa added as a fertilizer. Turnips, radishes and lettuces require little in the way of maintenance, grow quickly, and don't need much water and were all grown successfully in Martian regolith.

There is a catch though: fresh water was also needed. Based on previous experiments, the briny water available on Mars could be treated with a type of marine bacteria and then filtered through volcanic rock in order to produce the fresh water needed for crop growth at Mars (David Nield et al., 2022) condition.

Future martian habitation will be inadequate without these kinds of nourishing foods. We have selected coriander to be the test crop to grow in the mars simulant given based on the aforementioned considerations.

THEORY:

Coriander is a tropical crop and can be grown throughout the year except in very hot seasons i.e. March-May. A dry and cold weather free from frost especially during flowering and fruit setting stage favors good grain production. Heavy rain affects the crop. As an irrigated crop, it can be cultivated on almost all types of soils provided sufficient organic matter is applied. Black cotton soils with high retentivity of moisture are best under rainfed conditions.

The Martian regolith is extremely salty and contains uncommon salts like sulphates and perchlorates, making its use in agriculture extremely difficult (Kasiviswanathan P 2022).

According to Seiferlin et al., 2008 organic matter and biotic activity is not found in martial soil. Therefore, all macro- and micronutrients, which are derived solely (N), largely (P, S), or partially (K, Ca, Mg, Fe, Zn, Cu, Mn, B, Cl, and Ni) from the breakdown of organic components, are possibly inadequate in Martian. Regolith simulants therefore are unable to be sustainable for crop production in the absence of exogenous inputs of organic matter or inorganic fertilizers.

These simulations generally have properties that are detrimental to plant health, such as an high salt content (Ramírez et al., 2019) alkaline pH, high Na availability (Zeng et al., 2015; Kölbl et al., 2017), low mineral component cohesiveness, and a preference for macro-vs. micropores and limited water storage capacity. Additionally, hazardous perchlorates (Eichler et al., 2021) can occasionally be found in the Martian regolith.

Developing techniques to eliminate or reduce salt toxicity is crucial, but it is also important to increase the fertility of Martian regolith by including organic materials reused from solid waste composting processes from the human habitat.

The use of microorganisms to degrade organic matter including nanoparticles for soil remediation. (Ramírez et al., 2017).

The regolith may also play host to several potentially growth limiting characteristics: High salinity, phytotoxic substances (E.g. perchlorate, heavy metals, etc.), low water

holding capacity (WHC), nutrients present in non-bioavailable forms or at toxic concentrations, and extremely low availability of organic matter. In its raw form, Martian regolith would likely be inhospitable for growing plants, but there is potential to treat it to make it suitable. (Fackrell, 2018).

Khan et al. (2014) found that sodium chloride (NaCl) decreased all of the measured metrics, such as seed germination, plant length, number of leaves, plant strength, number of roots, length of roots, and percentage of survival.

Any plants growing on Martian soil will be under significant stress from the extreme salinity of the soil. To maximize production in a protected, controlled environment with a pressurized atmosphere, it will be essential to combine the right sowing technique with tolerant genotypes and soil management.

Coriander crop requires NPK in the ratio 10:40:20 (Nitrogen: Phosphorus: Potassium). This is for 1 hectare of area requirement. It has been calculated for a single pot (1 liter in size) with 350g soil and added as a treatment.

MEASUREMENTS AND METHODS:

- Initial samples were analyzed in Mars Simulant and Earth Soil before proceeding with the experiment.
- Test crop: Coriander (*Coriandrum sativum*)
- Number of sets- 2
- SET 1 - Earth soil
- SET 2 - Mars regolith
- **Treatment 1:** Mars regolith 350g without adding any amendments is taken as control for the experiment.
- **Treatment 2:** Mars regolith 350g, coir pith 150g, Vermicompost 100g, Biochar 5g, Gypsum 1.25g and Inorganic fertilizers Urea 0.04g, Single super phosphate 0.05, and Murate of potash 0.07g is added.
- **Treatment 3:** Mars regolith 350g, Coir pith 150g, Vermicompost 100g, Waste Decomposer, Gypsum 1.25g.
- **Treatment 4:** Mars regolith 350g, Coir pith 150g, Vermicompost 100g, Biochar 5g, Gypsum 1.25g, Bio dynamic manure 1.5g.

- Another set uses Earth soil with the same treatment.
- The total of 12 pots in each set, three replicas of each treatment. For the experiment, a total of 24 pots were used.
- 2 ml of Salinity reducing bacteria is added as an amendment in all mars regolith pots. It is provided by the School of Bio Sciences and Technology (SBST), Vellore Institute of Technology, Vellore, India.
- Vermicompost used for the experiment were collected from the Vermicomposting unit, VIT School of Agricultural Innovations and Advanced Learning (VAIAL), Vellore, India. It is made from decomposing waste using earthworms in our unit. It helps in plant growth and improving soil qualities.
- Biodynamic manure used for the experiment was collected from our faculties. It is made from cow dung from a lactating cow of average 50-150 g dung per horn, which is fermented and is buried in the soil for 6 months. It helps to stimulate root growth, increases germination and humus formation. The BD 500 - Combination Manure (Cow horn + Cow dung + Flower manure) has its Nitrogen, Potassium, Phosphorus and organic carbon content at 1.6%, 1.1%, 2.5%, 24.50% respectively which will increase on the period basis. It helps to increase the root growth and increase the germination (Vatsala et al., 2013)
- Quality coriander seed of the variety **Divya** has been used for sowing.

PROJECT HYPOTHESIS:

The hypothesis of the project is to improve soil quality by adding amendments and nutrients. The main objective of the experiment is to develop a healthy crop and overcome crop establishment and growth issues. The primary goal of this research is to develop a better strategy for increasing coriander growth and yield in Martian soil. One of the driving questions is whether the amendments and supplements are effective as a potential media for the mars soil. Furthermore, testing the potential of amendments to reduce the salinity of mars soil is a driving research force.

EXPERIMENTAL DESIGN AND PROCEDURES:

- In a contained environment, experimental pots are placed in the shade net house, VIT school of Agricultural Innovations and Advanced Learning, Vellore Institute of Technology, Vellore, India. To lower temperature and maintain humidity inside the polyhouse, jute bags were placed on all sides and were routinely irrigated.
- The pots were filled with regolith, soil, amendments, treatments before sowing and were placed in an order. One table had Earth soil pots, and another table had Mars regolith pots.
- A transparent polythene sheet was covered in the top of the shade net to protect the crops from rain.
- Seeds were broken and soaked for 6 hours in water for uniform germination of seeds.
- After setting up pots with treatments, 50 seeds in each pot were sown and 100 ml of distilled water was added using measuring cylinders.
- The experimental setup is shown as follows.

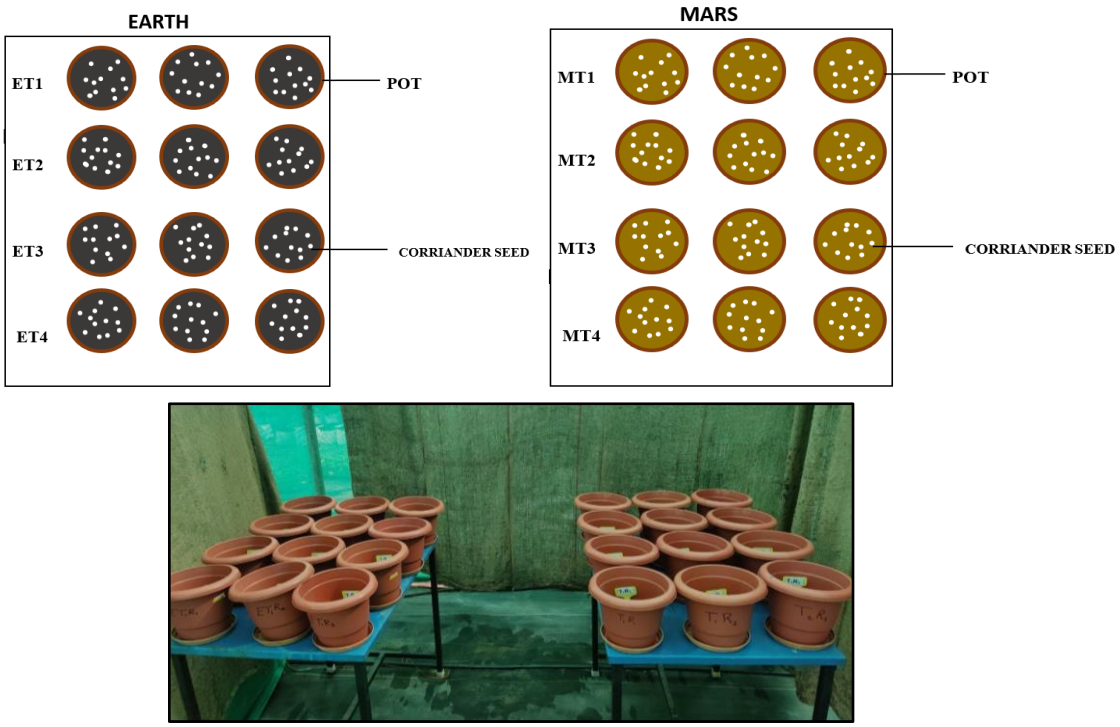


Figure 1. Picture of the pot setup in the polyhouse

- Waste decomposing bacteria is mass multiplied by taking 2 kg of jaggery in 200 L of water in a drum and mixing 1 bottle of waste decomposer. This mixture was stirred regularly with a wooden stick and closed tightly. After 5 days, the solution turns creamy and can be used for application.
- Waste decomposing bacteria were added at a ratio of 1:5 (waste decomposing bacteria: distilled water) one time per week for five weeks. pH was recorded in the first week as initial reading, later at the end of the study pH was analyzed.
- Harvesting was carried out the 60th day after sowing on all the replications and the total biomass was recorded.

PLANT ANALYSIS:

The plant growth in the mars stimulant was recorded through various agronomic parameters, among them plant height and root length. Root length was recorded by pulling out a plant in 1 pot for every pot during week 1 and a week before harvest. This value is averaged and represented in the graph.

Three analysis were carried out on the harvested plant samples i.e. Microbiome, Gas Chromatography (GC) and Plant growth hormone analysis. For GC analysis 100 mg samples from all the treatments of Earth and Mars soil were taken. For the Microbiome and Hormone analysis 100 mg plant samples from four treatments were chosen. Plants harvested from treatment 2 and 4 of earth soil were sent for analysis of 430 mg each. Plants harvested from treatment 1 and 4 of the mars stimulant were sent for analysis of 321 mg, 871 mg respectively. The results are yet to come, which will be further incorporated in our future study report.

RESULTS AND DISCUSSION:

Initial chemical analysis was done on mars regolith simulant using LaMotte soil testing kit before the start of the growth period. The results revealed a neutral pH of 7.8 and an electrical conductivity of 8.05 mS. The primary nutrient concentration was estimated as Nitrogen at 0.03 ppm (low), Potassium at 17.8 ppm (very high), phosphate at 0.18 ppm (very low).

Table 1. Initial Mars regolith analysis

Component	Result
Electrical Conductivity	8.05 mS
pH	7.81
Nitrogen	0.03 ppm
Phosphorous	0.18 ppm
Potassium	0.25 ppm

Other growth supporting nutrients like copper was estimated at 11.8 ppm, chloride, iron and magnesium at higher levels. The pH of the soil samples was tested at various stages of the growth period. The initial pH of the mars simulant was alkaline. After the amendments, the pH was reached near neutral and suitable for growth of coriander cultivation under optimum pH 6-7 and electrical conductivity levels (Rabiei et al., 2020)

Table 2. Initial and Final pH of Mars regolith and Earth soil at the end of study

Treatments	Earth soil		Mars regolith	
	Initial pH	Final pH	Initial pH	Final
T1	8.2	7.3	8.4	7.9
T2	8.2	7.4	8.0	7.6
T3	8.2	7.2	8.1	7.8
T4	8.5	6.8	8.5	7.6
Mean	**8.3**	**7.2**	**8.3**	**7.7**

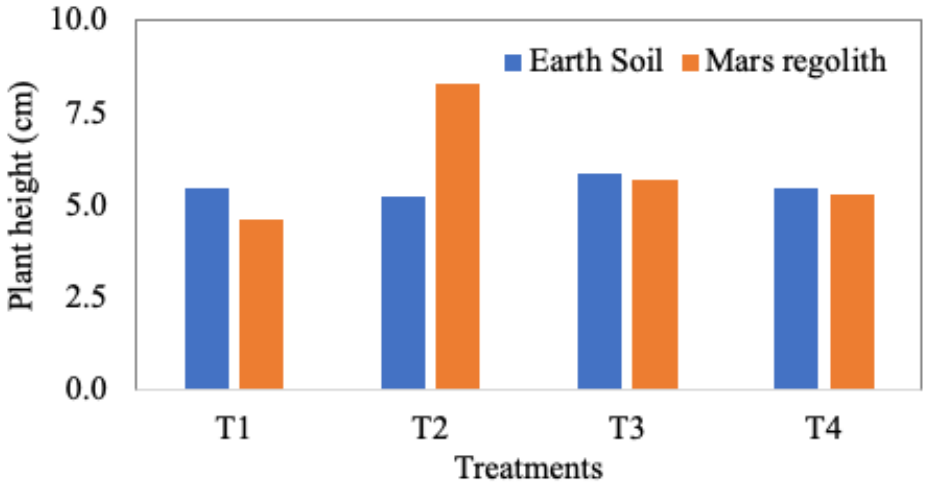
Table 3. Electrical conductivity (mS) of samples at the end of the study

Treatments	Earth Soil	Mars regolith
T1	0.23	0.25
T2	0.29	0.15
T3	0.35	0.39
T4	0.24	0.31
Mean	**0.28**	**0.27**

Table 4. Germination percentage and plant height of samples in the first week

Treatments	Earth soil		Mars regolith	
	Germination percentage (%)	Plant height (cm)	Germination percentage (%)	Plant height (cm)
T1	10.0	5.5	14.7	4.2
T2	13.0	5.2	50.7	6.9
T3	23.0	5.9	54.7	6.7
T4	13.3	5.5	19.3	4.7
Mean	**14.8**	**5.5**	**34.8**	**5.6**

The highest plant height in the mar simulant was recorded in treatment 2, containing Biochar + Gypsum + NPK. The additional supplement of nitrogen, phosphorus and potassium was given through inorganic fertilizers such as Urea 0.04g, Single super phosphate 0.05 g, and Murate of potash 0.07g per pot. The increase in plant height with NPK fertilization can be attributed to the fact that macro elements, particularly nitrogen, promotes plant growth which ultimately increases the number and length of the internodes which results in progressive increase in plant height (Kwon et al., 2019).



The highest root length was observed in treatment 3 containing Gypsum + Biochar + Waste Decomposing Bacteria. It can be inferred that the microbial consortium applied was effective in reducing the salinity of the mars stimulant, which paved the way for the better establishment of the plant and better root development. In addition, the biodynamic manure helps in increasing root activity, stimulates/increases soil-micro life, regulates trace elements, and helps in increasing germination percentage (Zaller et al., 2004).

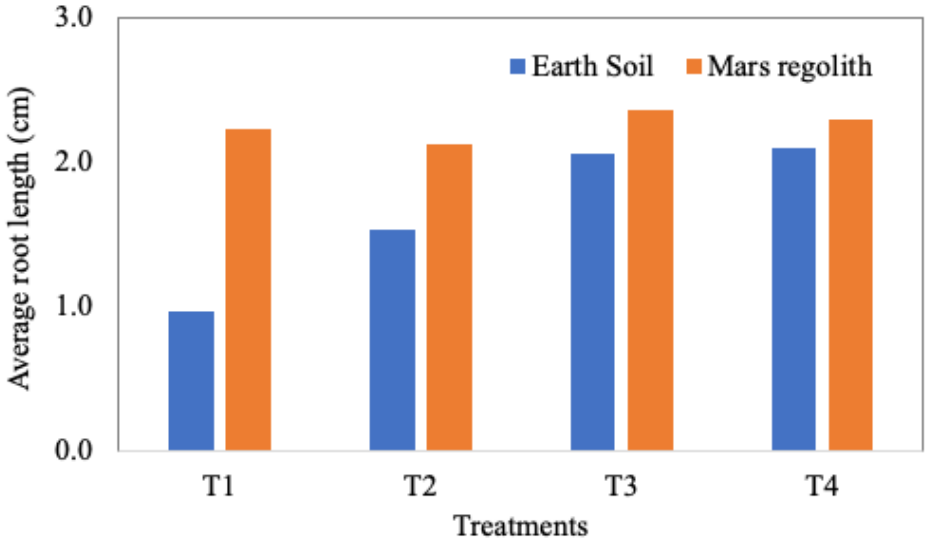


Figure 2. Root length of plants on Mars regolith and Earth soil at the end of study

Total biomass of the crop was recorded after harvest. The highest biomass was observed in treatment 2 i.e., Mars simulant with Biochar, Gypsum and Inorganic fertilizers (Urea, Single super phosphate, and Murate of potash) followed by treatment 4 containing Biochar, Gypsum, Bio dynamic manure. In T4, the biodynamic manure BD 500 cow horn manure has the potential microbes which were growth promotion capacity reasoned for the increased biomass (Vatsala et al., 2013). The microbiome analysis of plant samples is yet to be completed for deeper understanding. In T2, Inorganic fertilizers, biochar played a major role in increasing vegetative growth and improving harvest biomass (Saleem et al., 2021).

Table 5. Total biomass of harvested plant samples

Treatments	Earth Soil	Mars regolith
T1	0.05	0.17
T2	0.09	0.26
T3	0.14	0.10
T4	0.25	0.20
Mean	**0.13**	**0.18**

The nutrient analysis was done at the end of the growth period and observed that of the considerable increase in the nitrogen content in all the treatments of the mars regolith except the control, the highest nitrogen content was found in treatment 3, whereas potassium content was recorded the highest in treatment 2. In T3, the use of waste decomposing bacteria promoted the growth of bacterial population in soil and is considered an effective method for improving mars regolith for plant growth. The organic amendments present (Biochar, Gypsum, Coir pith, Vermicompost) in T2, provided favorable growth, nutrient availability to the coriander plants by making the media suitable for the Martian regolith.

Table 6. Analysis of soils at the end of study

Earth				Mars			
Treatments	N (ppm)	P (ppm)	K (ppm)	Treatments	N (ppm)	P (ppm)	K (ppm)
ET1	5.7	41.86	112.7	MT1	0.4	26.48	39.1
ET2	4.5	33.82	125.6	MT2	3.2	21.82	102.2
ET3	5.8	30.2	101.3	MT3	3.4	20.84	24.14
ET4	4.8	32.04	109.5	MT4	2.4	17.46	43.17

Based on an analysis of the data, it can be concluded that the addition of different amendments, such as gypsum, biochar, inorganic fertilizers, microbial consortiums, and biodynamic manure, will optimize the development of coriander in the mars regolith. The information and findings from this preliminary trial, which was conducted to gather information, served as the basis for our present efforts to maximize the growth and for our future undertaking on optimizing the growth of amaranthus which was sowed using the aforementioned methods in the future in order to create a more suitable set of cultivation techniques for growing coriander in the martian regolith.

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APPENDICES

Table 1. Initial and Final pH of Mars regolith and Earth soil at the end of study

Earth Samples	Initial pH	Final pH	Mars Samples	Initial pH	Final pH
ET1R1	8.2	7.47	MT1R1	8.5	7.87
ET1R2	8.1	7.35	MT1R2	8.4	7.94
ET1R3	8.4	7.15	MT1R3	8.4	7.92
ET2R1	8.3	7.34	MT2R1	8	7.71
ET2R2	8.2	7.4	MT2R2	8	7.84
ET2R3	8.2	7.4	MT2R3	8.1	7.16
ET3R1	8.1	7.38	MT3R1	8.4	7.9
ET3R2	8.3	7.25	MT3R2	7.9	7.79
ET3R3	8.2	7.05	MT3R3	8	7.65
ET4R1	8.4	7.3	MT4R1	8.4	7.7
ET4R2	8.6	6.7	MT4R2	8.5	7.71
ET4R3	8.4	6.3	MT4R3	8.5	7.3

Table 2. Electrical conductivity at the end of the study

Earth Soil	Electrical Conductivity (mS)	Mars Soil	Electrical Conductivity (mS)
T1R1	0.23	T1R1	0.26
T1R2	0.26	T1R2	0.28
T1R3	0.21	T1R3	0.20
T2R1	0.27	T2R1	0.18
T2R2	0.21	T2R2	0.14
T2R3	0.38	T2R3	0.13
T3R1	0.38	T3R1	0.42
T3R2	0.38	T3R2	0.38
T3R3	0.29	T3R3	0.36
T4R1	0.25	T4R1	0.34
T4R2	0.2	T4R2	0.39
T4R3	0.28	T4R3	0.21

Table 3. Germination percentage and plant height of samples in the first week

Treatment (Earth)	Germination percentage (%)	Avg. height of plants (cm)	Treatment (Mars)	Germination percentage (%)	Avg. height of plants (cm)
ET1R1	7	6.1	MT1R1	12	3.6
ET1R2	15	4.7	MT1R2	22	4.7
ET1R3	8	5.6	MT1R3	10	4.2
ET2R1	14	5.6	MT2R1	68	6.3
ET2R2	6	4.4	MT2R2	40	7.7
ET2R3	19	5.8	MT2R3	44	6.8
ET3R1	12	5.7	MT3R1	48	6.8
ET3R2	33	6.1	MT3R2	52	7.3
ET3R3	24	5.9	MT3R3	64	6.0
ET4R1	14	5.5	MT4R1	18	6.3
ET4R2	14	4.7	MT4R2	10	4.1
ET4R3	12	6.1	MT4R3	30	3.8

Table 4. Total biomass of harvested plant samples

Earth Soil	Total Biomass (g)	Mars Regolith	Total Biomass (g)
T1R1	0.051	T1R1	0.005
T1R2	0.041	T1R2	0.224
T1R3	0.062	T1R3	0.266
T2R1	0.176	T2R1	0.18
T2R2	0.057	T2R2	0.457
T2R3	0.034	T2R3	0.142
T3R1	0.368	T3R1	0.185
T3R2	0.024	T3R2	0.064
T3R3	0.021	T3R3	0.051
T4R1	0.32	T4R1	0.396
T4R2	0.245	T4R2	0.023
T4R3	0.195	T4R3	0.191

Table 5. Plant height at the end of study in Mars regolith and Earth soil

Earth Soil	Average plant height (cm)	Mars Soil	Average plant height (cm)
ET1R1	6.1	MT1R1	3.6
ET1R2	4.7	MT1R2	4.7
ET1R3	5.6	MT1R3	5.6
ET2R1	5.6	MT2R1	9.7
ET2R2	4.4	MT2R2	8.2
ET2R3	5.8	MT2R3	7.0
ET3R1	5.7	MT3R1	4.7
ET3R2	6.1	MT3R2	7.3
ET3R3	5.9	MT3R3	5.1
ET4R1	5.5	MT4R1	6.3
ET4R2	4.7	MT4R2	4.1
ET4R3	6.1	MT4R3	5.5

Table 6. Root length of plants on Mars regolith and Earth soil at the end of study

Earth Soil	Average root length (cm)	Mars Soil	Average root length (cm)
ET1R1	0.8	MT1R1	0.9
ET1R2	1.0	MT1R2	1.8
ET1R3	1.1	MT1R3	4.0
ET2R1	1.3	MT2R1	2.1
ET2R2	1.6	MT2R2	2.1
ET2R3	1.7	MT2R3	2.2
ET3R1	2.2	MT3R1	1.8
ET3R2	2.7	MT3R2	1.8
ET3R3	1.3	MT3R3	3.5
ET4R1	2.6	MT4R1	2.1
ET4R2	1.8	MT4R2	3.2
ET4R3	1.9	MT4R3	1.6

PHOTOS

Mars Global Simulant (as received)

Earth soil we used

Setting up the pots for treatment

Vermicompost from VAIAL, VIT,Vellore

Weighing and preparing the pot mixture

Experimental Setup with pot mixtures

Seeds used for sowing in the pots

Sowing seeds in pot

Seeds were sown into the pot

Mass multiplication of waste decomposing bacteria

Preparation of waste decomposer for the treatment

Adding waste decomposing bacteria to the soil

Salinity reducing bacteria

Irrigation done by Team members

Team members performing Soil Tests

Soil Potassium testing

Soil pH testing

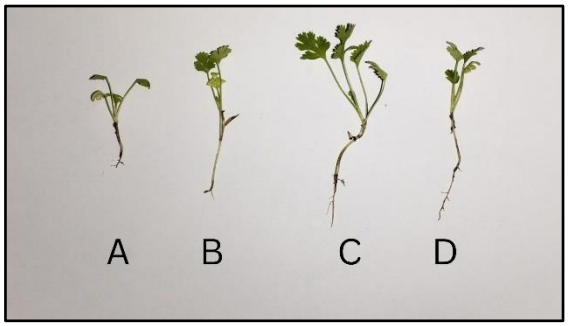
Coriander Germination in Earth soil

Coriander Germination in Mars soil

Coriander plant in Mars soil

Coriander plant in Earth soil

Harvested Plant samples



Grown Coriander Plant (A-T1, B-T2,C-T3, D-T4)

Student team involved in the Project