Original Research Article

Statistical Evaluation of Microbial Additives and Fertilizers on Bush Bean Growth in Simulated Lunar Soil

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ABSTRACT

A significant challenge in establishing sustainable human settlements on the Moon lies in cultivating food within extraterrestrial environments. Lunar regolith, the Moon's surface material, is composed of rock fragments, minerals, and volcanic glass, and poses substantial limitations for plant growth due to its lack of essential nutrients. This study employs statistical regression analysis to evaluate the correlation and impact of microbial additives and nitrogen, phosphorus, potassium (N-P-K) fertilizers on the growth of bush bean (*Phaseolus vulgaris*) seeds in a lunar soil simulant. The microbial additive used, TPS Plant Foods Billions of Microbes, contains five strains of *Bacillus* bacteria, four strains of mycorrhizae, and one strain of *Trichoderma*. The fertilizer applied was Fruit & Bloom Booster NPK 2-15-15. Bush beans were chosen for their rapid germination, resilience to temperature fluctuations, and disease resistance. Specific regression analyses were conducted to assess the individual and combined effects of nitrogen, phosphorus, potassium, and microbial inputs on plant growth. The results demonstrated that both TPS Billions and nitrogen significantly enhanced plant growth, as evidenced by a 500–2000% increase in number of leaves and leaf area in the 60% lunar soil mixture. These findings suggest that targeted microbial and nitrogen-based amendments can effectively support plant cultivation in lunar regolith, offering valuable insights for future lunar agricultural systems and long-term human habitation.

Keywords: TPS Billions; Lunar regolith; Mycorrhizae; NPK fertilizer; Weighted Least Squares; Plant height; Leaf area

INTRODUCTION

As NASA's Artemis program (1) advances toward a sustained human presence on the Moon and future missions to Mars, ensuring a reliable food supply in extraterrestrial environments is a critical challenge. Lunar regolith (2), a mixture of rock fragments, minerals, volcanic glass, and agglutinates, lacks essential nutrients and organic matter, and may contain toxic heavy metals that inhibit plant growth. While studies, including those by Anna-Lisa Paul (3), show that germination is possible in lunar soil, plants exhibit stress responses, indicating regolith alone is inadequate for healthy, sustained growth. This highlights the need for soil amendments to enhance regolith's agricultural potential.

This study examines the impact of additives on lunar soil, aiming to minimize reliance on Earth-based potting soil and reduce logistical constraints for Moon missions. Bush Bean seeds were selected due to their rapid

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sprouting of 7–10 days, short maturation of 55 days, tolerance to temperature fluctuations, and resistance to foliar diseases and root rots (4).

We compared two soil supplements: TPS Plant Foods Billions of Microbes ("TPS Billions") and Fruit & Bloom Booster (NPK 2-15-15). TPS Billions contains a diverse microbial consortium, including five strains of *Bacillus*, four strains of mycorrhizae, and one strain of *Trichoderma*. As summarized by Chauhan *et al*. (5), diverse soil microbial populations including rhizospheric bacteria, fungi, and endophytes enhance nutrient availability, improve root colonization by beneficial microbes, and promote plant growth, particularly under stress conditions.

We hypothesize that TPS Billions and nitrogen will significantly improve bush bean growth in a lunar soil simulant, reducing dependency on Earth-derived soil. Pot #5, containing 100% nutrient-rich potting soil with both additives, serves as the control and is expected to show the most vigorous growth. Pot #1, a 60:40 mixture of lunar simulant and potting soil with both additives, is anticipated to exhibit substantial growth including increased leaf development and plant height. Pot #4, with the same soil mixture but no additives, is expected to show minimal growth demonstrating the importance of microbial and nutrient supplementation. Predicted growth performance is: Pot #5 > Pot #1 > Pot #2 > Pot #3 > Pot #4.

This study evaluates whether microbial and nitrogen-based additives can enhance plant growth in simulated lunar regolith, providing insight into sustainable crop production for extraterrestrial habitats.

METHODS AND MATERIALS

Experimental Design and Material Preparation

To conduct this study, Lunar regolith simulant was obtained from Space Resource Technologies, as actual samples are not readily available. Table 1 presents a list of all materials and equipment used in the experiment, along with their respective vendors.

Five pots were used to plant the bush bean seeds. Pots #1 through #4 were filled with a mixture of 60% lunar soil and 40% potting soil by volume. Pot #5 was the control pot, and was filled 100% potting soil by volume. Four bush bean seeds were planted in each pot at a depth of 1 inch. Due to the clay-like nature of lunar regolith, which contains rock chips, mineral fragments, and glass, we opted not to use 100% lunar soil for the pot. We could explore this as a potential avenue for future research based on the findings of this study. Table 2 displays the experimental set up.

Bush beans are known for fixing nitrogen and restoring nitrogen-deficient soil, which is why a low-nitrogen fertilizer was selected. Additives used were TPS Billions and NPK (2-15-15) fertilizer. These additives were prepared as below and were labeled and stored separately.

TPS Billions Preparation: 1/4 teaspoon of TPS Billions was measured using a measuring spoon, weighing 0.85 grams on a scale. This amount was dissolved in 1 gallon (~3.785 liters) of water.

NPK Fertilizer Preparation: 1/2 teaspoon of NPK fertilizer was measured using a measuring spoon, weighing 1.7 grams on a scale. It was then mixed into 1 gallon of water.

The pots were kept indoors in a room where the temperature was consistently maintained at 70°F, and the relative humidity ranged from 45% to 60%. Each pot received a total of 5 ml of water per week, which was based on our research into optimal conditions for growing bush beans. The additives were added to each pot as per Table 2. Table 3 below shows the amount added in grams for each pot each week. Regarding the nutrient additions, the weekly inputs may appear extremely small when viewed individually; however, these quantities

Table 1. Materials, Apparatus & Vendors Used

Item	Vendor	
Lunar Highlands (LHS-1E) Regolith Simulant	Space Resource Technologies	link
Pots	Amazon	link
Greenhouse box with light & humidity control	Amazon	link
2-15-15 NPK fertilizer	Walmart	link
Bush beans seeds	Amazon	link
Grow lights	Amazon	link
Weighing scale for measuring powders	Amazon	link
Measuring spoons	Amazon	link
Tape measure (for measuring L/W of leaf)	Amazon	link
Measuring cups for regolith	Home Depot	link
Medicine measuring cups (for 2.5/5 ml)	Home	

reflect the use of Fruit & Bloom Booster 2-15-15, a super-concentrated fertilizer. The manufacturer recommends diluting just 1 teaspoon per 2 gallons of water, which naturally results in very small nutrient masses per application. Although each weekly dose is modest, the additions accumulate over the course of the experiment, providing a meaningful and deliberately controlled supply of fertilizer to avoid oversaturating the nutrient-poor lunar simulant. Accordingly, the values presented in the table are consistent with the product's specifications and the experimental design.

All five pots were supplied with artificial grow lights, powered by a USB connection to a timer that ensures 12 hours of light each day. The timer automatically turned the lights off from 8 PM to 8 AM. By Week 2 we replaced the grow lights with taller ones to accommodate the plants' healthy growth.

There were 8 Independent variables and 6 dependent variables along with 1 calculated variable. The 8 Independent variables were "Pot Number", "Days of growth", "Did Pot have additives (1 or 0)", "Total amount of N added in g", "Total amount of P added in g", "Total amount of K added in g", "Amount of lunar soil in pot as %", and "Total amount of TPS Billions added in g". The 6 dependent variables were "Plant Height (in)", "Stem width (in)", "Number of leaves", pH, "Length of biggest leaf (in)", and "Width of biggest leaf (in)". Area of the leaf was then calculated by using the formula: 0.68 * "length of biggest leaf" * "width of biggest leaf." This methodology for calculating leaf area was adapted from the research study by He, Reddy, *et al*. (6), who used a Montgomery parameter of 0.68.

In addition to the quantitative data measured, visual inspections were conducted weekly, and qualitative observations were recorded to assess factors such as leaf size, bud development, and other noticeable characteristics.

Data was collected every Sunday afternoon. Each week, the number of germinated plants in each pot was recorded. For every germinated plant, additional measurements were taken, including the total number of leaves, plant height, stem width, length of biggest leaf, width of biggest leaf and soil pH. pH was measured using pH strips, while a measuring tape was used to

Table 2. Experimental Setup

Pot Number	Soil Mixture (by volume)	Amount of Additives
# 1	60% lunar soil + 40% potting soil	2.5 ml of NPK Fertilizer (2-15-15) + 2.5 ml of TPS billions every week
# 2	60% lunar soil + 40% potting soil	5 ml of TPS billions every week
# 3	60% lunar soil + 40% potting soil	5 ml of NPK Fertilizer (2-15-15)
# 4	60% lunar soil + 40% potting soil	None
# 5	100% potting soil	2.5 ml of NPK Fertilizer (2-15-15) + 2.5 ml of TPS billions every week

Table 3. Weekly Additive Application in g per Pot

Pot Number	Total N/wk added (in g)	Total P/wk added (in g)	Total K/wk added (in g)	Total TPS Billions/wk added (in g)
# 1	0.000022	0.00017	0.00017	0.00056
# 2	0	0	0	0.00112
# 3	0.000045	0.00034	0.00034	0
# 4	0	0	0	0
# 5	0.000022	0.00017	0.00017	0.00056

Note: Nutrient quantities were calculated as follows-Nitrogen (2%): 2.5 ml is (1.7*2% *2.5)/3785 = 0.000022;Phosphorus (P_2O_5) (15%): 2.5 ml is (1.7*15% *2.5)/3785 = 0.00017;Potassium (K_2O) (15%): 2.5 ml is (1.7*15% *2.5)/3785 = 0.00017;TPS Billions: 2.5 ml is (0.85*2.5)/3785 = 0.00056.

measure plant height, stem width, and leaf dimensions. Every week, a medicine measuring cup was used to determine the quantity of additives to be added to each pot, as outlined in Table 2.

Statistical analysis

Statistical analysis was conducted using the Analysis ToolPak in Microsoft Excel. A one-way Analysis of Variance (ANOVA) Single-factor was performed to compare the sum, average, and variance across the 8 independent variables and 6 dependent variables. The F-statistic and p-value were used to assess whether significant differences existed between the groups. A p-value less than 0.05 ($p < 0.05$) was considered statistically significant. Out of the 6 dependent variables, regression and correlation analyses were conducted on three: "Plant Height", "Number of Leaves", and "Area of the Biggest Leaf". The "Area of the Biggest Leaf" was derived from the "Length" and "Width" of the largest leaf, serving as a substitute for these individual measurements. Other dependent variables, such as "Stem Width" and "pH" showed minimal change over the study period.

Three separate regression and correlation analyses were carried out to evaluate the impact of the independent variables on the three dependent variables, using a combination of Xrealstats and the Analysis ToolPak in Excel. The first analysis examined the relationship between the independent variables, "Days of Growth," "Did the Pot Have Additives (1 or 0)", "Amount of Lunar Soil in Pot as %" and the dependent variable "Area of the Biggest Leaf". The second analysis assessed the same independent variables as the dependent variable "Number of Leaves". Finally, the third analysis focused on the same independent variables in relation to the dependent variable "Plant Height"

Following these, another set of regression and correlation analyses was conducted to understand the impact of the variables, "Total Amount of N added in g", "Total Amount of P added in g", "Total Amount of K added in g", and "Total Amount of TPS Billions added in g", on each of the three dependent variables. The findings from these analyses prompted further regression analyses on the variables that showed an impact on the dependent variables. Successive statistical analyses were conducted to isolate the X-variables that significantly impacted the three Y-variables

To assess the relationship between the observed and predicted values from the regression models, the Multiple R (correlation coefficient) was calculated. R Square (coefficient of determination) was used to determine the proportion of variance in the dependent variable explained by the independent variables. Residuals versus predicted value plots were generated to check for heteroskedasticity, and more robust tests, including the White test and Breusch-Pagan test, were performed using the XRealStats add-in in Microsoft Excel.

Heteroskedasticity was addressed using the Weighted Least Squares (WLS) method. This involved calculating the residuals, squaring them, and then using the inverse of the squared values as weights. The square root of these weights was applied to both the X-values and the Y-values. All results, including Multiple R, R Squared, Adjusted R Squared, ANOVA, intercepts, coefficients for each of the 6 variables, and their p-values, were recorded.

RESULTS

Results of one-way ANOVA (Single factor) analysis is shown in Table 4 below. Total count of all recordings is 160 as there were 5 pots with 4 plants each and the measurements were taken every week for a total of 8 weeks.

Table 5 shows the summary of the results from running the regression and correlation analysis of the three X-variables - "Days of Growth", "Did the Pot Have Additives (1 or 0)", and "Amount of Lunar Soil in pot as (%)" on the three Y-Variables – "Area of the Biggest Leaf", "Number of Leaves", and "Plant height".

Based on the results presented in Table 5, successive statistical analyses were conducted on the four X-variables — "Total Amount of N added in g", "Total Amount of P added in g", "Total Amount of K added in g", and "Total Amount of TPS Billions added in g" with respect to the same three Y-variables. Table 6 summarizes the results of these analyses.

Based on the results presented in Tables 5 and 6, another set of statistical analyses was conducted on the four X-variables — "Days of Growth", "Total Amount of N Added in g", "Amount of Lunar Soil in Pot (%)", and "Total Amount of TPS Billions Added in g" with respect to the same three Y-variables. Table 7 summarizes the results of these analyses.

Based on the results presented in Tables 5, 6 and 7, another set of statistical analyses was conducted on the three X-variables — "Total Amount of N added in g", "Amount of lunar soil in pot as (%)", and "Total Amount of TPS Billions added in g" with respect to the same three Y-variables. Table 8 summarizes the results of these analyses.

The F and p-values from the results presented in Table 8 clearly indicate that the X-variables have a significant impact on the three Y-variables. This finding prompted a check for heteroskedasticity. Figure 1 shows the residuals of observed leaf area plotted against the predicted values from the regression model. Residuals were calculated as the difference between observed values and those predicted using the model's intercept and coefficients for the X-variables (Total Amount of N, TPS Billions, Lunar Soil). This plot allows assessment of model fit by highlighting deviations, patterns, and non-constant variance. Similarly, Figure 2 shows residuals for the number of leaves, and Figure 3 shows residuals

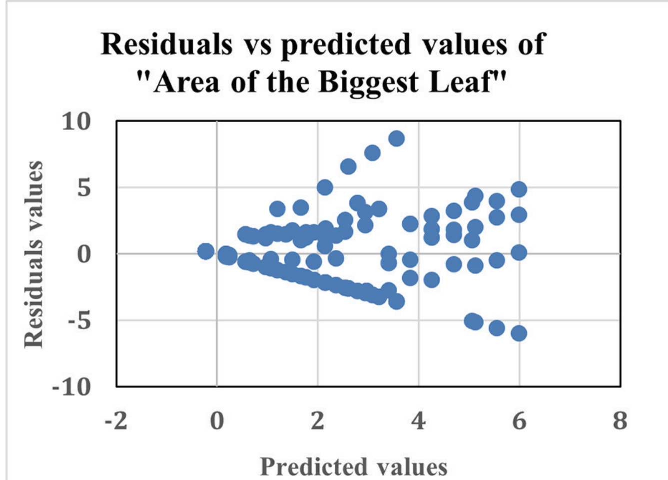

Figure 1. Leaf Area Residuals vs Predicted Values.

Table 4. One-way ANOVA Results of All Independent and Dependent Variables

Anova: Single Factor SUMMARY, Readings count - 160			
Groups	***Sum***	***Average***	***Variance***
Pot Number	480	3.0	2.01
Days of growth	5040	31.5	258.87
Did pot have additives (1 or 0)	128	0.8	0.16
Total amount of N added in g	0.013	0.00008	8.86E-09
Total amount of P added in g	0.097	0.00061	5.04E-07
Total amount of K added in g	0.097	0.0006	5.08E-07
Amount of lunar soil in pot as (%)	76.8	0.48	0.058
Total amount of TPS Billions added in g	0.32	0.002	5.57E-06
Plant Height (in)	783.3	4.90	36.44
Stem Width (in)	13.3	0.083	0.011
Number of leaves	607	3.80	27.33
pH	1164	7.27	0.20
Length of biggest leaf (in)	201.3	1.26	2.95
Width of biggest leaf (in)	161.55	1.009	1.69
Area (sq in)	301.36	1.88	8.27
Source of Variation	***SS***	***F***	***P-value***
Between Groups	142500.7	451.7	0
Within Groups	53743.9		

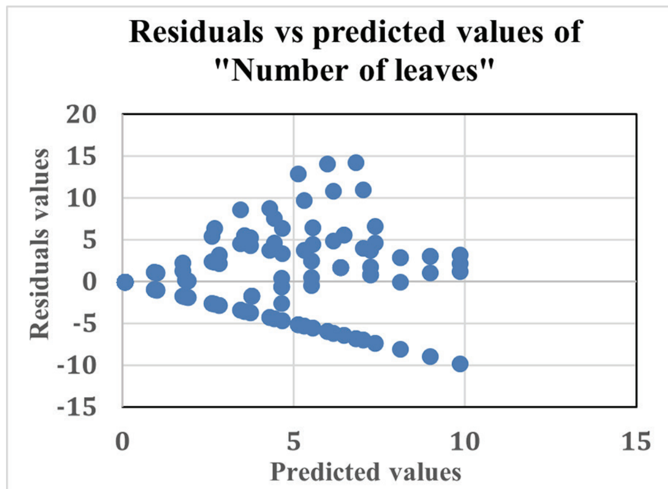

Figure 2. Number of Leaves Residuals vs Predicted Values.

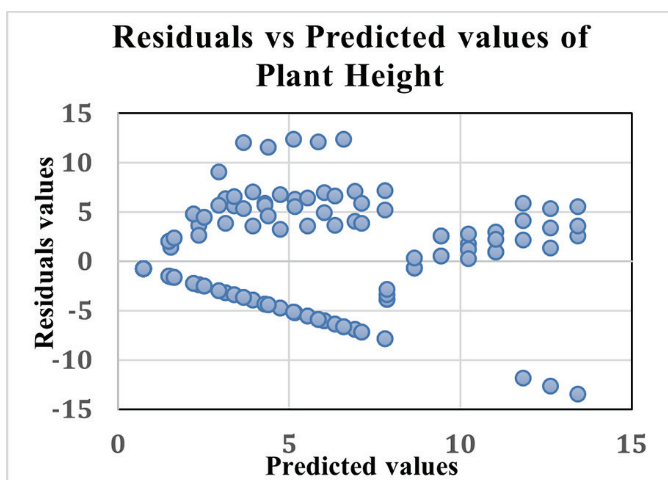

Figure 3. Plant Height Residuals vs Predicted Values.

Table 5. Summary of Regression and Correlation Between X-Variables (Days of Growth, Did the Pot Have Additives, Amount of Lunar Soil) and Key Plant Growth Variables

Y variable	X variables	Multiple R	R Squared	F	p-value	correlation coefficient	p-value of each X variable
Area of the Biggest Leaf	Days of Growth	0.59	0.34	27.85	1.77E-14	0.31	4.28E-06
	Did the Pot Have Additives (1 or 0)					0.33	0.00074
	Amount of Lunar Soil in pot as (%)					**-0.45**	2.01E-08
Number of Leaves	Days of Growth	0.51	0.26	18.74	1.96E-10	0.30	2.16E-05
	Did the Pot Have Additives (1 or 0)					0.36	2.11E-05
	Amount of Lunar Soil in pot as (%)					**-0.29**	0.0032
Plant height	Days of Growth	0.59	0.35	28.5	9.52E-15	0.19	0.0028
	Did the Pot Have Additives (1 or 0)					0.41	8.23E-06
	Amount of Lunar Soil in pot as (%)					**-0.48**	1.15E-08

Table 6. Summary of Regression and Correlation Between X-Variables (Total Amount of N, P, K, TPS Billions) and Key Plant Growth Variables

Y variable	X variables	Multiple R	R Squared	F	p-value	correlation coefficient	p-value of each X variable	Regression coefficient
Area of the Biggest Leaf	Total Amount of N Added in g	0.52	0.27	14.63	3.70E-10	0.27	0.0032	**-407632.28**
	Total Amount of P Added in g					0.28	**0.34**	45227.19
	Total Amount of K Added in g					0.28	**0.84**	10058.71
	Total Amount of TPS Billions added in g					0.34	0.00022	354.01
Number of Leaves	Total Amount of N added in g	0.52	0.27	14.08	8.01E-10	0.33	0.047	**-500342.85**
	Total Amount of P added in g					0.34	**0.36**	79512.16
	Total Amount of K added in g					0.34	**0.91**	**-10366.99**
	Total Amount of TPS Billions added in g					0.30	0.0002	650.29
Plant height	Total Amount of N added in g	0.48	0.23	11.52	3.23E-08	0.31	0.0105	**-764781.56**
	Total Amount of P added in g					0.31	**0.23**	123876.40
	Total Amount of K added in g					0.31	**0.86**	**-19549.65**
	Total Amount of TPS Billions added in g					0.25	0.0083	650.29

Table 7. Summary of Regression and Correlation Between X-Variables (Days of Growth, Total Amount of N, TPS Billions, Lunar Soil) and Key Plant Growth Variables

Y variable	X variables	Multiple R	R Squared	F	p-value	correlation coefficient	p-value of each X variable
Area of the Biggest Leaf	Days of growth	0.61	0.37	23.06	5.74E-15	0.30	**0.50**
	Total amount of N added in g					0.27	0.0014
	Amount of lunar soil in pot as (%)					**-0.45**	1.19E-08
	Total amount of TPS Billions added in g					0.34	0.00012
Number of Leaves	Days of growth	0.54	0.29	15.64	8.98E-11	0.30	**0.90**
	Total amount of N added in g					0.33	2.13E-05
	Amount of lunar soil in pot as (%)					**-0.29**	0.0023
	Total amount of TPS Billions added in g					0.30	7.73E-05
Plant height	Days of growth	0.60	0.36	22.02	2.06E-14	0.19	**0.299**
	Total amount of N added in g					0.30	1.84E-05
	Amount of lunar soil in pot as (%)					**-0.48**	4.30E-09
	Total amount of TPS Billions added in g					0.25	0.00023

Table 8. Summary of Regression and Correlation Between X-Variables (Total Amount of N, TPS Billions, Lunar Soil) and Key Plant Growth Variables

Y variable	X variables	Multiple R	R Squared	F	p-value
Area of the Biggest Leaf	Total amount of N added in g	0.61	0.37	30.70	1.18E-15
	Amount of lunar soil in pot as (%)				
	Total amount of TPS Billions added in g				
Number of Leaves	Total amount of N added in g	0.53	0.29	20.98	1.79E-11
	Amount of lunar soil in pot as (%)				
	Total amount of TPS Billions added in g				
Plant height	Total amount of N added in g	0.60	0.36	28.99	5.89E-15
	Amount of lunar soil in pot as (%)				
	Total amount of TPS Billions added in g				

for plant height, both against their respective predicted values from the regression models.

Table 9 summarizes the results of the Breusch–Pagan and White tests for heteroskedasticity based on the data set used in the analyses reported in Table 8.

Based on Figures 1, 2, and 3, as well as the results from Table 9, heteroskedasticity was confirmed. Further details are discussed in the Discussion section. To address the issue of heteroskedasticity, the Weighted Least Squares (WLS) method was employed. Table 10 presents a summary of the regression and correlation results obtained after applying WLS.

Table 9. Breusch-Pagan and White Tests for Heteroskedasticity on Key Plant Growth Variables

Y variable	Breusch-Pagan				White Test			
	LM stat	p-value	F stat	p-value	LM stat	p-value	F stat	p-value
Area of the Biggest Leaf	0.13	0.99	0.04	0.99	6.47	0.04	3.31	0.04
Number of Leaves	10.88	0.01	3.80	0.01	32.89	0.00	20.32	0.00
Plant height	26.90	0.00	10.51	0.00	38.68	0.00	25.03	0.00

Table 10. Summary of Regression and Correlation Between X-Variables (WLS values of N, TPS Billions, Lunar Soil) and Key Plant Growth Variables

Y variable	X variables	Multiple R	R Squared	F	p-value	correlation coefficient	p-value of each X variable
Area of the Biggest Leaf	weighted - N	0.99	0.99	590842.8	0	0.69	1.63E-25
	weighted - Lunar soil					**-0.046**	2.97E-21
	weighted - TPS					0.99	1.92E-42
Number of Leaves	weighted - N	0.98	0.96	1183.2	4.8E-107	0.86	3.34E-58
	weighted - Lunar soil					**-0.054**	2.21E-22
	weighted - TPS					0.88	2.5E-60
Plant height	weighted - N	0.93	0.87	344.2	1.53E-68	0.88	2.9E-11
	weighted - Lunar soil					**-0.33**	0.063
	weighted - TPS					0.86	1.0E-08

DISCUSSION

The results from Table 4's ANOVA analysis show that the F-statistic of 451.69 exceeds the critical F value of 1.69, and the p-value is 0, indicating that the groups are significantly different.

The results presented in Table 5 show R-squared values of 0.34, 0.26, and 0.35, indicating that the three independent variables "Days of Growth", "Did Pots Have Additives (1 or 0)", and "Amount of Lunar Soil in pot as (%)" collectively explain approximately 25–35% of the variance in the dependent variables "Area of the Biggest Leaf", "Number of Leaves", and "Plant height". The F-statistics and corresponding p-values (< 0.05) indicate that the overall model is statistically significant. The negative correlation coefficient associated with "Amount of Lunar Soil in pot as (%)" ($p < 0.05$) suggests a negative relationship with the dependent variables, whereas the positive correlation coefficients for "Days of Growth" and "Did Pots Have Additives (1 or 0)" ($p < 0.05$) suggest positive relationships. Given that the presence of additives positively influences plant growth, further analysis was conducted to determine which additive - Nitrogen (N), Phosphorus (P), Potassium (K), or TPS Billions exerts the greatest effect.

Table 6 summarizes the results of two sets of statistical analyses. The first section presents the R-squared values, F-statistics, p-values, and correlation coefficients from the correlation analysis. The R-squared values (0.27, 0.27, and 0.23) indicate that the four independent variables—"Total Amount of N Added in g", "Total Amount of P Added in g", "Total Amount of K Added in g" and "Total Amount of TPS Billions Added in g"—collectively explain approximately 23–27% of the variation observed in the dependent variables ("Area of the Biggest Leaf", "Number of Leaves" and "Plant Height").

Within this analysis, the p-values for "Total Amount of P Added in g" and "Total Amount of K Added in g" exceed 0.05, indicating that these variables do not significantly contribute to plant growth. In contrast, "Total Amount of N Added in g" and "Total Amount of TPS Billions Added in g" show p-values below 0.05, confirming their statistically significant effects. All correlation coefficients are positive, ranging from 0.25 to 0.34; however, only nitrogen and TPS exhibit both significant p-values and positive correlations, identifying them as the only two variables with a meaningful influence on plant growth.

The second section of Table 6 reports the regression coefficients for the four independent variables. These results reveal contrasting effects on the dependent variables. The regression coefficients for "Total Amount of N Added in g" are strongly negative (–407,632.28; –500,342.85; –764,781.56), indicating that, when holding other variables constant, a 1-g increase in nitrogen is associated with a decrease of approximately 407,000 to 765,000 units in the dependent variables. This negative association may reflect nitrogen toxicity at higher application levels, which can inhibit plant growth through osmotic stress, impaired root function, or disrupted nutrient uptake. Conversely, the positive regression coefficients for "Total Amount of TPS Billions Added in g" suggest that increasing TPS is associated with higher values of the dependent variables when other factors are controlled. The strong positive effect of the TPS Billions additive suggests its benefits extend beyond simple nutrient supplementation. A likely mechanism is enhanced root–microbe symbiosis: microbial amendments can stimulate root exudation, increase root surface area, and promote colonization by beneficial microorganisms (e.g., arbuscular mycorrhizal fungi (AMF) or plant growth-promoting rhizobacteria), thereby improving nutrient uptake and stress resilience. For instance, Ma *et al*. (7) demonstrated that AMF significantly increased root vigor and N, P, and K accumulation in maize, while Li, Zhou, *et al*. (8) reported that AMF inoculation enhanced maize seedling growth, increasing biomass by 42.7%, chlorophyll content by 13.4%, and antioxidant capacity. These findings highlight the potential of microbial supplementation to improve soil function and plant performance in suboptimal environments.

Taken together, these findings help reconcile the regression patterns. Although nitrogen is fundamentally essential for plant growth, the highly negative regression coefficients suggest that beyond an optimal threshold, it becomes rapidly detrimental unlike TPS, which maintains a positive influence even at higher concentrations. In our study, TPS likely acted as a microbial-friendly substrate (or delivered microbial inoculants) that improved soil structure and root–soil interface function, enhancing nutrient uptake from otherwise limiting pools and promoting growth.

Overall, the contrasting regression outcomes indicate that in suboptimal substrates, plant growth may depend more on root–microbe–soil dynamics than on the sheer quantity of added nutrients, highlighting the unique growth-promoting role of TPS relative to high nitrogen inputs.

Given these insights, it is clear that the two variables with notable impacts on plant growth are "Total Amount of TPS Billions added in g" and "Total Amount of N added in g." The remaining variables, "Days of Growth" and "Amount of Lunar Soil in pot as (%)" are also statistically significant based on results from Table 5.

Table 7 presents the regression results for the four variables in relation to plant growth. Two key findings emerge from these results. First, the "Days of Growth" variable has p-values above 0.05, indicating that it is not a statistically significant factor in plant growth. Second, "Amount of Lunar Soil in pot as (%)" exhibits a negative correlation coefficient with p-values < 0.05, indicating a significant negative effect on plant growth. These results suggest that the three variables with a significant impact on plant growth are "Total Amount of N added in g", "Amount of Lunar Soil in pot as (%)", and "Total Amount of TPS Billions added in g". Consequently, regression analyses were conducted on these variables against the Y-variables, and the results are presented in Table 8.

Table 8 results show R-squared values of 0.37, 0.29, and 0.36, indicating that the three independent variables "Total Amount of N added in g", "Amount of lunar soil in pot as (%)", and "Total Amount of TPS Billions added in g" collectively explain approximately 29–37% of the variance in the dependent variables "Area of the Biggest Leaf", "Number of Leaves", and "Plant height". The F-statistics and corresponding p-values (< 0.05) indicate that the overall model is statistically significant.

Given that these three variables significantly impact plant growth, it was necessary to assess whether the assumption of constant variance of errors (homoskedasticity) holds. Figures 1, 2, and 3 present the residual plots for the three dependent variables. As shown in the figures, the residuals exhibit a "fanning" pattern as predicted values increase, suggesting the presence of heteroskedasticity. To confirm this observation, Breusch–Pagan and White tests were conducted, and the results are presented in Table 9.

From Table 9, for "Number of Leaves", the p-values are < 0.05 in both the Breusch–Pagan and White tests, providing strong evidence of heteroskedasticity and indicating that the variance of residuals is not constant. Similarly, for "Plant Height", p-values < 0.05 in both tests also indicate clear heteroskedasticity. In contrast, the results for "Area of the Biggest Leaf" are mixed: the Breusch–Pagan test yields a p-value > 0.05, whereas the White test yields a p-value < 0.05. Considering these results alongside the residual plots for the three dependent variables, it is reasonable to conclude that heteroskedasticity is present.

To overcome heteroskedasticity, WLS method was applied and the results are presented in Table 10. Table 10 results show R-squared values of 0.99, 0.96, and 0.87 indicating that the weighted values of the three independent variables collectively explain approximately 87-99% of the variance in the dependent variables "Area of the Biggest Leaf", "Number of Leaves", and "Plant height". The F-statistics and corresponding p-values (< 0.05) indicate that the overall model is statistically significant. The negative correlation coefficient for the "weighted lunar soil composition" variable indicates that higher the percentage of lunar soil regolith in the pot negatively impacts plant growth. The weighted variable for TPS Billions has a correlation coefficient of 0.99, 0.88, and 0.86, closer to 1 clearly indicating a very positive and significant contribution to plant growth.

CONCLUSION

The regression analyses indicate that both nitrogen and TPS Plant Foods Billions of Microbes significantly influence bush bean growth in a lunar soil simulant. Our results show that excessive amounts of nitrogen can negatively affect plant growth, whereas higher amounts of TPS Billions exhibit a strong positive correlation with leaf development—likely attributable to improvements in soil structure and root–soil interface function. Among all treatments, Pot #2, which received a higher concentration of the TPS microbial additive, showed the most vigorous growth. Notably, correlation coefficients for the microbial additive were consistently higher across all three growth parameters—plant height, leaf area, and leaf count—than those for nitrogen, suggesting that microbial activity exerted a more pronounced effect on plant performance. In contrast, P and K showed no measurable influence on plant development.

While the initial hypothesis predicted the growth ranking Pot #5 (control) > Pot #1 > Pot #2 > Pot #3 > Pot #4, the observed pattern was Pot #5 > Pot #2 > Pot #1 > Pot #3 > Pot #4. The unexpectedly strong performance of Pot #2 reinforces the conclusion that microbial amendments have a greater impact on plant growth than nitrogen fertilization alone.

Overall, this study provides early quantitative evidence that microbial soil additives can enhance crop growth in extraterrestrial regolith simulants, bridging plant biology and space resource utilization.

A key limitation in our experimentation set up is the small sample size (n = 5 pots per treatment), which may reduce statistical power and generalizability. It would be valuable to explore the impact of these additives in a larger number of pots with varying lunar soil compositions, such as 70%, 80%, and 90% and that is one area of recommendation for future work.

CONFLICT OF INTEREST

The authors declare no conflict of interests related to this work.

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