

# The Effect of Biochar Feedstock on the Remediation of Acidic Soils and Improvement of *Pisum sativum* v. *saccharatum* Growth

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Intensive agriculture has led to depletion of nutrients in soils and increasing soil acidity. Biochar has often been considered an effective soil amendment; however, its characteristics can change depending on feedstock. Therefore, the purpose of this experiment was to observe whether biochar made from different feedstocks could remediate acidic soil and improve *Pisum sativum* v. *saccharatum* growth. The hypothesis was that a combination of arugula and pine bark biochar would lead to the greatest remediation of acidic soil and *Pisum sativum* v. *saccharatum* growth. This experiment was done by creating arugula and pine bark biochar using the retort method. Then, biochars were added to soil in a 72-Cell Tray at total 5 % concentrations: 5 % pine bark, 5 % arugula, or 2.5 % pine bark and 2.5 % arugula. A control group with no biochar addition was also created. Afterward, one *P. sativum* seed was planted into each cell, and a water and vinegar solution at pH 5.5 was used to water plants over 41 days. Soil NPK and pH, stem lengths, and number of plants alive were recorded throughout experimentation. Results found that there was no statistical significance between trial groups on soil NPK and pH levels ( $p > 0.05$ ,  $\alpha = 0.05$ ). However, a one-way ANOVA of *P. sativum* stem lengths indicated statistical significance among trial groups (ANOVA[F(3, 413) = 29.69,  $p < 0.001$ ]). A post hoc Tukey Test found that there was statistical significance between Control vs. Arugula, Pine Bark vs. Arugula, Pine Bark vs. Combined, and Control vs. Combined groups. Survivorship curves indicated that addition of biochars increased *Pisum sativum* yield. This experiment found that both Arugula and Combined groups were more effective in increasing *P. sativum* growth than Pine Bark, due to increased nutrient capacity of arugula biochar. The experiment also showed that feedstock is essential in determining overall characteristics of biochar.

## Introduction

Soil acidity has risen as a substantial issue in agriculture, leading to reduced crop growth as well as decreased soil nutrients and microbial activity<sup>1</sup>. Soil acidity is a naturally occurring process due to the gradual increase of hydrogen ions in the soil, but this has been accelerated by intensive agriculture and the use of ammonium-based fertilizers. Acidic soils also lead to an increase in the concentration of soluble aluminum in the soil, which can further restrict root growth, water accessibility, and nutrient accessibility due to aluminum toxicity. Methods have been found in order to increase the pH of soils, one such process being liming<sup>1</sup>. However, natural alternatives are being researched as well.

One such alternative is biochar. Biochar is a substance made from the burning of organic matter at high temperatures under anaerobic conditions<sup>2</sup>. Biochar has often been posited as a beneficial soil amendment due to its high porosity and ability to increase water and nutrient retention in soil. Previous research has shown the useful effects of biochar on plants and soil as well. Gao et al. tested the effects of biochar made from timber, with and without poultry litter, on soil quality<sup>3</sup>. Results found that the combination of biochar and poultry litter produced the highest soil nutrient concentration, while biochar itself resulted in the highest deep soil nutrient concentration. Additionally, biochar overall increased carbon sequestration within soils and the availability of beneficial ions to plants. Another study by Hagemann et al. noted biochar's high nutrient retention in soils and found it to be a valuable slow-release nitrate fertilizer<sup>4</sup>. Slow-release fertilization is the ability of a substance to absorb nutrients from the soil and release them back at a lower rate, ultimately reducing nitrate leaching as plants are more readily able to absorb a greater amount of nutrients over time.

Furthermore, biochar has shown potential in improving the quality of poor soils. Gas-flared soils are known for having highly acidic pHs and increased depletion of nutrients<sup>5</sup>. Akachukwu et al. used palm fiber biochar pyrolyzed at 450 °C to remediate gas-flared soil and improve fluted pumpkin growth<sup>5</sup>. Results found that the application of biochar increased fluted pumpkin vitamin composition and leaf ash content, in both the intermediate and highest concentrations.

Research has also shown that the effects of biochar on plants and soil is dependent on biochar's feedstock and pyrolyzation temperature. Bhattarai et al. investigated the effects of five different types of biochars on soil and garden pea properties<sup>6</sup>. Feedstocks included poultry manure, rice husk, sheep manure, farmyard manure, and wood. Results showed that all biochars exhibited a significant positive effect in increasing soil quality and garden pea yield. Specifically, rice husk biochar had the greatest effect on garden pea yield; poultry manure and sheep manure biochar had similar effects in increasing soil nitrogen levels; and sheep manure biochar most significantly increased carbon soil percentage. Additionally, Domingues et al. tested the environmental and agricultural effects of biochars made from five different feedstocks, each at three different pyrolyzation temperatures<sup>7</sup>. Feedstocks included coffee husk, chicken manure, eucalyptus sawdust, pine bark, and sugarcane bagasse, and temperatures included 350 °C, 450 °C, and 750 °C. Results showed that biochar made from wood and sugarcane bagasse had the most potential in improving the carbon storage of soils. Also, the high amounts of nutrients present in coffee husk and chicken manure made these biochars most able to improve soil quality. Although pyrolyzation temperature was not as integral in determining characteristics of biochar, higher temperatures did show a decrease in the cation exchange capacity of biochar, which could ultimately decrease the amount of beneficial ions biochar can provide to plants.

Though feedstock and pyrolyzation temperature play an important role in determining the characteristics of biochar, the concentration of biochar applied to the soil is also essential in providing advantageous effects to both the soil and plants. Prapagdee and Tawinteung tested the effects of cassava stem biochar in concentrations of 0 %, 5 %, and 10 % on the qualities of soil and mung bean plants<sup>2</sup>. Results showed that biochar at 5 % concentration significantly increased mung bean growth, while biochar at 10 % concentration increased the mass of the bean plants. The application of biochar generally contributed to increased nutrients in the soil as well, though with 10 % cassava stem biochar potassium levels were also increased within plants.

One plant that can benefit from the application of biochar to soil is *Pisum sativum* var. *saccharatum*, more commonly known as snow peas. Snow peas are a cool season vegetable from the *Leguminosae* family. They have high vitamin A, vitamin C, iron, potassium, dietary fiber, and folic acid contents<sup>8</sup>. Additionally, snow peas are known for their beneficial effects in increasing digestive processes, bone mineral density, and vision<sup>9</sup>.

Biochar's inherent alkalinity and ability to increase water and nutrient retention may lessen the detrimental effects of soil acidity. Additionally, the effects of a combination of biochars made from different feedstocks may provide increased benefits that should be further researched. Therefore,

the purpose of this experiment was to investigate whether the application of biochar or a combination of biochars may remediate acidic soils and improve the quality of *Pisum sativum var. saccharatum*. The independent variable in this experiment was the type of biochar applied to the soil. Biochars included pine bark, arugula, and a combination of the two biochars. The dependent variables in this experiment were *Pisum sativum var. saccharatum* stem lengths (cm), soil pH, soil NPK levels (ppm), and the number of plants alive every other day. It was hypothesized that if pine bark biochar, arugula biochar, and a combination of pine bark and arugula biochar were applied to acidic soil, then the trial group with both pine bark and arugula biochar would lead to the highest soil pH and NPK levels, as well as the highest *Pisum sativum var. saccharatum* growth rates. This experiment was done by creating arugula and pine bark biochar using the retort method. Then, pine bark biochar and arugula biochar were applied to separate soil mixtures at 5 % concentrations for their respective trial groups. In the combined trial group of arugula and pine bark biochar, a 2.5 % concentration of pine bark biochar and a 2.5 % concentration of arugula biochar were applied to the soil. Afterward, 40 mL of the respective soil mixture was used to fill each of fifteen cells in a 72-Cell Tray, creating 60 trial cells overall. Once *Pisum sativum var. saccharatum* seeds were planted, stem lengths and the number of plants alive were measured every other day, and soil pH and NPK levels were measured weekly over the course of 41 days. A solution of vinegar and water was used to simulate acid rain and create acidic soils throughout the experiment.

## Methods

Before experimentation, pine bark and arugula biochars were prepared using the retort method. Pine bark and arugula were collected and bought, respectively. Both substances were cut into 2 cm<sup>3</sup> pieces and heated separately in the oven for two hours at 150 °C. Then, arugula and pine bark pieces were separately wrapped in tin foil and placed in a metal box with an attached vent. The box was then grilled for three hours at 360 degrees Celsius with a closed lid. After allowing the chars to cool for two hours, arugula and pine bark chars were placed in separate plastic bags and crushed.

Experimental set-up followed the creation of the biochar. Forty mL of soil were added to each of the 15 cells in one 72-Cell Tray; this was labelled as the Control group (C). Then, 30 mL of pine bark biochar was mixed into 570 mL of soil, creating an overall 5 % pine bark biochar concentration. Forty mL of this soil was then added to each of 15 more cells in the 72-Cell Tray; these cells were then labelled as the Pine Bark group (PB). This process was repeated with arugula biochar in order to create soil with a 5 % arugula biochar concentration, and the resulting trial group was labelled as the Arugula group (A). Afterward, 15 mL of pine bark biochar and 15 mL of arugula biochar was added to 570 mL of soil, creating soil with a 5 % mixed concentration of pine bark and arugula biochar. Forty mL of this soil mixture was then added to each of 15 more cells in the 72-Cell Tray, creating 60 trial cells overall. The final 15 cells were labelled as the Combined trial group (PBA). One *Pisum sativum* seed was then planted into each cell. Once adult leaves began growing on the plants, stem lengths and the number of plants alive were recorded every other day. Weekly soil pH and NPK levels were also recorded from the beginning to end of experimentation, over the course of approximately 41 days. Nitrate, phosphorus, and potassium values were initially measured in pounds per acre for a 6-inch soil depth using a LaMotte NPK Soil Test Kit. However, a table was used to convert values into parts per million<sup>10</sup>. Soil pH levels were measured using a Rapitest Soil pH Test Kit. An acidic vinegar solution was used to water the plants throughout the growing period in order to simulate acid rain and create acidic soils. This solution was created by adding vinegar in drops to 1 L of water until the solution reached the desired pH of 5.5. Data was analyzed using Version 14 and Version 19 of Minitab. Figure 1 shows the Experimental Design Diagram for this experiment.

## Results

Table 1 shows the descriptive statistics of *Pisum sativum* stem lengths among all trial groups. The table shows that the Control group had a mean of 21.096 and a standard deviation of 4.201; the Pine Bark group had a mean of 21.748 and a standard deviation of 3.460; the Arugula group had a mean of 23.909 and standard deviation of 4.333; and the Combined group had a mean of 24.918 and standard deviation of 3.971. Figure 2 shows boxplots of all *Pisum sativum* stem lengths among the different trial groups. It can be seen on Figure 2 that the medians of the Arugula and Combined groups are higher than the medians of the Control and Pine Bark groups. A potential outlier can also be seen in the Pine Bark group.

Figure 3 shows the survivorship curves of *Pisum sativum* plants among all trials. It can be seen that Pine Bark, Arugula, and Combined trial groups all maintained their number of plants alive through the duration of the experiment, while the Control group lost one plant during the experiment. Additionally, both Pine Bark and Combined biochars maintained the life of all plants. The Control group maintained the life of 14 plants initially, and the Arugula biochar maintained the lives 11 plants throughout the experiment.

Table 2 shows a Repeated Measures Two-Way ANOVA of *Pisum sativum* stem lengths for the interaction between time and trial group. The Day\*Group analysis failed to reject the null hypothesis with  $F(21, 385) = 0.38, p = 0.995 > \alpha = 0.05$ , indicating that there was no statistical significance between the interaction of time and trial group in the experiment. However, the Day analysis rejected the null hypothesis with  $F(7, 385) = 50.36, p < 0.001 < \alpha = 0.05$ . The Group analysis also rejected the null hypothesis with  $F(3, 385) = 46.48, p < 0.001 < \alpha = 0.05$ .

After the Repeated Measures Two-Way ANOVA was run, a One-Way ANOVA was run as well (Table 3). With  $F(3, 413) = 29.69, p < 0.001 < \alpha = 0.05$ , the null hypothesis was rejected, indicating there was statistical significance among the data for *Pisum sativum* stem lengths. A post hoc Tukey Test was run as well (Table 4). These data indicated that there was no statistical significance between the Control vs Pine Bark and Arugula vs Combined groups. However, statistical significance was found between Control vs Arugula, Control vs Combined, Pine Bark vs Arugula, and Pine Bark vs Combined groups.

Table 5 shows the raw data table for soil nitrate levels, while Figure 4 shows a time-series plot of soil nitrate levels among all trial groups over five weeks. The data show that the Control group maintained a level of 20 ppm throughout the experiment. The Pine Bark, Arugula, and Combined biochar groups rose to 50 ppm at Week 2, but maintained a level of 20 ppm for all other weeks.

Table 6 shows the raw data of soil phosphorus levels among all trial groups, whereas Figure 5 shows a time-series plot of the soil phosphorus levels in all trial groups throughout the experiment. It can be seen that the Control and Pine Bark groups do not exceed soil phosphorus levels over 10 ppm (Table 6). On the other hand, the Arugula and Combined groups both exceed these levels towards Weeks 3 and 4 (Figure 5). However, it can be seen that the Arugula group maintains 32 ppm for Weeks 4 and 5, while the Combined group reaches 32 ppm on Week 3, drops to 4 ppm on Week 4, and increases back to 32 ppm on Week 5.

Table 7 shows the raw data of soil potassium levels, while Figure 6 shows the time-series plot of soil potassium levels over time. Table 7 shows that both Arugula and Combined groups had the same soil potassium concentration throughout the experiment. These values were generally higher than either the Control or Pine Bark groups, and were maintained for a longer duration in the experiment than either Control or Pine Bark groups.

Table 8 shows the raw data for soil pH levels, while Figure 7 shows a time-series plot of soil pH levels of all trial groups throughout the duration of the experiment. No descriptive trends can be observed within or among trial groups. The Pine Bark trial group can be seen to decrease to 5.0 in Week 5, while Control and Combined groups were able to reach a pH of 6.5 in Week 5 and Weeks 5 and 6, respectively.

As soil nitrate, phosphorus, potassium, and pH data sets were found to be non-normal, non-parametric tests were run in order to determine significance in soil levels. Friedman tests were run on soil N, P, K, and pH levels. The results of these tests can be found in Tables 9, 10, 11, and 12, respectively. The p-values of 0.392 for nitrate, 0.392 for phosphorus, and 0.559 for pH are all greater than  $\alpha = 0.05$ , failing to reject the null hypothesis and indicating no statistical significance in the data. On the other hand, the p-value for potassium soil levels is 0.015, which is less than  $\alpha = 0.05$ , rejecting the null hypothesis. However, the p-value that was not adjusted for ties is 0.092, which is greater than  $\alpha = 0.05$ , failing to reject the null hypothesis. The results of the Friedman test for soil potassium levels prompted the running of a series of Wilcoxon Signed Rank tests on the differences between potassium levels of individual trial groups to find significance.

Table 13 shows the summary table of a series of Wilcoxon Signed Rank tests between trial groups on soil potassium levels. As the difference between the Control group and Arugula, Control and Combined, Pine Bark and Arugula, and Pine Bark and Combined groups all had a p-value of 0.100, which was greater than  $\alpha = 0.05$ , the null hypothesis failed to reject for these differences. Additionally, as the difference between the Control and Pine Bark group had a p-value that was greater than 0.999, which was also greater than  $\alpha = 0.05$ , the null hypothesis failed to reject. This indicated that there was no statistical significance between trial groups for soil potassium levels. A Wilcoxon Signed Rank test could not be run on the difference between Arugula and Combined trial groups as both trial groups had the same potassium values throughout the experiment.

## Discussion

The purpose of this study was to test the effect of a combination of biochars on *Pisum sativum* growth and soil NPK and pH levels in acidic soils. The hypothesis was that if different feedstocks and combinations of biochars were added to acidic soil, then the combination of pine bark and arugula biochar would lead to the most significant *Pisum sativum* growth and highest soil NPK and pH levels. The One-Way ANOVA (Table 3) indicated that there were significant differences in *Pisum sativum* stem lengths among trial groups. Comparing this with the Tukey Test (Table 4) and boxplots (Figure 2), it can be seen that the Arugula Biochar and Combined Biochar trial groups had higher stem lengths than the Pine Bark Biochar or Control groups. This partly supported the first part of the hypothesis, that the Combined Biochar trial group would have the highest *Pisum sativum* stem lengths. Though the Combined biochar had higher *Pisum sativum* growth than the Control and Pine Bark groups, there was no statistical significance between the Combined Biochar and Arugula Biochar trial groups. The survivorship curves (Figure 3) also indicated that the addition of biochar improved *P. sativum* yield, as the Control group was the only group unable to maintain the number of plants alive throughout the experiment. However, the Friedman test for soil nitrogen (Table 9), phosphorus (Table 10), and pH levels (Table 12), along with the Wilcoxon Signed Rank tests for potassium (Table 13) found no significant differences in these levels between the trial groups throughout the experiment. These data failed to support the second part of the hypothesis, that the Combined trial group would have the highest soil NPK and pH levels. Thus, the data only partially supported the overall hypothesis.

While there was no statistical significance in soil levels, descriptive trends show potential increases in soil nutrients due to addition of biochar. The increase of N levels to 50 ppm in Week 3 in Biochar, Pine Bark, and Combined groups (Figure 4) indicate that the application of biochar into soil may have increased soil N levels. Additionally, the fact that Arugula and Combined trial groups reached higher phosphorus values than Pine Bark and Control groups (Figure 5) indicate that the addition of arugula biochar was able to increase phosphorus levels in the soil. Furthermore, Figure 6 indicates that arugula biochar was able to increase potassium levels in the soil as well, as the Arugula and Combined trial groups reached and maintained higher potassium levels than Pine Bark or Control groups. However, due to the statistical results and equipment used in this experiment, further research would be needed in order to explore this.

The fact that arugula biochar increased *Pisum sativum* growth in acidic soils compared with Akachukwuet al.'s study, which found that palm fiber biochar significantly improved the quality of *Telfairia occidentalis* in gas-flared soils<sup>5</sup>. The reason why the *P. sativum* stem lengths were higher in the Arugula and Combined trial groups than the Pine Bark or Combined trial groups may have been due to the increased nutrient absorption capabilities of arugula biochar. Domingues et al. cited in their experiment that increased biochar nutrient absorption could be seen in biochars pyrolyzed from feedstocks with high amounts of nutrients<sup>7</sup>. As arugula has a high amount of nutrients<sup>8</sup>, biochar made from this feedstock may have been able to more effectively provide nutrients to plants. This may also explain why both Arugula and Combined biochar had higher *Pisum sativum* stem lengths, as both trial groups contained arugula biochar.

The results also showed that pine bark biochar was not as effective in increasing *P. sativum* growth, but was still effective in increasing *Pisum sativum* yield. This compared with Bhattarai et al., who stated that in their experiment wood waste biochar had the least positive effect on soil levels and garden pea yield<sup>6</sup>. The reason for why pine bark biochar may not have been able to increase *Pisum sativum* stem lengths may have been because the pine bark itself did not have as many beneficial nutrients. This meant that the biochar did not have as high of a nutrient retention capacity, and was unable to provide as many beneficial nutrients to *P. sativum*.

The results of soil NPK and pH levels in this experiment were major sources of uncertainty. The results of the experiment found that there was no statistical significance in soil nitrate (Table 9), phosphorus (Table 10), potassium (Table 13), and pH levels (Table 12). These results contrasted with Obia et al., who found that the addition of biochar made from a combination of rice husk and cacao shell increased the pH of acidic soils<sup>11</sup>. The results of this experiment also contrasted with results from Eykelboshet al., who found that the addition of sugarcane filter cake biochar increased the availability of soil nutrients, such as phosphorus, potassium, and calcium, to plants<sup>12</sup>. One reason for why these soil levels were found to be statistically insignificant could be due to the equipment used. Testing of soil NPK levels and pH was done using color charts, and determination of the colors could have differed based on the time of day and the amount of light passing through the sample. This could have led to inaccurate results in the experiment.

Some aspects of this experiment could have been modified for the improvement of the study. One modification is the method of application of biochar to the soil. As biochar was added to soil in a large bucket and then distributed across the cells, instead of each cell being applied with biochar, there could have been slightly different concentrations of biochar among trial cells, which could have affected individual stem lengths of *Pisum sativum*. This could have been improved by adding the exact amount of biochar to each cell in order to create a 5% concentration. Additionally, the solution used to water the plants was created by adding vinegar to water until the pH of the solution reached 5.5. The determination of the pH was done using a color chart, which could have led to differences in the acidity among the solutions used to water the plants throughout the experiment. The soil NPK and pH levels were also determined using color charts, which could have affected the results depending on the amount of light passing through the sample. Thus, another improvement to this study would be using more precise equipment in

order to gain exact values of soil NPK, water pH, and soil pH.

There are numerous venues for future studies. The effect of biochar feedstock could be tested in increasingly acidic soils, in order to determine the extent to which biochar can improve plant growth in poor soils. Additionally, studies have found that wood biochar can improve carbon storage of soils and increase carbon sequestration<sup>7</sup>. Thus, another future study could include the testing of soil carbon storage and plant growth in acidic soils with or without the treatment of different biochars. This could thereby show potential benefits of pine bark biochar and further support the implementation of a combination of biochars into soil. Further, this study could be repeated with another combination of biochars, such as from two feedstocks that have high concentrations of nutrients. This could be studied in order to determine whether the diversity of feedstocks that are high in nutrients could improve plant growth and soil quality.

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## Tables and Figures

**Figure 1. Experimental Design Diagram**

<b>Title of the Experiment:</b> The effect of biochar feedstock on the remediation of acidic soils and improvement of <i>Pisum sativum v. saccharatum</i> growth				
<b>Hypothesis:</b> If biochar is added to soil affected by acid rain, then the trial group with both pine bark and arugula biochar will lead to the highest soil NPK and pH levels and highest <i>Pisum sativum v. saccharatum</i> growth rates.				
<b>Independent Variable:</b> Biochar Feedstock				
<b>Levels of Independent</b>	Control	Pine Bark	Arugula	Combined
<b>Number of Repeated</b>	15	15	15	15
<b>Dependent Variable:</b> <i>Pisum sativum</i> stem lengths (cm), pH of soil, soil NPK levels (ppm), amount of plants alive on every measurement day				
<b>Constants:</b> Water, biochar concentration, number of trials, light, temperature, soil				
<b>Control:</b> The control group is the trial group with no addition of biochar into the soil.				

**Table 1. Descriptive statistics for *Pisum sativum* stem lengths (cm) in all trial groups**

Variable	Mean	Standard	Minimum	Q1	Median	Q3	Maximum	Range	IQR
<b>Control</b>	21.096	4.201	8.400	18.025	21.000	24.875	28.500	20.100	6.850
<b>Pine Bark</b>	21.748	3.460	8.800	18.525	21.300	24.375	28.100	19.300	4.850
<b>Arugula</b>	23.909	4.333	12.000	21.500	24.100	27.600	31.000	19.000	6.100
<b>Combined</b>	24.918	3.971	14.200	22.100	25.500	28.175	34.000	19.800	6.075

Table 1 shows the descriptive statistics of *Pisum sativum* stem lengths in all trial groups. These values include means, five-number summaries, standard deviations, and ranges. All values were recorded in centimeters.

Figure 2. Boxplot of *Pisum sativum* stem lengths (cm)

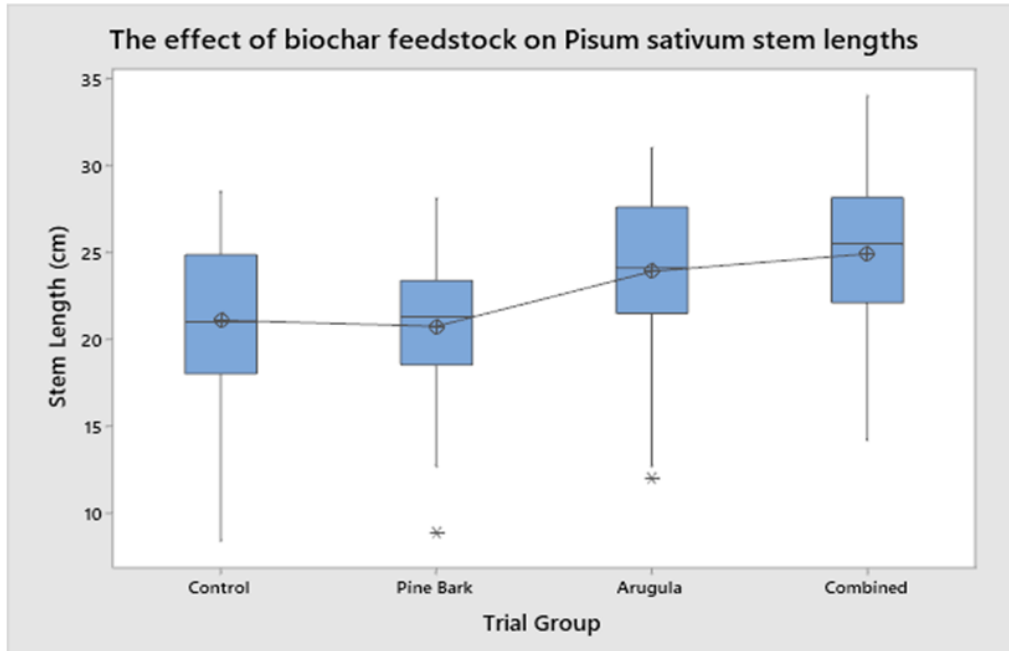


Figure 2 shows *Pisum sativum* stem lengths in all tested trial groups. It can be seen that Arugula and Combined trial groups seem to have higher medians and stem lengths than Control or Pine Bark trial groups. A potential outlier can be seen in the Pine Bark group.

Figure 3. Survivorship curves of *Pisum sativum* among trial groups

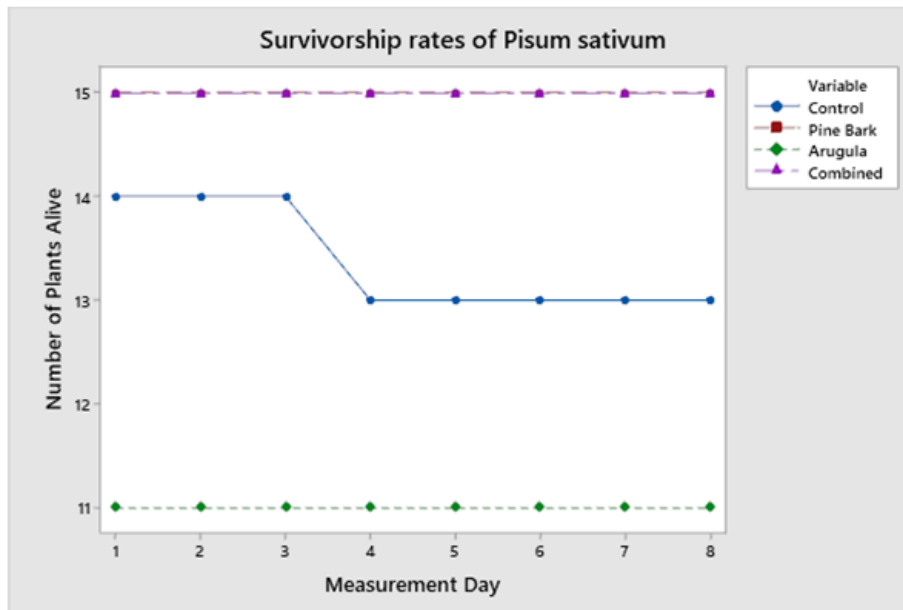


Figure 3 shows the survivorship curves of *Pisum sativum* among all trial groups for the duration of the experiment. The graph shows that the Pine Bark, Arugula, and Combined trial groups all maintained plant numbers through the experiment, while the Control trial group lost one plant between Measurement Days 3 and 4.

Table 2. Two-Way Repeated Measures ANOVA summary table for *Pisum sativum* stem lengths

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Day	7	3039.65	2915.53	416.50	46.48	<0.001
Group	3	1349.33	1353.73	451.24	50.36	<0.001
Day*Group	21	71.08	71.08	3.38	0.38	0.995
Error	385	3449.69	3449.69	8.96		
Total	416	7909.75				

This table shows the Two-way Repeated Measures ANOVA summary table for all *Pisum sativum* stem lengths. According to the table, the null hypothesis for the Day\*Group interaction failed to reject, as ANOVA[F(21, 385) = 0.38, p = 0.995]. However, the Group analysis rejected the null hypothesis, as ANOVA[F(3, 385) = 50.36, p<0.001]. This prompted a subsequent One-way ANOVA and post hoc Tukey Test. The Day analysis shows a rejection of the null hypothesis as well, as ANOVA[F(7, 385) = 46.48, p < 0.001].

Table 3. One-Way ANOVA for *Pisum sativum* stem lengths

Source	DF	Adj SS	Adj MS	F-value	P-value
Group	3	1403.1	467.7	29.69	<0.001
Error	413	6506.7	15.8		
Total	416	7909.7			

Table 3 shows that the results of the one-way ANOVA are ANOVA[F(3, 413) = 29.69, p < 0.001]. This led to rejection of the null hypothesis, and a post hoc Tukey Test was run to test statistical significance between trial groups.

Table 4. Tukey test for *Pisum sativum* stem lengths among all trial groups

Group	N	Mean	Grouping
4	120	24.918	A
3	85	23.909	A
1	96	21.096	B
2	116	20.748	B

Table 4 shows the post hoc Tukey Test that was used to determine statistical differences between trial groups for *Pisum sativum* stem lengths. The number '1' corresponds with the Control trial group, '2' with the Pine Bark trial group, '3' with Arugula, and '4' with Combined. The test shows that there was no statistical significance between Control versus Pine Bark trial groups or Arugula versus Combined trial groups. However, there was statistical significance in the Control versus Arugula, Control versus Combined, Pine Bark versus Arugula, and Pine Bark versus Combined trial groups.

Table 5. Raw data of soil nitrate levels (ppm)

	Control	Arugula	Pine Bark	Combined
Week 0	20	20	20	20
Week 1	20	20	20	20
Week 2	20	50	50	50
Week 3	20	20	20	20
Week 4	20	20	20	20
Week 5	20	20	20	20

This table shows the raw data table of soil nitrate levels, measured in ppm, among all trial groups over five weeks. It can be seen that all groups maintained a level of 20 ppm throughout the experiment. However, in Week 2, the Pine Bark, Arugula, and Combined Biochar trial groups all peaked at 50 ppm.

Figure 4. Time-Series plot of soil nitrate levels (ppm) among all trial groups

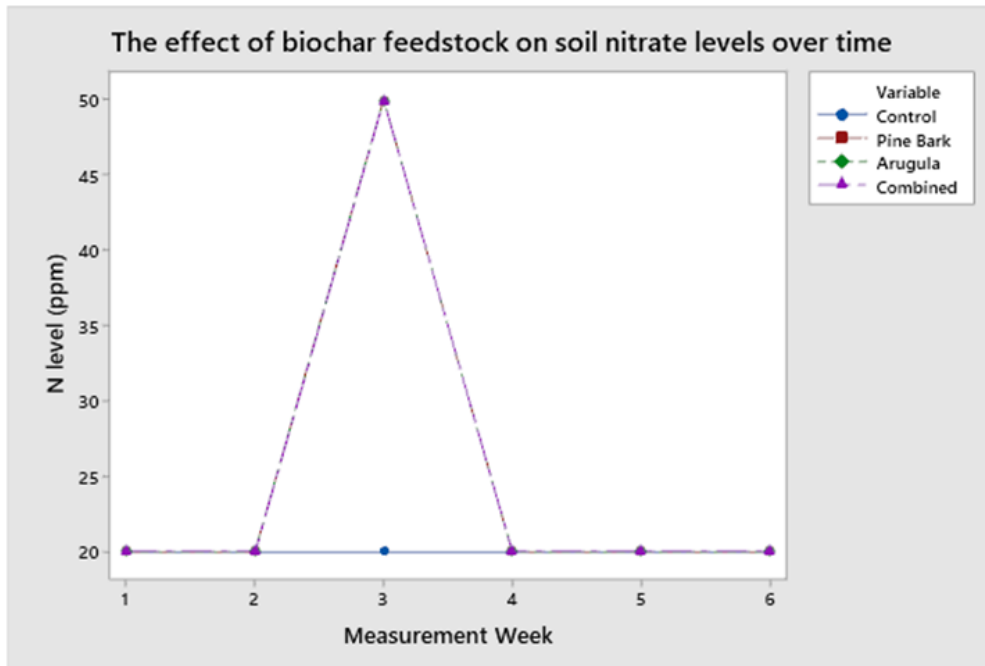


Figure 4 shows a time-series plot of soil nitrate levels in all trial groups over a period of five weeks. Measurement Week 1 corresponds with values from Week 0 on the raw data table. It can be descriptively determined that the Control group maintained a level of 20 ppm throughout the experiment, whereas the remaining groups rose to 50 ppm in Week 2, but otherwise maintained a concentration of 20 ppm throughout the experiment.



Table 6. Raw data of soil phosphorus levels (ppm)

	Control	Pine Bark	Arugula	Combined
Week 0	0	4	4	4
Week 1	7	10	10	10
Week 2	10	4	4	4
Week 3	4	4	4	32
Week 4	10	4	32	4
Week 5	4	4	32	32

Table 6 shows the raw data of soil phosphorus levels among all trial groups. It can be seen that in the first week, the Control group had 0 ppm of phosphorus in soil, and the level in the soil did not rise above 10 ppm. Both the Arugula and Combined trial groups reached a level of 32 ppm during Weeks 4 and 3, respectively; however, Arugula maintains this level in the last two measurements, whereas the Combined group's level is 32 ppm on Week 3, decreases to 4 ppm on Week 4, and increases again to 32 ppm on Week 5.

Figure 5. Time-Series plot of soil phosphorus levels (ppm) in all trial groups

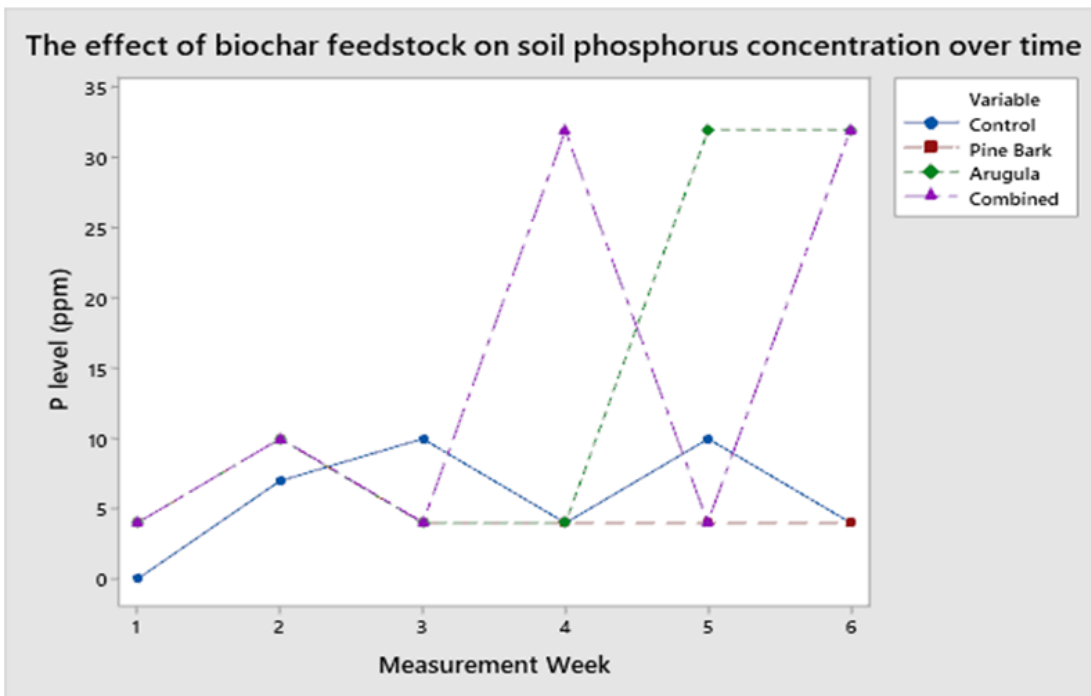


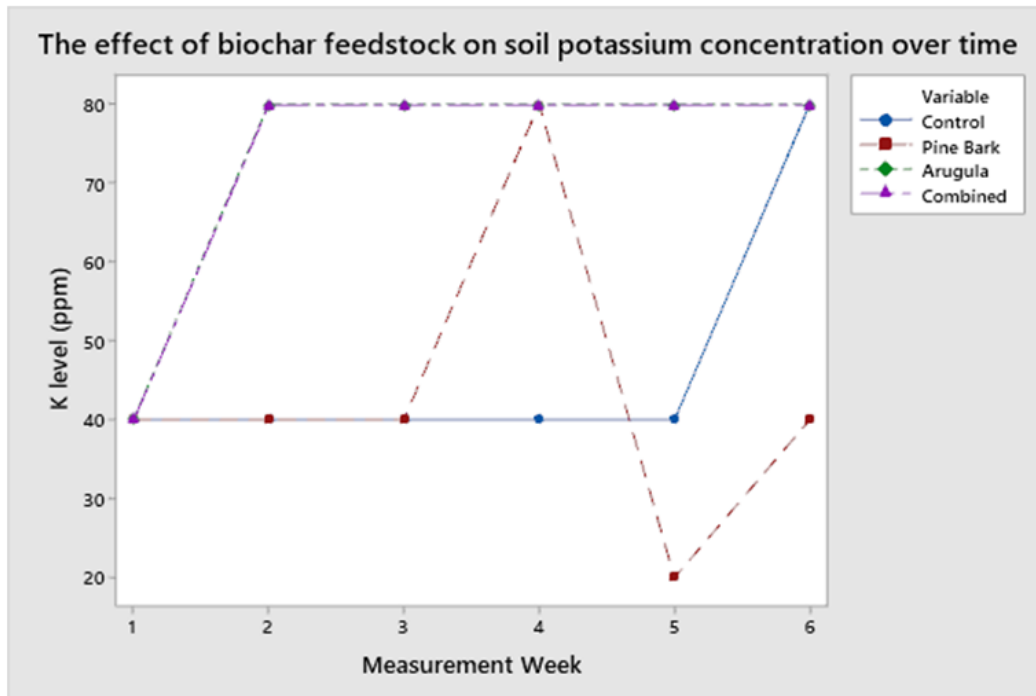
Figure 5 shows a time-series plot of soil phosphorus levels among all trial groups over time. Measurement Week 1 corresponds with values from Week 0 on the raw data table. It can be seen descriptively that both the Control and Pine Bark groups maintain lower levels of phosphorus, whereas the Arugula and Combined groups have higher soil phosphorus levels near the second half of the measuring period.

Table 7. Raw data of soil potassium levels (ppm)

	Control	Pine Bark	Arugula	Combined
Week 0	40	40	40	40
Week 1	40	40	80	80
Week 2	40	40	80	80
Week 3	40	80	80	80
Week 4	40	20	80	80
Week 5	80	40	80	80

Table 7 shows the raw data of soil potassium levels, measured in ppm. The table shows that both Arugula and Combined groups reached 80 ppm earlier than the Control and Pine Bark groups and were able to maintain these levels for most of the experiment.

Figure 6. Time-Series plot of soil potassium levels (ppm) in all trial groups



This figure shows a time-series plot of soil potassium concentration among all trial groups throughout the duration of the experiment. Measurement Week 1 corresponds with values from Week 0 on the raw data table. It can be seen descriptively that the Combined and Arugula groups maintained the same soil K concentration throughout the experiment. These values were overall higher than the Pine Bark and Control group K levels; however, the Pine Bark group reached 80 ppm once on Week 4, and the Control reaches 80 ppm on Week 5.

**Table 8. Raw data of soil pH levels**

	Control	Pine Bark	Arugula	Combined
Week 0	5.75	5.75	5.5	5.5
Week 1	5.5	6.0	5.5	5.5
Week 2	6.0	6.0	6.0	6.0
Week 3	6.0	5.5	6.0	5.5
Week 4	6.5	5.0	5.5	6.5
Week 5	6.0	6.0	5.5	6.5

Table 8 shows the soil pH levels of all trial groups throughout the experiment. All values ranged between values of 5.5 to 6.5.

Figure 7. Time-Series plot of soil pH levels in all trial groups

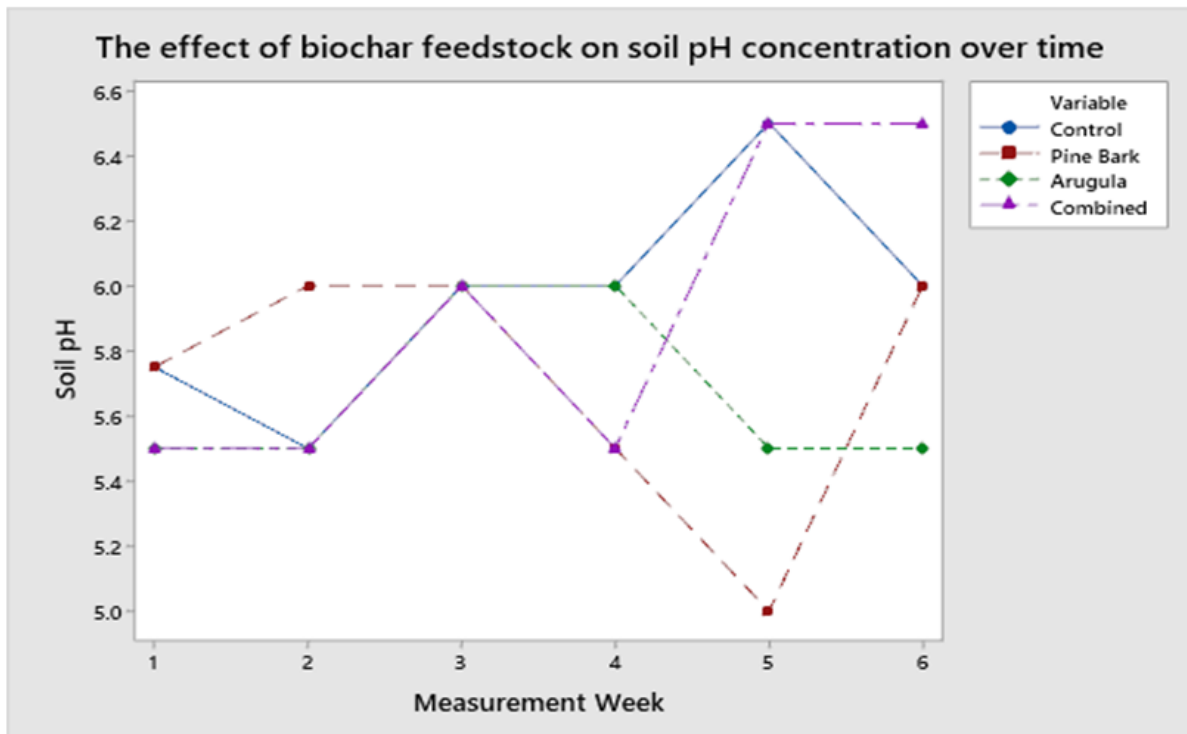


Figure 7 shows the soil pH levels of all trial groups throughout the experiment. Measurement Week 1 corresponds with values from Week 0 on the raw data table. There are no descriptive trends that can be observed in or among trial groups.

Table 9. Summary table of Friedman Test for Soil N Levels

Method	DF	Chi-Square	P-value
Not adjusted for ties	3	0.30	0.960
Adjusted for ties	3	3.00	0.392

Table 9 shows a summary table for the Friedman Test for soil N levels. As  $p = 0.392$  is greater than  $\alpha = 0.05$ , the null hypothesis fails to reject, indicating there was no significance in soil nitrate levels among trial groups over the duration of the experiment.

Table 10. Summary table of Friedman Test for Soil P Levels

Method	DF	Chi-Square	P-value
Not adjusted for ties	3	2.05	0.562
Adjusted for ties	3	3.00	0.392

Table 10 shows a summary table for the Friedman Test for soil phosphorus levels. As  $p = 0.392$  is greater than  $\alpha = 0.05$ , the null hypothesis fails to reject, indicating there was no significance in soil phosphorus levels among trial groups over the duration of the experiment.

Table 11. Summary table of Friedman Test for Soil K Levels

Method	DF	Chi-Square	P-Value
Not adjusted for ties	3	6.45	$P = 0.092$
Adjusted for ties	3	10.46	$P = 0.015$

Table 11 shows a summary table for the Friedman Test for soil potassium levels. As  $p = 0.015$  is less than  $\alpha = 0.05$ , the null hypothesis is rejected, indicating there was potential statistical significance in soil potassium levels among trial groups. This prompted the running of a series of Wilcoxon Signed Rank tests among the differences between each trial group.

Table 12. Summary table of Friedman Test for Soil pH Levels

Method	DF	Chi-Square	P-Value
Not adjusted for ties	3	1.25	$P = 0.741$
Adjusted for ties	3	1.87	$P = 0.559$

Table 12 shows a summary table for the Friedman Test for soil pH levels. As  $p = 0.559$  is greater than  $\alpha = 0.05$ , the null hypothesis fails to reject, indicating there was no significance in soil pH levels among trial groups over the duration of the experiment.

Table 13. Summary table of Wilcoxon Signed Rank tests for potassium values among differences of all groups

Sample	N for Test	Wilcoxon Statistic	P-Value
Co-A	4	0.00	0.100
Co-PB	3	3.50	>0.999
Co-C	4	0.00	0.100
PB-A	4	0.00	0.100
PB-C	4	0.00	0.100

Table 13 shows the summary table on a series of Wilcoxon Signed Rank tests run on the mean of differences between trial groups for soil potassium levels. Co stands for the Control group, PB stands for the Pine Bark trial group, A for Arugula, and C for Combined. As  $p = 0.100 > \alpha = 0.05$ , the null hypothesis failed to reject for differences between Control and Arugula, Control and Combined, Pine Bark and Arugula, and Pine Bark and Combined. Additionally, as the p-value for the difference between the Control and Pine Bark groups was greater than 0.999, which is greater than  $\alpha = 0.05$ , the null hypothesis failed to reject for this difference as well. The difference between Arugula and Combined could not be tested, as all values were the same between the two trial groups throughout the experiment.

### Appendix A

Table 10. Day 15 *Pisum sativum v. saccharatum* stem lengths (cm)

Trial	Control	Pine Bark	Arugula	Combined
1	12.8	16.70	*	20.6
2	*	12.7	18	21.4
3	19.2	8.8	19.8	22
4	16	14.4	*	20.2
5	41.6	17.5	*	19.5
6	17.6	15.8	18.9	18.2
7	19.1	20.3	17.7	20.1
8	14.8	19.0	19.8	22
9	4.7	17.1	16	14.2
10	17.2	18.9	*	19.1
11	8.40	15.7	17.6	17.3
12	16.4	19.4	6	16.2
13	16.1	16.5	17.9	22.2
14	15.5	17.7	18.3	20
15	16.6	20.3	20.1	15

Table 10 shows *Pisum sativum v. saccharatum* stem lengths on Day 15, the first day the plants began showing adult leaves. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.

**Table 11. Day 17 *Pisum sativum* v. *saccharatum* stem lengths (cm)**

Trial	Control	Pine Bark	Arugula	Combined
1	15.8	18.60	*	25.5
2	*	15.1	22.8	21.9
3	25.4	12.8	22.1	22
4	22.2	18.2	*	22.7
5	49.2	18.5	*	22.1
6	18.8	17.7	20.5	25.5
7	17.50	22.3	23.8	22.6
8	17.6	18.0	21.6	23.4
9	4.8	18.6	19.4	16
10	20.5	19	*	22.5
11	11.30	19.8	19.8	21.6
12	21.5	21.3	6.8	19.9
13	19	19.8	22	22.2
14	16.6	21.6	23.5	26.6
15	17.5	23.5	25.3	24.1

Table 11 shows *Pisum sativum* v. *saccharatum* stem lengths on Day 17. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.

**Table 12. Day 20 *Pisum sativum* v. *saccharatum* stem lengths (cm)**

Trial	Control	Pine Bark	Arugula	Combined
1	16.1	20.00	*	23.8
2	*	16.8	24.2	22.9
3	25.9	14.1	21.3	24
4	25.5	18.1	*	23.8
5	47	18.5	*	23.8
6	18.1	16	21.5	20.2
7	20.90	21.6	25.4	24.9
8	15	21.4	22.6	24.6
9	4.7	19.8	21.5	17.9
10	23.5	19.5	*	25.5
11	15.10	22.9	21.9	22.1
12	22	22.8	10.1	24.6
13	19.8	21.6	22.5	26.1
14	17.9	23.2	21.7	29.6
15	17.8	24.7	27.5	25.2

Table 12 shows *Pisum sativum* v. *saccharatum* stem lengths on Day 20. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.

**Table 13. Day 24 *Pisum sativum v. saccharatum* stem lengths (cm)**

Trial	Control	Pine Bark	Arugula	Combined
1	18.1	19.60	*	26
2	*	17.7	24.2	24.5
3	25.5	15.2	24.8	23.2
4	24.5	19.5	*	21
5	48	18.9	*	22.5
6	18.0	18.6	23.5	22.1
7	22.70	21.5	23.2	24.5
8	21.5	21.3	24	26.7
9	*	21.8	22.6	18
10	23.3	19.1	*	23.6
11	22.50	18.1	27.6	21.9
12	24.2	22	12	23.6
13	19.1	17.1	23	26.2
14	17.2	20.5	24.1	26.8
15	16	23.5	24.7	23.5

Table 13 shows *Pisum sativum v. saccharatum* stem lengths on Day 24. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.

**Table 14. Day 29 *Pisum sativum v. saccharatum* stem lengths (cm)**

Trial	Control	Pine Bark	Arugula	Combined
1	19.5	20.00	*	29.1
2	*	*	23.9	26.6
3	25.7	15.5	25.1	26.9
4	27.8	19.9	*	25.7
5	53.6	21.9	*	25.7
6	20.1	20.6	23.4	23.3
7	24.10	22.2	24.1	27.3
8	21.1	23.3	23.7	26.5
9	*	20.1	23.5	18.2
10	25	22.5	*	24.6
11	26.70	21.6	29.1	26
12	23.6	19.8	12.7	26
13	19.1	22.2	27.6	25.5
14	18.1	21.6	27.2	28.6
15	19.1	24.5	25.5	26.5

Table 14 shows *Pisum sativum v. saccharatum* stem lengths on Day 29. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.

**Table 15. Day 31 *Pisum sativum* v. *saccharatum* stem lengths (cm)**

Trial	Control	Pine Bark	Arugula	Combined
1	21.2	22.4	*	29.7
2	*	*	24.3	26.5
3	26.1	16.1	27.8	28.3
4	27.5	22.8	*	27.7
5	54.8	24	*	27.9
6	20.5	20.1	24.6	23.2
7	20.8	22.6	28.8	29.3
8	24.5	25	27.6	27.5
9	*	21.4	26.6	20.2
10	27.3	17.9	*	23.02
11	18.7	23.4	28.5	27.8
12	25.6	23.8	13.9	27.1
13	21.5	22.7	24.4	28.1
14	20.3	21.5	28.5	29.5
15	19.3	26.5	29.7	26.8

Table 15 shows *Pisum sativum* v. *saccharatum* stem lengths on Day 31. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.

**Table 16. Day 34 *Pisum sativum* v. *saccharatum* stem lengths (cm)**

Trial	Control	Pine Bark	Arugula	Combined
1	23.7	24.3	*	30.3
2	*	*	25.6	29.1
3	26.2	17.7	28.7	29.3
4	27.9	21.6	*	29.1
5	56.5	24.1	*	28.6
6	19.3	22.6	24.3	26.4
7	26.2	25.1	29	31.7
8	26	24.5	28.2	28.5
9	*	20.1	26.3	20.4
10	27.7	23.3	*	28.9
11	21.1	26.2	30.2	27
12	26.9	24	16.6	26.8
13	20.6	23.7	27.4	30.2
14	19.4	20.7	29.1	31.3
15	21.1	28.1	30.6	28.2

Table 16 shows *Pisum sativum* v. *saccharatum* stem lengths on Day 34. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.



**Table 17. Day 38 *Pisum sativum v. saccharatum* stem lengths (cm)**

Trial	Control	Pine Bark	Arugula	Combined
1	23.5	25.7	*	31
2	*	*	29	30.2
3	27.9	18.7	28.6	29
4	28.5	24.6	*	28.9
5	57.3	25.3	*	30
6	20.3	23.5	27.1	29.6
7	27.6	27.5	31	34
8	25.1	26.1	29	30.6
9	*	22.8	27.6	21.3
10	28.1	23.8	*	28.9
11	21.1	26.6	16.8	25.1
12	25.2	23.9	19.2	30.3
13	21.2	23.4	29.2	27.5
14	22.7	23.9	29.7	30.8
15	21.1	28.1	29.9	28.3

Table 17 shows *Pisum sativum v. saccharatum* stem lengths on Day 38. All stem lengths are measured in centimeters. Asterisks indicate empty cells with no plants.

### Appendix B

**Table 18. Raw data of amount of *Pisum sativum v. saccharatum* plants alive on each measurement day**

Day	Control	Pine Bark	Arugula	Combined
15	14	15	11	15
17	14	15	11	15
20	14	15	11	15
24	13	15	11	15
29	13	15	11	15
31	13	15	11	15
34	13	15	11	15
38	13	15	11	15

Table 18 shows the raw data collected on each measurement day of the amount of plants alive in each trial group. The Arugula trial group started at 11 plants and the Control group started at 14 plants on Day 15 as some seeds were non-germinating and did not grow when planted.