The Effects of *Rhizobium* sp. Inoculants on Non-Legumes’ Germination and Growth Rates

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Abstract

As the number of organic farmers and other individuals interested in preserving the environment is increasing, alternative agricultural practices are being developed. Consequently, farmers are relying less on inorganic fertilizers and are turning to biofertilizers instead (Adiguzel et al., 2010, p. 5017). *Rhizobia* sp. are an example of how this environmental friendly practice is utilized. *Rhizobia* sp. are gram negative bacteria found in the soil that form a symbiotic relationship with legumes by infecting their roots and subsequently engaging in nitrogen fixation. This process involves the conversion of atmospheric nitrogen into ammonia which is used by the legumes to produce protein and ultimately grow faster (Graham & Vance, 2000, p. 94). While nodules present themselves in legumes to increase nitrogen production and overall plant growth, research involving the possible association of *Rhizobia* sp. with the roots of non-legumes has not been thoroughly explored. Nevertheless, research has demonstrated that *Rhizobia* sp. have been successfully colonized in different crops such as corn (*Zea mays*), consequently promoting growth, quality and product yield (Mehboob, Ahmad Zahir, Arshad, Tanveer, & Khalid, 2012, p. 37). Additional research indicates that *Rhizobium* strains have supported the growth and quality of tomato seeds (*Solanum lycopersicum*) (García-Fraile et al., 2012, p. 1). With the projected world population to exceed eight billion by 2025, the increased demand for food sources is inevitable (Graham & Vance, 2000, p. 94). Due to these increases, the continuous depletion of nitrogen in soils, and the antagonistic effect of pesticides on *Rhizobia* sp., alternatives for increasing nitrogen and faster crop turnovers are potential methods of mitigating future food shortages. This project examined the possibility of using *Rhizobia* sp. in non-legumes for increasing crop productivity; specifically germination and growth rates.

Inoculants containing various strains of *Rhizobia* were applied to radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*), corn (*Zea mays*), and tomato (*Solanum*
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lycopersicum) seeds and planted in vermiculite. The planters were watered as needed with deionized water and germination records were kept for two weeks. The corn plants were also measured to determine if their growth had been affected by the application of the different strains of *Rhizobia sp.*

*Keywords*: Biofertilizers, Non-Legumes, Nitrogen, Nodules, *Rhizobium, Rhizobia*

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**Background Information**

Legumes have long been planted to replace nitrogen in soils which has been depleted of this element by other crops. *Rhizobium* sp., which converts nitrogen to ammonia, is responsible for this process and does so by forming a symbiotic relationship with its host (Shahzad et al., 2012, p. 522). Legumes are responsible for approximately 46 million tons of fixed nitrogen annually; which represents almost half of all nitrogen used in agriculture (Adiguzel et al., 2010, p. 5017). Apart from its nitrogen fixing benefits, *Rhizobium* sp. is allowing farmers interested in providing eco-friendly legumes to grow their crops without using harmful fertilizers. Plant Growth Promoting Rhizobacteria (PGPR) is a term widely used for any bacteria inhabiting the soil which forms a symbiotic relationship with surrounding plants by colonizing the rhizosphere, rhizoplane, or the actual root; consequently, promoting plant growth. The most studied and longest exploited form of PGPR are *Rhizobia* sp. due to their ability to fixate nitrogen in *only legume* host plants (Mehboob, Ahmad Zahir, Arshad, Tanveer, & Khalid, 2012, p. 37). In contrast to this well known technique, PGPR research and its application to non-legumes is relatively limited. This experiment incorporated the latter application to further investigate the effects of PGPR on germination and growth rates in non-legumes. For this project, various non-legumes were selected and coated with *Rhizobium* sp. strains, allowed to dry
and then planted. The objective of this project was to grow non-legumes coated with \textit{Rhizobium} sp. strains and compare the germination and growth rates to control seeds which were not treated. Average germination and growth rates were then recorded and compared to the control seeds.

\textbf{Research Question}

Will the application of \textit{Rhizobium} sp. inoculants affect the germination and growth rate of non-legumes?

\textbf{Hypothesis}

By coating (inoculating) seeds of non-legumes with \textit{Rhizobium} sp. strains prior to planting, non-legumes will germinate and grow faster than uncoated (untreated) seeds.

Table I

\textbf{Variable Table}

<table>
<thead>
<tr>
<th>Name</th>
<th>I/D/C</th>
<th>Identifier</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coated Non-legume Seeds</td>
<td>I</td>
<td>Scientific Name</td>
<td>\textit{Rhizobia} sp.</td>
</tr>
<tr>
<td>Germination Rate</td>
<td>D</td>
<td>#</td>
<td>Mean Days</td>
</tr>
<tr>
<td>Growth Rate</td>
<td>D</td>
<td>mm</td>
<td>Mean Length</td>
</tr>
<tr>
<td>Uncoated Seeds</td>
<td>C</td>
<td>Scientific Name</td>
<td>Uncoated</td>
</tr>
</tbody>
</table>
Apparatus

A model of a lighting incubator which was used for growing the plants is depicted below along with an actual display of one used in the laboratory. Other basic supplies used were potting plants, vermiculite, coffee filters, Erlenmeyer flasks, tweezers, spatulas, respiratory masks, and non-legume seeds: radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), carrot (*Daucus carota*), corn (*Zea mays*), and tomato (*Solanum lycopersicum*) seeds, sterile water, 70% isopropyl alcohol, and inoculants containing various strains of *Rhizobium* sp.

![Figure 1. Lighting Incubator](image1)

![Figure 2. Actual Display Panel](image2)

Procedures

**Seed Preparation**

1. Approximately 60 seeds of each plant variety were soaked in 20 ml of 70% isopropyl alcohol for one minute in 100 ml beakers, poured into a coffee filter, allowing the alcohol to pass and the seeds to dry.
2. Half of the seeds were then mixed with approximately .25 grams of *Rhizobium* sp. strains (specifically prepared and mixed for garden peas, sweet peas, lima beans and soy beans) and 3 milliliters of sterile water. More water was added if necessary to completely coat all seeds. The remaining seeds were rinsed with sterile water. All seeds were allowed to dry.

**Planter Preparation**

1. 30 planters were filled with approximately ¾ full of vermiculite.

2. Three seeds of each plant were placed on top of the vermiculite; except for the corn in which only one seed was planted. All seeds were then coated with additional layer of vermiculite.

3. Each planter was then watered with two liters of deionized water.

4. The planters were placed in a lighting incubator set @ approximately 26° C due to varying optimal germination temperatures, and watered as needed.

5. Germination records were kept daily or as laboratory and weekend schedules permitted.

6. The corn plants were measured after a two week period.

**Data**

Data on germination was taken on seven different days for the various iterations conducted. In an effort to maintain consistency, lab hours were maintained at approximately the same intervals; however, as the project advanced, proficiency by repetition allowed for quicker data collection. In addition, all corn plants were measured on day 12. Due to the sheer number of data points, which exceeded 5000, actual data tables are not included in this report; however, before data compilation, basic tables (10 columns x 5 rows) were maintained to track
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germination. Upon completion of the experiment, all of the data was then entered into several excel spreadsheets and different calculations were performed including but not limited to mean germination, mean grown, and paired t-tests to determine potential statistical differences between the means of the dependent variables. In an effort to mitigate confounding variables, protocols were strictly adhered to and the paired t-test was chosen to increase the statistical power of the results provided as well as take into account these unknown variables. In total, six iterations were completed for this project; however due to a micro-environment present in the incubator, growth measurement data for the corn was not available for one set.

**Analysis**

![Graph Indicating Statistical Significance](image)

Indicates a Significant Statistical Difference Between *Rhizobium* and Control

Graph 1

![Radish Germination Graph](image)

Paired T-Test comparing *Rhizobium* application to Control resulted in $\alpha=0.0287$ indicating a statistical significant difference.
Paired T-Test comparing *Rhizobium* application to Control resulted in $\alpha=0.024369$ indicating a statistical significant difference.
Paired T-Test comparing *Rhizobium* to Control resulted in α=0.006412 indicating a statistical difference.

Paired T-Test comparing *Rhizobium* to Control resulted in α=0.583343 indicating no statistical difference.
Paired T-Test comparing *Rhizobium* to Control resulted in a $\alpha=0.006197$ indicating a statistical difference.

Paired T-Test comparing *Rhizobium* to Control resulted in $\alpha=0.00363$ indicating a statistical difference.
Figure 3. Corn Growth Day **Seven Control**

Figure 4. Corn Growth Day **Six Rhizobium**

Figure 5. Control Day **12 Example**
Figure 6. Day 12 Rhizobium Example

Figure 7. Day 12 Rhizobium Example
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Figure 8. Day 12 Rhizobium Example
Conclusion

By performing paired t-tests, significant differences between the Rhizobium and control for all intervals were found. In three of the cases, tomato and carrot germination and corn growth, confidence intervals surpassed 99%. The remaining seeds, with the exception of corn, were shown to be statistically different at a 95% confidence interval. With this specific statistical analysis, confounding variables are mitigated, and the statistical results are strengthened by its own results. Consequently, the null hypothesis which would indicate that the difference between the means would be zero can be rejected by the confirmation that the application of Rhizobium sp. inoculants did have a positive impact on plant germination and growth. Evidence of this claim is clearly illustrated in the bar graphs and the paired t-tests support the claim even further. All graphs are uniform and compare Rhizobium application to control and data points were taken on the same days to maintain consistency. In addition, ‘p’ values were added to each graph to
determine whether or not a significant statistical difference was observed. The various pictures, although not quantitative in nature, also show a visual difference between the *Rhizobium* application and control. Nevertheless, all 'p' values and corresponding confidence intervals are relative only to the specific interval measured. In other words, it is a common mistake to assume that since these intervals demonstrated a statistical difference, future rounds would as well.

Although PGPR has been around for centuries, the term was not coined until the early 1990’s and it was still limited to *Rhizobium* which formed a symbiotic relationship with legumes. Nevertheless, recent research has demonstrated that *Rhizobia* sp. have been successfully colonized in different crops such as corn (*Zea mays*), consequently promoting growth, quality and product yield (Mehboob, Ahmad Zahir, Arshad, Tanveer, & Khalid, 2012, p. 37). Additional research indicates that *Rhizobium* strains have supported the growth and quality of tomato seeds (*Solanum lycopersicum*) (García-Fraile et al., 2012, p. 1). Although this research project was primarily based and limited to Open Educational Resources and databases to which various libraries subscribe, additional research supporting this practice was scarce. Consequently, further investigation is needed to determine if final yield, weight, and quality would be enhanced as well. In addition to this, different *Rhizobium* species can be applied to an array of seeds to determine its effects. The combinations are far reaching and while the scientific community supports this research project, much is still to be explored. By exploiting this endeavor, the scientific community will not only further promote environmentally conscious farming by replenishing and augmenting depleted nitrogen, it will potentially eliminate the use of harmful pesticides as well. In addition, since current population and food demand already outweighs supply, additional research, application, and techniques, are not only prudent choices, they are also a necessity.

**References**


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