Running Head: THE EFFECT OF MYCORRHIZAE ON D. CAROTA

The Effect of Arbuscular Mycorrhizal Fungi on the Growth of Daucus carota.

Word Count: 4752

Abstract

Arbuscular mycorrhizal fungi have been proven to increase the growth of plants, but are not commonly inoculated in agricultural fields. Using these fungi, the agricultural plant *Daucus carota* could increase in biomass and, in turn, produce larger crops. Larger crops could be used to feed more people, especially in impoverished areas, and fight against malnutrition and starvation that affects millions of people worldwide. Different species of mycorrhizal fungi will be added to seedlings of *Daucus carota* and grown for 54 days. After the growth period, the growth of the different experimental groups will be compared to each other and to the control to see which increased crop biomass the most as well as other features of the plant like stem length. *Daucus carota* plants with the addition of the high mulch inoculant will be most successful due to the increased amounts of connections that the fungi would have with those plants as well as other hopeful bacteria that could be present in the inoculant.

Introduction

Most ecosystems are thought of as large interconnected webs between plants and animals. However, what is not commonly thought of is the far more complex underground systems that form between plants (Kurth et al., 2013). Underground there is an abundance of connections made between different plants and between plants and fungi. The fungi play a key role in the formation of large common mycorrhizal networks because of their ability to connect with several host plants, that can be of the same species or different species, due to their broad host specificity (Qiao et al. 2016). This broad host specificity makes them crucial to the survival and success of the plants in many ecosystems, as well as allowing for more connections with plant species, which will be beneficial for both plant and fungus. It will be helpful for both of them because they form a connection that is symbiotic (Thonar et al., 2010). In the symbiotic relationship, fungi act as another root system for the plants that increase the uptake of nutrients and water and, in return, the fungi retain a steady source of carbon (Bennett et al., 2013). The most common nutrients that the fungi help the plants attain are phosphorous and nitrogen, which help the plants' roots and ability to flower. Overall, the benefits on the nutrient uptake of the plant increase its growth rate, the rate of photosynthesis, and increased resistance to pathogens (Mandal et al., 2013). The resistance to pathogens can be seen by the reduced symptoms of infected plants with mycorrhizal connections as compared to those without, as well as decreased presence of the DNA of the virus in different sections of the plant (Maffei et al., 2013). Viruses aren't the sole negative circumstance that mycorrhizal fungi help plants overcome. Mycorrhizal fungi can help plants survive in soils that aren't as promoting of plant growth, as seen by plant ability to improve in growth in soils under salinity stress because of the connects with the fungi

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(Mohammadi, Khalesro, Sohrabi, and Heidari, 2011). Similar success thanks to mycorrhizal fungi has been seen by the improved nutrient intake of plants in water-stressed soils (Tobar, Azcon, and Barea, 1994). So, mycorrhizal fungi help improve the growth of plants in good or bad conditions, however, the benefits of the fungi extend even further than that. In addition to all the positive effects mycorrhizal fungi have on plants when they are growing, mycorrhizal fungi also have an ability to increase the decomposition rate of the plants into the soil, which further replenishes the soil with more nutrients (Schädler, Brandl, and Kempel, 2009). The overall helpful effect of mycorrhizal fungi on an ecosystem is clear, yet there are certain studies that have proven aspects of the fungi that can harm certain members of an ecosystem.

These symbiotic relationships that are formed are not always beneficial, however. When there are plants that are unable to make mycorrhizal connections in an ecosystem, they are at a disadvantage because they are unable to uptake as many nutrients as those plants with mycorrhizal connections (Veiga et al., 2013). Non-mycorrhizal plants could also exhaust themselves by using their plant defense systems because they feel threatened (Veiga et al., 2013). In this way, mycorrhizal fungi can have a negative effect on specific plants in an ecosystem. There are other studies that have also seen how mycorrhizal fungi had primarily negative effects on specific plant species. One of the studies concluded that the fungi caused iron deficiency in *Eucalyptus tetrodonta* plants, which inhibited their success in their environment (Janos, Scott, Aristizábal, and Bowman, 2013). While this is a negative effect caused by mycorrhizal fungi, it is arguable that such effects could be a way that some ecosystems avoid or fight against the colonization of invasive species. Another study discovered one other negative effect of mycorrhizal fungi on ecosystems, which was seedlings struggled to grow in an environment with

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preexisting mycorrhizal connections (Merrild, Ambus, Rosendahl, and Jakobsen, 2013). This counterintuitive aspect of mycorrhizal fungi may have some repercussions on natural ecosystems but would have little impact on their use as an agricultural farming technique, a modern focus of mycorrhizal fungi, due to the uprooting of crops at harvest.

One of the specific focuses is which fungal species can help increase the different aspects of different agricultural crops and the presence of these fungal species in modern agricultural soils. Of these aspects looked for, the most common is crop biomass, which would increase thanks to the mycorrhizal fungi if it makes those connections. However, there is little worry about whether or not they will make the connections because arbuscular mycorrhizal fungi have been found to be connected to nearly all different species of grain crops, vegetables, and fruits (He and Nara, 2007). In fact, 95% of vascular plants contribute to the diversity of ecosystems with the mycorrhizal fungi additions that they use to help grow (Filion, St. Arnaud, and Fontin, 1999). Daucus carota is one of those many vegetables that are able to establish and usually take advantage of symbiotic connections made with arbuscular mycorrhizal fungi by increasing the phosphatase chemicals taken up by the roots of *Daucus carota*, which were colonized 70% by Glomus intraradices (Wang, Tong, Shi, Xu, and He, 2011). While Glomus intraradices has been proven to help increase the biomass of *Daucus carota* and there are other species that have a likelihood of helping which could be found in healthy soils. However, not much has been done to try and exploit this advantage that nature gives farmers to help them grow larger, more successful crops. This is in large part due to the fact that large companies give reasoning for 21% of their GDP to be fertilizers, which they then use along pesticides and other harmful farming techniques which keep beneficial fungi and microorganisms from forming in the soil and require

more and more fertilizer to remain at a constant production level (Chakrabarty et al., 2014). These other harmful farming techniques include the use of fungicides, many of which greatly hinder the colonization of mycorrhizal fungi on plants, and greatly altered the entire arbuscular mycorrhizal fungi community (Jin, Germida, and Walley, 2013). On the other hand, numerous organic farms use the mycorrhizal fungi to help their crops grow and only experience a minor decrease in crop yield but require, according to one study, 97% less pesticide and 34 to 53% percent less fertilizer (Mäder et al., 2002). It has also been concluded that using organic techniques, such as using mulch, increases the root colonization of plants by 40% as compared to traditional agricultural methods (Mäder et al., 2002). Using mulch as a single method has been proven to increase the variety of mycorrhizae in the soil as well as increase the ability of the mycorrhizal fungi to make connections (Derkowska, Sas-Paszt, Sumorok, Szwonek, and Gluszek, 2008). Despite all of this research showing the effects of different soil types and different fungi on a variety of plants, little of it studied their effects on Daucus carota. For this reason, it is pertinent to expand this field by comparing the growth of *Daucus carota* in different types of soil, which either promote or inhibit mycorrhizal variety, to inoculants proven to increase the growth of *Daucus carota*. So, the experiment will be testing the mycorrhizal fungi, mentioned above, as well as high mulch soil and traditional agricultural soil to see which of the inoculant will cause the most significant growth in plant biomass, as well as which may have a negative effect on plant biomass.

Purpose

The purpose of the experiment is to determine the benefits of using different soil inoculants or mycorrhizal fungi inoculants to promote an increase in biomass of *Daucus carota*.

The results may show us that there could be a more effective way to grow *Daucus carota*, which would show how it may be beneficial to rethink how we grow *Daucus carota* in order to increase crop yield. An increased crop yield can then go towards helping to feed impoverished areas with high percentages of malnourished people.

Hypothesis

The *Daucus carota* being inoculated with the high mulch soil inoculant will have the greatest effect on the plant biomass due to the mycorrhizal fungi present in the soil as well as other helpful bacteria. *Glomus intraradices* will have the second greatest effect on the growth of *Daucus carota* and its biomass. *Glomus intraradices* has been previously proven to be the most influential in the growth of *Daucus carota*. The plants with the traditional agricultural soil inoculant will have similar or worse biomasses than the control carrots in plain potting soil due to harmful bacteria or other negative aspects of the soil.

Null Hypothesis

The inoculants, neither the high mulch, traditional agricultural, nor *Glomus intraradices*, will not have a significant effect on the plant biomass, plant length, or seed germination of *Daucus carota*.

Safety Issues

The drill is a very powerful and dangerous tool, which could cause a number of injuries if not used properly, so it should be handled with care by an adult who knows the piece of equipment and can make the incisions into the water bottles. Being cut by the scissors is also a potential safety hazard, so they should be handled with care by those cutting off the tops of the water bottles. Accidental consumption of the Mykos fertilizer is also a possible safety issue,

however unlikely. The growth chamber is also a piece of equipment that should be used with care due to its potential to malfunction and tendency to get reach high temperatures on the side of the chamber, which could cause burns if skin is touching the surface for too long.

Materials

- Drill and drill bit (Rigid brand)
- Scissors
- Sterilized soil (Edna's Best)
- Thermo Scientific Precision Model 815 Refrigerated Incubator Microprocessor Controlled
- 1 lb of high mulch soil (to act as an inoculant)
- 1 lb of traditional agricultural soil (to act as an inoculant)
- Approximately 60 grams (more in case of mistakes) *Glomus intraradices* spores from a purchased one pound bag of Mykos brand fertilizer
- 24 small Trader Joe's alkaline water bottles
- 120 *Daucus carota* seeds (OSH)
- PVC Hardware Cloth (Home Depot)
- 4 trays for bottle storage and water drainage
- Beakers for watering

Methods

Collection of Soil Inoculants:

Both the high mulch soil and traditional agricultural soil inoculants were collected from a local farm owned by Limoneira in Ojai one day prior to the planting of the *Daucus carota* in

order to keep these inoculants as fresh as possible. The high mulch soil inoculant was collected from in between the rows of lemon trees at 5 different locations, approximately 1 to 3 steps from one another, in order to improve the inoculum diversity from across the field. The first 5 to 10 cm of topsoil was collected from underneath the layers of mulch, which had recently been added, and added to a gallon Ziploc bag container. The traditional agricultural soil inoculant was collected in a similar manner, except it consisted of soil from the edges of the farm at the end of the rows of trees, where there was less mulch. 4 gallon Ziploc bags of each inoculant were collected and labeled.

Programming the Growth Chamber:

A growth chamber was used in this study to make sure each plant were under the same conditions in terms of temperature and availability of light. The growth chamber was programmed to have the same daily schedule. Every day from 7:00 am to 6:00 pm the light would be on in the growth chamber and the temperature would be 24.0 degrees unless the door of the chamber was opened which caused some variance. At night the light would be off and the temperature would drop to 18 degrees until the next cycle at 7:00 the next morning. This information is described below.

Day	Hr. 1 (AM)	Minutes 1		U	Hr. 2 (PM)	Minutes 2	-	Light 2 (On/Off)
Sunday	7	:00	24	On	6	:00	18	Off
Monday	7	:00	24	On	6	:00	18	Off
Tuesday	7	:00	24	On	6	:00	18	Off
Wednesday	7	:00	24	On	6	:00	18	Off
Thursday	7	:00	24	On	6	:00	18	Off
Friday	7	:00	24	On	6	:00	18	Off
Saturday	7	:00	24	On	6	:00	18	Off



Table 1: Shows the information put into program the growth chamber. Fig. 1: Growth Chamber

Preparation of Plant Pots:

Since no pots could be found to accommodate for the limited space to store the plants and for the height of the container needed for the carrots to grow, water bottles of about 8 inches in height were used because they would be most appropriate replacement to allow the plants to mature. The water bottles were altered in order to make them suitable for the growth of *Daucus carota*. First, the bottles were marked at the 8 inches from the bottom of the bottle. The tops of the bottles were then cut off by creating a ¼" hole using a drill at the 8-inch mark and cutting around the bottle along the mark. Finally, drainage holes were then made using the drill at the bottom of the bottle to allow water to drain out of the soil, promoting healthy root growth and reducing the risk of the growth of mold in the soil.



Fig. 2: Drilling drainage holes in bottles.



Fig. 3: Finalized water bottle pots.

Planting of *Daucus carota*:

Purchased sterile soil from a local plant nursery was added until each water bottle was about halfway full. The soil was then watered until water came out of the drainage holes, which amounted to approximately 20 mL, in order to ensure proper dampness of the soil throughout the

entire bottle. Soil was then again added until about an inch below the edge of the bottle to allow for space for the inoculants. The inoculants were then prepared to be added to their labeled water bottles. The Glomus intraradices inoculant was measured out at the recommended amount of about a teaspoon or 5 grams. The high mulch soil inoculant was put through a metal cloth, which was placed on the top of a bucket, by moving the dirt around until large mulch pieces were left and those were discarded. The inoculant that made it through the sieve into the bucket was stirred and mixed together to make the inoculum diversity of mycorrhizal fungi constant throughout. The traditional agricultural inoculant was prepared in the same manner using a different metal cloth and bucket to safeguard from the contamination of the inoculants by one another. Next, the bottles were appropriately inoculated with either, the high mulch soil, traditional agricultural soil, or *Glomus intraradices* inoculants. There were 6 plastic bottles which did not receive any inoculant and made up the control group, 6 plastic bottles inoculated by *Glomus intraradices* which made up the first experimental group, 6 plastic bottles inoculated with the high mulch soil which made up the second experimental group, and 6 plastic bottles inoculated with the traditional agricultural farm soil which made up the third and final experimental group. Half an inch of the prepared high mulch soil and traditional agricultural soil inoculants were added to each of their six bottles. About a teaspoon of the mykos inoculant was added to each of its six bottles and lightly stirred into the top inch of the soil. Then five Daucus *carota* seeds were added about a centimeter below the surface of the soil in every water bottle. Another 20 mL of water was added to dampen the topsoil for the seeds to grow. All of the plants were then positioned in trays labeled high mulch soil, traditional agricultural soil, G.

intraradices, or control, and finally placed in the programmed growth chamber to be at constant

conditions.



Fig. 4: Sieving the soil inoculants.

Watering and Keep of *Daucus carota*:



Fig. 5: Adding potting soil to bottles.

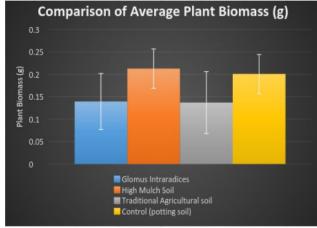
During the course of plant growth, the watering schedule changed according to the plant's necessity for water which was determined by the dampness of the soil, but the amount of water added to the bottles stayed consistent at 50 mL of water. The dampness of the soil was determined by submerging a finger in the first centimeter of soil. The plants were watered the first few days after being planted in order to make sure the seeds would have plenty of moisture over the weekend. The plants were then watered Tuesday and Friday of the next three weeks and approximately every five days until the end of the sixty days of growth. After the first two weeks, the seedlings will be thinned out so there are only one per pot to eliminate competition for space and resources between plants within each bottle.

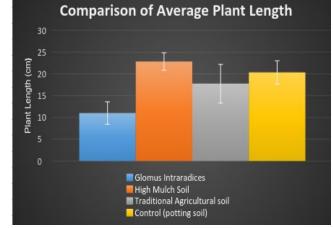
Data Collection:

In order to collect the plants for data analysis, the plants were uprooted from their pots so plant length can be measured. In order to keep the plants' roots from breaking off, the plants were removed from the pots by slowly shaking out the dirt and plant into a tray. Also, the

biomass of the entire plant was taken after the roots were cleaned to remove any additional dirt

particles that could have remained attached to the plant roots.

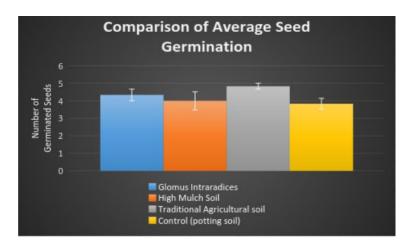




Data Table and Graphs

Fig. 6: Graph of the average plant biomass.

Fig. 7: Graph of the average plant length.



Inoculant	Glomus intraradices								
Plant Pot	Pot 1	Pot 2	Pot 3	Pot 4	Pot 5	Pot 6	Average		
Plant Biomass (g)	0.2216	0.0699	0.0867	0.4135	0.0137	0.0298	0.1392		
Plant length (cm)	16.5	11	11.5	24.5	7.5	9	11		
Number of germinated seeds	5	4	3	4	5	5	4.333		

Inoculant	High Mulch Soil								
Plant Pot	1	2	3	4	5	6	Average		
Plant Biomass (g)	.1199	.2052	.3626	.2763	.0650	.2466	.2126		
Plant Length (cm)	18	22	29	27	16	25	22.83		
Number of germinated seeds	3	2	5	4	5	5	4		
Inoculant	Traditional Agricultural Soil								
Plant Pot	1	2	3	4	5	6	Average		
Plant Biomass (g)	.0190	.0405	.4666	.1414	.1241	.0296	.1369		
Plant length (cm)	10.5	12	32	22	19	11	17.75		
Number of germinated seeds	5	5	5	4	5	5	4.833		
Inoculant	Plain Potting Soil (Control)								
Plant Pot	1	2	3	4	5	6	Average		
Plant Biomass (g)	.1241	.0530	.2468	.1543	.2380	.388	.2007		
Plant Length (cm)	19	17	26	10	22	28	20.33		
Number of germinated seeds	4	5	4	3	3	4	3.833		

Table 2: All the data from the experiment: plant biomass and length and germinated seeds.

Results

Plant Biomass:

The *Daucus carota* inoculated with the high mulch soil did the best out of the three inoculants and had a 5.9% increase in biomass as compared to the control group. The *Glomus intraradices* inoculant had a negative effect on plant biomass and did 30.6% worse than the control group. The agricultural soil had the most negative effect on *Daucus carota* and had an

average biomass that was 31.8% worse than the control group's. Each of the p-values was .858, .454, and .467 respectively, meaning that the results were insignificant and therefore the null hypothesis can be accepted that the inoculants would not have a significant effect on *Daucus carota*.

Plant Length:

The high mulch soil inoculant, again, proved to be most effective in improving the length of the *Daucus carota* with an average plant length that was 12.2% greater. The traditional agricultural soil inoculant had an average plant length that was 12.9% worse than that of the control group. *Glomus intraradices* had the most negative effect on plant length with an average that was 45.9% worse than the control groups. Each of the p-values was .478, .566, and .087, respectively, showing that the results are statistically insignificant.

Number of Germinated Seeds:

Each of the inoculants was more effective at allowing seed germination than the control *Daucus carota* with no inoculant. The traditional agricultural soil inoculant was the most effective at allowing the seeds to germinate 26.1% more successfully than that of the control group and a total percentage of the number of seeds germinated of 96.7%. The *Glomus intraradices* had the second most successful seed germination with 86.7% of the seeds germinating, which was 13.0% better than the control. The high mulch soil inoculant had a more successful germination of seeds then the control with 80% of seeds germinating, but only by 4.3%. Only 76.7% of the seeds in the control group germinated.

Discussion

The Daucus carota with traditional agricultural soil was most successful at germinating the greatest number of seeds per pot with 96.7% of the seeds germinating. The reasoning for this is likely the fact that this inoculant was a little looser due to the method of putting this inoculant through a metal sieve, which caused partial clumping of the soil. The clumping could have allowed for more open space for the seeds to sprout and could have provided greater aeration for the germination process. The use of this soil for agricultural purposes may stem from the fact that more seeds germinated on average, however, this analysis may be incorrect because the clumping of this soil experienced from these methods would not occur in any agricultural field. 86.7% of the Daucus carota inoculated with Glomus intraradices successfully germinated. Mycorrhizal fungi often have the greatest effect on plant growth, if they make the symbiotic connections with the plant early in development. So these seeds successfully germinated due-to symbiotic connections made with the mycorrhizal fungi inoculant because it gave the seeds more access to nutrients. The inoculant was also made up of small granules which did not impede the sprouting process, allowing for seed germination. 80% of the Daucus carota inoculated with the high mulch soil germinated successfully, which is lower than the percentages shown by the traditional agricultural soil inoculant and the *Glomus intraradices* inoculant, but still higher than the percentage of the control group. This inoculant likely experienced this percentage due to multiple factors that either helped or hindered seed germination. The helpful factor that probably promoted the seed germination was the presence of mycorrhizal fungi and bountiful nutrients, which would have given the seeds a greater likelihood of absorbing these nutrients. However, germination was hindered by the top layer of high mulch inoculant which did not clump as the

traditional agricultural soil did, so it created a denser layer of inoculant for the seeds to sprout through. The control group, with no inoculant, had the worst percentage of germinated seeds at 76.7%. The control group was least successful due to its lack of helpful properties, which would have increased the chance of seed germination. However, according to the t-tests, only the difference between the traditional agricultural soil and the control was statistically significant. The significance of the other results could be proven, but more experimentation with larger experimental and control groups must first occur.

The *Daucus carota* with the high mulch inoculant grew the most in terms a biomass, with an average biomass 6% greater than that of the control group. This inoculant worked the best because it provided a greater variety of mycorrhizal fungi thanks to the organic matter that had covered the soil while at the farm. Each of the species of mycorrhizal fungi in the soil may have made symbiotic connections with the roots of the *Daucus carota*, which allowed it to grow as much as it did. However, a t-test determined that this result is likely statistically insignificant due to its high p-value (.858). Such a high p-value stems from the likelihood that not all of the plants measured had germinated at the same time nor survived the entire course of the experiment. These factors caused a higher standard deviation between results, which skewed the t-test's accuracy. In order to correct this and collect measurements that are significant, a greater population of each experimental and control group should be grown and measured. The *Daucus* carota inoculated with Glomus intraradices did worse than expected and harmed the biomass of the plants, making it 68% lower than that of the control. Since Daucus carota have been proven to make connections with mycorrhizal fungi, it is unlikely that the inoculant directly worked against the plants in some way, but instead worked in a way that had some unintended

repercussions. There are two main possibilities could have caused the growth deficiency seen in this experimental group. One is that the roots simply didn't make connections with the fungi, since it requires the roots to come directly in contact with it and the fungi were evenly spread through the topsoil. If the inoculant were applied in the soil immediately around the seeds it would have been more likely to make the connections with the fungi. The second possibility is that the fungi did work and made connections with some if not all of the seedlings, but since they made connections with some of the seedlings that were taken out of the soil, they made the roots harder to extract causing them to snap and compete with the seedlings that were left to grow. Despite the general trend of these plants showing negative signs of the fungi's effectiveness, the plant from pot 4 had one of the highest biomasses. This could prove that the mycorrhizal fungi did make connections with this plant and could be helpful overall. These inconsistent results once again contributed to a high p-value (.454). The traditional agricultural soil inoculant had the worst average biomass, which was 69% worse than that of the control group. The low biomass can be attributed to the lack of beneficial nutrients that would have been in the soil and the minimal, if any, concentration of mycorrhizal fungi. The soil could have also had remnants of pesticides or insecticide, which could have negatively affected plant growth. This inoculant, once again, had a large p-value (.467) meaning that the difference is insignificant despite the large discrepancy. Once again the best way to fix the large p-value would be to have larger experimental groups with more plants.

The high mulch inoculant allowed for the longest average plant length from roots to the end of the longest stem, which was 12.2% better than the control group. The increased length of the carrots is due, for the most part, to the connections made with mycorrhizal fungi, which gave

the plant more nutrient, allowing the stem and roots to grow out more than the control. These connections could have also come up with the plant when they were uprooted, giving them a longer length overall. The *Daucus carota* inoculated with traditional agricultural soil had an average plant length 12.9% worse than the control's. As it was discussed in terms of its negative effects on the plant's biomass, the length of the carrots with this inoculant is likely lower due to the lack of nutrients or mycorrhizal fungi in the soil and the possibility of harmful pesticides that could have hindered the plant growth. The *Glomus intraradices* inoculant had the worst average plant length, which was 45.9% worse than the control. Since the average biomass of this inoculant was greater than that of the traditional agricultural soil inoculant, it does not quite make sense that the average length would be so much worse. However, a possible explanation for this could be that mycorrhizal connections made with these roots caused them to snap, which would have shortened the average plant length.

Conclusion

According to the results collected, *Daucus carota* inoculated with high mulch soil proved to be most successful, showing that the use of a thin layer of mulch over the soil used to grow carrots is an effective way to increase the yield of carrots by promoting the growth of numerous species of mycorrhizal fungi and other helpful bacteria in the soil. The use of traditional agricultural soil should be discontinued due to its negative effects on the plant's growth and yield. However, the high p-values show that the statistical differences between the results are insignificant, which is probably due to the lack of time that the plants had to grow. So more conclusive and significant values could be achieved through redoing the experiment with more time for the plants to grow and more plants as a part of each control and experimental group.

Further Work

There are many ways that this experiment could be redone and improved upon. If this experiment were to be expanded or repeated, several improvements could be made, as follows: use of better designed pots to ensure plenty of space for growth, longer time for plant growth, use of different conditions to put the plants in during plant growth, the staining and microscopy of the roots to look for the connections made by mycorrhizal fungi, inoculation of different amounts of mycorrhizal fungi, and the use of different fungi.

There is also a number of other related studies that could be conducted in order to expand the knowledge of the field. For one, different agricultural plants could be tested to see how they do with the mycorrhizal fungi. The mycorrhizal fungi can also be used to see if they could increase the medicinal properties or the production of medicinal substances of medicinal plants, which could be used to help cure a number of illnesses in a more natural way.

Acknowledgements

Special thanks to my instructor Dr. Nikki Malhotra, who helped me work through some of my issues with my project as well as stay on track to be the most successful in the science fair. Special thanks to my mentor Dr. Edith Allen as well, who worked with me so much, even when I wasn't being exactly open-minded, to improve and clarify my project. Thanks to the stores from which I purchased my materials, including Home Depot and OSH, as well as thanks to Kinkos and Cybercopy for printing my black and white poster proof and final poster board for science fair, respectively.

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