An examination of the effectiveness of MYKE® on growth of potted American elm seedlings under greenhouse conditions

By

Liam McFarlane

Faculty of Natural Resources Management

Lakehead University

Thunder Bay, Ontario

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Abstract

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Keywords: American elm, MYKE®, mycorrhizal fungi, *Ulmus americana,* urban environment, urban forestry

This thesis is an examination of the commercial mycorrhizal additive known as MYKE®, and the impact upon growth that it has on American elm seedlings grown under greenhouse conditions.

Seedlings of American elm were grown in a plug tray for several months and then transferred into four-inch pots which contained various amounts of the product MYKE®. Ten pots had no product to serve as a control, ten pots had 1 gram, ten pots had 5 grams, and ten pots had 25 grams. These seedlings were grown for another three months under greenhouse conditions with regular watering. Upon being harvested, soil was carefully removed from the roots. The roots and shoots were visually inspected, photographed, and then separated at the root collar and placed into paper bags and dried at 100 degrees Celsius for three days. After drying, the stems and roots were weighed to the nearest milligram and then statistical analyses were performed on the data to see if there were statistically significant differences. The results found that there were no significant differences between the 25 gram and control in the root and shoot, and no significant differences between the 1 and 5-gram trials in the root and shoot measurements. However, there were significant differences between the two groups as illustrated by the LSD test performed. In the combined weights there were no significant differences between the control and 25 gram trials. However, the 1 gram trial was significantly different from these two in addition to the 5 gram trial. The 5 gram trial was significantly different from the control, 1 gram and the 25 gram trial. These results show that this product does work as intended and could assist in growth in an urban environment.

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INTRODUCTION

Mycorrhizas are mutualisms formed between fungi and plant roots. They are one of, if not the most important relationship that plants are involved in. There is evidence that up to 95% of all plants across the world are involved in mycorrhizal relationships (Brundrett 2002), and many of them would not survive without their fungal counterparts. Mycorrhizas have existed for a very long time and there is evidence of this relationship in some of the fossils of the earliest land plants (Malloch 1987, Brundrett 2002). There is even some speculation that the earliest plants were not able to colonize onto land until they had developed these mycorrhizal relationships (Pirozynski and Malloch 1975). It is considered to be a mutualistic relationship because both the plants and the fungi are benefiting. The fungi attach to the roots of the plant and spread their hyphae outwards, into the soil as extramatrical hyphae, increasing the surface area of the roots. This increases the nutrient uptake potential of the roots, allowing the plant to access more micro and macro nutrients, the most important of which are phosphorus and nitrogen which are typically the most limiting nutrients to the growth of a plant. The plant then in return provides some of the energy it creates through photosynthesis, to the growth of the fungi.

It is also generally thought that over time, different types of mycorrhizas have evolved with plants involving several different taxonomic groups of fungi to date (Malloch 1987, Brundrett 2002). The two most commonly studied kinds of mycorrhizas are vesicular-arbuscular mycorrhizas and ectomycorrhizas (Peterson *et al*, 2004). A vesicular-arbuscular mycorrhiza is categorized by the fact that the fungus penetrates the cortical cells of the roots of a vascular plant. They then form unique structures such as arbuscules and vesicles and are formed by fungi of the Phylum Glomeromycota. These structures in turn help the plant to capture nutrients and micronutrients from the soil (Peterson *et al*, 2004). Ectomycorrhizas on the other hand consist of a hyphal sheath, or mantle, which covers the root tip, and a Hartig net of hyphae surrounding the plant cells within the root cortex (Peterson *et al*,

2004). In some cases, the hyphae may also penetrate the plant cells. If this occurs, the mycorrhizas are known as ectendomycorrhizas (Peterson *et al*, 2004). Outside the root, the fungus forms a hyphal network within the soil and leaf litter, connecting various plants and facilitating the flow of nutrients. This network also has been shown to move carbon between trees of various species, which can promote succession of slower growing trees (Simard *et al*, 1997).

There have been numerous and significant studies into the other potential benefits provided by mycorrhizas throughout the years that have proven to be quite interesting. It has been observed that having a mycorrhiza association can help protect a plant from parasitic root-infecting fungi, and potentially dangerous nematodes that attack the plant roots in the soil. This kind of protection against pathogens is usually observed in ectomycorrhizas (Smith and Read, 1997). There are various ways that the mycorrhizas protect their host plants. Some of the most interesting found include simply outcompeting the invading pathogen for colonization sites, indirect initiation of the plants defence responses, and altering the other rhizosphere biota (Sikes, 2010). Of these three strategies, the most common is the initiation of the plants natural defences. This is also likely the most effective, as it essentially allows the plant to fight off the invading pathogen before it takes hold. The mycorrhizas do this by sharing many of its cell surface molecules with the invading pathogen. These molecules act as signals that trigger the production of plant defensive compounds such as phytoalexins, and phenolics which will be able to fend off the invading pathogen before it infects the plant (Sikes,2010). They also provide protection against nematodes, which are parasitic, microscopic worms (Schouteden *et al*, 2015). Many nematodes are major pests of many plants and can cause serious damage and even death to plants through the roots. Protection was suggested as being provided through induced resistance in the plants, direct competition for nutrients and space, and altered rhizosphere interactions (Schouteden *et al*, 2015).

Another major function of mycorrhizas apart from the previously mentioned is the fact that they have been shown to suppress the growth of other competing plants (Rinaudo *et al*, 2010). This is a phenomenon frequently observed in agriculture where there are small plants attempting to seed into an area, along with a large amount of "weedy" species. In a study performed to test this trait (Rinaudo *et al*, 2010), sunflowers were grown with six widespread agricultural weeds in a controlled environment. The results were quite significant. Without the mycorrhizas, the weeds would overtake the sunflowers in growth. However, when grown with the mycorrhizas, the biomass of two of the weed species was reduced by up to 66%, and the other four species were reduced by up to 37% (Rinaudo *et al*, 2010). These results suggest that these mycorrhizal associations may be major players in the suppression of many agricultural weeds that would otherwise overtake many crops (Rinaudo *et al*, 2010). The full extent of how exactly these fungi perform this is still unknown but is being studied and even considered as an alternative method as a biological control, as opposed to using things such as pesticides.

The final main function of mycorrhizas is that a network of fungal hyphae that is connected to large, dominant trees, can help transport the nutrients and energy produced by the "parent" trees to the younger, establishing saplings that are in the shade of the larger plants (Bingham and Simard, 2012). This can also occur when a large tree dies in the forest and begins to decompose. The mycorrhizas that were established with the larger tree can form new relationships with new trees and help move nutrients from the deceased adult to the young (Bingham and Simard, 2012). This nutrient transfer isn't the only thing that the mycorrhizas transfer between trees either. As previously mentioned, the fungi can help protect from other pathogens, but when a pathogen claims a tree, its mycorrhizas can send signals to other trees that they are linked with, allowing those trees to prepare their defences to protect themselves from the coming danger (Song *et al*, 2015). The nutrient transfer is a newly discovered property of the fungi and wasn't confirmed until Dr. Suzanne Simard and her colleagues (Teste *et al.*, 2009) performed a test at the University of British Columbia, where they injected a large "parent" tree

with a radioactive isotope that was trackable with a Geiger counter into its tissues. They then came back several days later and discovered that the “parent” tree had moved the isotope from its tissues, into its roots, and then the mycorrhizas had moved it into the surrounding younger seedlings (Teste *et al*, 2009). This was a major breakthrough which solidified the fact of this nutrient sharing theory.

Soils in urban environments are often lacking in nutrients, and microbial diversity, particularly mycorrhiza forming fungi (Danielson, 1989; Stabler *et al*. 2001). As such, trees growing in these conditions face tough challenges with regards to growth, nutrient uptake, and water uptake and retention. Therefore, adding mycorrhizal forming fungi to the soil when planting can help trees and shrubs to alleviate such problems.

The product MYKE® consists of spores of the fungus *Glomus intraradices* N.C. Schenck and G.S. Sm. which forms vesicular-arbuscular mycorrhizas (VAM). This product does not work with conifers in the Pinaceae, blueberries or rhododendrons as they cannot be colonized by this fungus present in MYKE®. MYKE® also contains perlite and peat to give it a granular texture. The purpose of this product is to quickly form VAM associations with the newly planted tree or shrub, in order to increase the chances of their survival. Garden soils are typically not of the highest quality in terms of nutrients, and are typically lacking in already established fungal networks, so this product, in theory, significantly increases the amount of VAM fungi present in the soil, which in turn assists in the growth of all plants nearby. However, to test this claim, Devine (2017) used different amounts of MYKE® on seedlings of American elm (*Ulmus americana* L.) grown under aseptic conditions. The product failed to colonize the roots and as a consequence no conclusion could be made of its efficacy. Devine (2017) recommended that the experiment be repeated but in pots under greenhouse conditions which he felt would simulate a more natural situation, thus the rationale for this thesis project. The null hypothesis is that elm seedlings inoculated with MYKE® will not exhibit enhanced growth compared to the uninoculated controls.

MATERIALS AND METHODS

To begin the experiment, seeds of American elm (lot #9810006.3) were obtained from the National Tree Seed Centre in Fredericton, New Brunswick. On October 9th 2017, the seeds were planted in a small seed plug tray which was placed on a bench in the greenhouse for growing (Figure 1). They were watered two times every week on Tuesdays and Fridays.

Figure 1 Seed plug tray

These seeds germinated into small seedlings and grew for approximately two months until they had established some roots to hold onto the soil in the plug. Four-inch pots were prepared (Figure 2) with the MYKE® product in the following order: 10 pots with no product to serve as a control, 10 pots

with 1 gram of product, 10 pots with 5 grams of product, and 10 pots with 25 grams of product. Each pot had a total of 100 grams of planting material total, meaning that the contents of each pot was a combination of both the soil and the MYKE® and never exceeded 100 grams together, regardless of how much MYKE® was in the pot.

Figure 2 Pots prepared with MYKE® and seedlings

These seedlings grew for another three months in the greenhouse while the same watering schedule was maintained as before. Pots were randomly arranged on a regular basis. On March 12th, 2018 the seedlings were taken out of the greenhouse and brought back to the forest pathology lab, where they were very carefully removed out of the pots and soil removed from the roots, being careful not to damage the roots in the process. The roots were washed and placed in a Petri dish with water and examined under a dissecting microscope to remove any remaining soil particles left clinging to the

roots. The seedlings were placed on a special board with 1cm measurement lines to do a visual inspection of the root volume and length, and the shoot length. Following this, the roots were separated from the shoot at the root collar using a scalpel and placed into sperate, labelled paper bags for each of the seedlings (Figure 3). These bags were then placed into a drier at 100 degrees Celsius for three days to remove all moisture.

Figure 3 Paper bags containing shoots and roots of harvested seedlings

After being dried, the weights of both the stems and the roots of every sample were recorded to the nearest milligram. Anova tests were run to compare the statistical significance of the data, and also an LSD test to present a visual display of the difference in weights.

RESULTS

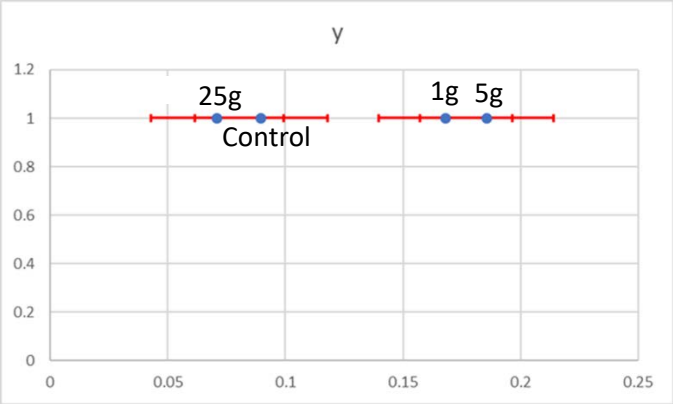
When the seedlings were harvested three months after inoculation, it looked as though there were differences in growth from a visual inspection (Figure 4). There were obvious visual differences in the volume of the roots in the 1 gram and 5 gram trials when compared with the control and 25 gram trials. During the growth of the seedlings, four in the control group died, and one seedling in each of the 1 gram and 5 gram trials were on the verge of death and showed almost no growth. Due to the deaths in the control group, the statistical analysis was run with only six randomly selected values from the other groups to keep the ANOVA tests equal. Upon comparing the weights of the dried samples (Table 1, Appendix 1) and performing both an ANOVA and LSD test (Figures 5-7,Appendices 2-4), there were statistically significant differences in all categories. In the root weight, there were no significant differences between the weights of the averages of the 1 and 5 gram trials, and there was also no difference between the control and the 25 gram trial. However, there was a significant difference between the 1 and 5 gram trials and the control and 25 gram trials. This same pattern also held true for the stem weight tests. However, when comparing the combined weights, the control, and 25 gram trials were not significantly different, but the 1 gram trial was significantly different from both the afore mentioned two, and the 5 gram trial. This also means that the 5 gram trial is significantly different from the control, the 1 gram and the 25 gram trial.

Table 1 Averages of stem, root, and combined weights

Trial	Stem dry weight (g) average	Root dry weight (g) average	Combined weight (g) average
Control	0.04	0.09	0.129
1g	0.064	0.143	0.2065
5g	0.063	0.156	0.219
25g	0.032	0.087	0.119

Figure 4 Growth of seedlings under various treatments

These results disprove the null hypothesis and show that the MYKE® product did in fact have a significant impact on growth and was able to form mycorrhizal associations with the elm roots.



Control = No MYKE®

1g = 1 gram of MYKE®

5g = 5 grams of MYKE®

25g = 25 grams of MYKE®

Figure 5 LSD Test (Root weight)

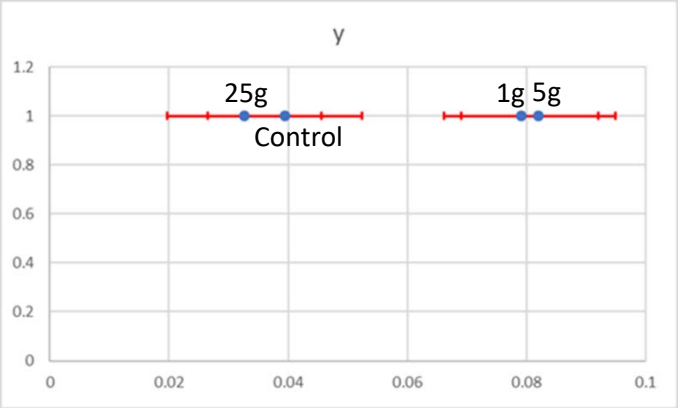


Figure 6 LSD Test (Stem weight)

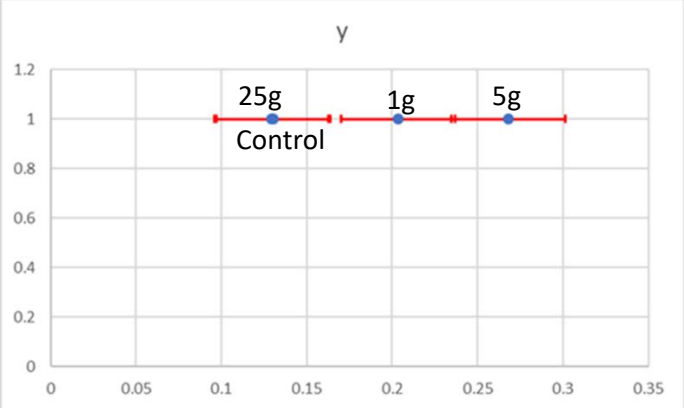


Figure 7 LSD Test (Combined weight)

DISCUSSION

Unlike Devine (2017), the present study did find significant results in the weights between the trials, and it appears as though the optimal ratio of MYKE® to soil in this small-scale trial, is 5 grams of MYKE® for every 95 grams of soil. This could be very important information in a nursery setting where small seedlings and saplings are grown, because by using this product it could lead to increased growth of both the stem and roots, and overall better health of the tree. As recommended by Devine (2017), the use of MYKE® in pots under greenhouse conditions may simulate a more natural setting, which probably explains the positive results in this current experiment.

It is felt that the reason those specific seedlings died, and a few others did not do so well in growth as compared to the others is simply that some were much better suited to growth than others, just like in the forest. Unfortunately, it was not possible to replant these missing seedlings as the experiment was too far along into the trial. Planting new seedlings would have skewed the result towards a smaller weight increment average. Trials grown with 25 grams of MYKE® actually had the smallest amount of growth and the lightest average weights compared to the other treatments. It might be that this is due to the fact that the MYKE® product consists of mostly a perlite-peat mixture to create a filler to carry the spores. While perlite does contain some nutrients, it does not contain all the nutrients that a soil mixture does, and since each pot only had 100 grams of material total, in the 25 gram trial there was only 75 grams of soil. It is possible that the nutrients missing from the 25 gram trial lead to the lesser growth because even though the fungus was able to colonize the roots and create the association, there were not the sufficient nutrients present in the soil for the fungus to assist in their uptake. This leads to the conclusion that in a closed growth environment where there is a limited amount of rooting volume, you can certainly have too much MYKE® in your soil and this will hamper the growth of your trees.

CONCLUSION

This product does work and could be used to increase the growth of many seedlings in preparation for planting in the field. This experiment has also proved that the product can have a beneficial effect provided the ratio of soil to product is correct and not too high. However, general use of this product is meant to be in a garden setting where you have a very high amount of soil, and no shortage of nutrients, so there is little risk of using too much product and having no beneficial effect such as what happened in the treatment with the 25-gram pots. Had there been more time, it would have been interesting to attempt to chemically test how much nutrients the colonized roots were actually taking up versus those roots that did not have the association just to get an idea of how significant an impact they could have in the long run.

LITERATURE CITED

Bingham, M.A. and S.W. Simard. 2012. Mycorrhizal networks affect ectomycorrhizal fungal community similarity between conspecific trees and seedlings. Mycorrhiza 22: 317-326

Brundrett, M.C. 2002. Coevolution of roots and mycorrhizas of land plants. Tansley Review No.134. New Phytologist 154: 275-304

Danielson, R.M. 1989. The ectomycorrhizal status of urban spruce. Mycologia 81: 335-341

Devine, D. 2017. Efficacy of MYKE® on in vitro growth of American elm (*Ulmus americana*) seedlings. HBScF thesis. Faculty of Natural Resources Management, Lakehead University. 29Pp.

Malloch, D. 1987. The evolution of mycorrhizae. Canadian Journal of Plant Pathology 9: 398-402

Peterson, R.L., H.B. Massicotte, L.H. Melville. 2004. Mycorrhizas: Anatomy and Cell Biology. NRC Research Press, Ottawa. 173Pp.

Priozynski, K.A. and D.W. Malloch. 1975. The origin of land plants: a matter of mycotrophism. Biosystems 6(3): 153-164

Rinaudo, V., P. Barberi, M. Giovannetti, M.G.A. van der Heijden. 2010. Mycorrhizal fungi suppress aggressive agricultural weeds. Plant and Soil 333 (1-2): 7-20

Schouteden, N., D. DeWaele, B.Panis and C.M. Vos. 2015. Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes:A review of the mechanisms involved. Frontiers in Microbiology 6: 1280

Sikes, B.A. 2010. When do arbuscular mycorrhizal fungi protect plant roots from pathogens? Plant Signaling and Behaviour 5(6): 763-765

Simard, S.W., D.A Perry, M.D. Jones, D.D. Myrold, D.M. Durall, and R. Molina. 1997. Net transfer of carbon between tree species with shared ectomycorrhizal fungi. Nature 388: 579-582

Smith, S.E. and D.J. Read. 1997. Mycorrhizal Symbiosis. Academic Press, London. 483 Pp.

Song, Y.Y, S.W. Simard, A. Carroll, W.W. Mohn and R.S. Zeng. 2015. Defoliation of interior Douglas-fir elicits carbon transfer and stress signaling to ponderosa pine neighbors through ectomycorrhizal networks. Scientific Reports 5: article number 8495.

Stabler, L.B., C.A. Martin, and J.C. Stutz. 2001. Effect of urban expansion on arbuscular mycorrhizal fungal mediation of landscape tree growth. Journal of Arboriculture 27(4): 193-202

Teste, F.P., S.W. Simard, D.M. Durall, R.D. Guy, M.D. Jones and A.L. Schoonmaker. 2009. Access to mycorrhizal networks and tree roots: importance for seedling survival and resource transfer. Ecology 90: 2808-2822

APPENDICES

APPENDIX 1, Raw Data

	Tree	Stem dry weight (g)	Root dry weight (g)	Combined weight (g)
Control				
	1	0.048	0.098	0.146
	2	0.022	0.07	0.092
	3	0.06	0.146	0.206
	4	0.046	0.1	0.146
	5	0.038	0.075	0.113
	6	0.023	0.049	0.072
	sum	0.237	0.538	0.775
	average	0.040	0.090	0.129
1g	1	0.063	0.145	0.208
	2	0.151	0.239	0.39
	3	0.039	0.078	0.117
	4	0.04	0.118	0.158
	5	0.045	0.121	0.166
	6	0.043	0.135	0.178
	7	0.041	0.114	0.155
	8	0.104	0.212	0.316
	9	0.04	0.085	0.125
	10	0.069	0.183	0.252
	sum	0.635	1.43	2.065
	average	0.064	0.143	0.2065

5g	1	0.097	0.226	0.323
	2	0.044	0.133	0.177
	3	0.101	0.296	0.397
	4	0.069	0.166	0.235
	5	0.103	0.231	0.334
	6	0.017	0.043	0.06
	7	0.061	0.152	0.213
	8	0.046	0.119	0.165
	9	0.084	0.187	0.271
	10	0.01	0.003	0.013
	sum	0.632	1.556	2.188
	average	0.063	0.156	0.219

25g	1	0.027	0.072	0.099
	2	0.019	0.048	0.067
	3	0.051	0.132	0.183
	4	0.02	0.035	0.055
	5	0.029	0.096	0.125
	6	0.03	0.067	0.097
	7	0.033	0.118	0.151
	8	0.057	0.158	0.215
	9	0.021	0.069	0.09
	10	0.031	0.075	0.106
	sum	0.318	0.87	1.188
	average	0.032	0.087	0.119

The highlighted values were those used in the statistical analyses to create the results

APPENDIX 2, ANOVA test on stem weights

Descriptive Statistics

Dependent Variable: stem weight (g)

Treat	Mean	Std. Deviation	N
1g	.07917	.041513	6
25g	.03267	.012863	6
5g	.08200	.023529	6
c	.03950	.014937	6
Total	.05833	.033214	24

Tests of Between-Subjects Effects

Dependent Variable: stem weight (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.012[a]	3	.004	6.025	.004
Intercept	.082	1	.082	122.552	.000
Treat	.012	3	.004	6.025	.004
Error	.013	20	.001		
Total	.107	24			
Corrected Total	.025	23			

a. R Squared = .475 (Adjusted R Squared = .396)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
stem weight (g)	24	.019	.151	.05833	.033214	1.125	.472	1.054	.918
Valid N (listwise)	24								

APPENDIX 3, ANOVA test on root weights

Descriptive Statistics

Dependent Variable: Root weight (g)

Treat	Mean	Std. Deviation	N
1g	.16800	.050990	6
25g	.07100	.033413	6
5g	.18550	.066564	6
c	.08967	.033482	6
Total	.12854	.067270	24

Tests of Between-Subjects Effects

Dependent Variable: Root weight (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.058[a]	3	.019	8.307	.001
Intercept	.397	1	.397	171.145	.000
Treat	.058	3	.019	8.307	.001
Error	.046	20	.002		
Total	.501	24			
Corrected Total	.104	23			

a. R Squared = .555 (Adjusted R Squared = .488)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
Root weight (g)	24	.035	.296	.12854	.067270	.792	.472	.177	.918
Valid N (listwise)	24								

APPENDIX 4, ANOVA test on combined weights

Descriptive Statistics

Dependent Variable: combined weight (g)

Treat	Mean	Std. Deviation	N
1g	.20367	.095261	6
25g	.13050	.058685	6
5g	.26817	.095851	6
c	.12917	.047709	6
Total	.18287	.093232	24

Tests of Between-Subjects Effects

Dependent Variable: combined weight (g)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.080[a]	3	.027	4.448	.015
Intercept	.803	1	.803	133.873	.000
Treat	.080	3	.027	4.448	.015
Error	.120	20	.006		
Total	1.003	24			
Corrected Total	.200	23			

a. R Squared = .400 (Adjusted R Squared = .310)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation	Skewness		Kurtosis	
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Std. Error
combined weight (g)	24	.055	.397	.18287	.093232	1.105	.472	.656	.918
Valid N (listwise)	24								

APPENDIX 5, LSD test on stem weight

Stem Weight

$$\sqrt{\frac{2\,MSerror}{n}}$$

$$=\sqrt{\frac{0.001*2}{6}}$$

$$=\sqrt{\frac{0.002}{6}}$$

$$=\sqrt{0.0003}$$

$$= 0.017$$

LSD= + 0.05, 20 * 0.017

= 2.086 * 0.017

= 0.035cm

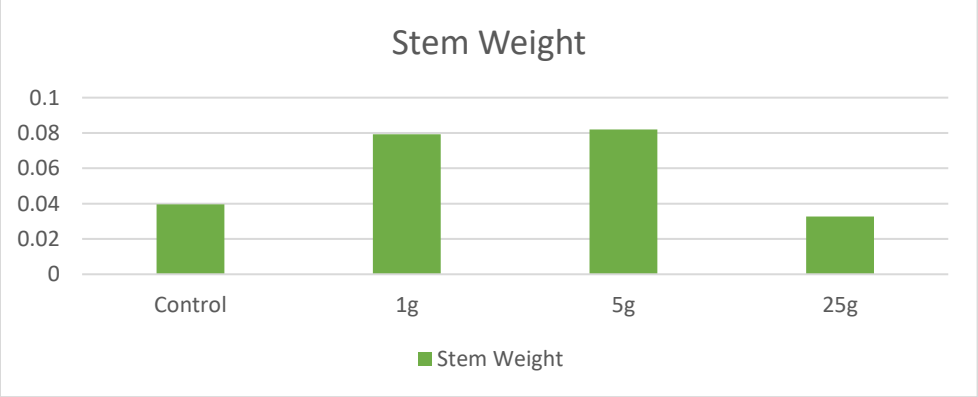
Means

C 0.03950 (2)

1g 0.07917 (3)

5g 0.08200 (4)

25g 0.03267 (1)



APPENDIX 6, LSD test on root weight

LSD= + 0.05, 20 * 0.025

= 2.086 * 0.025

= 0.052cm

Root Weight

$$\sqrt{\frac{2\,MSerror}{n}}$$

$$=\sqrt{\frac{0.002*2}{6}}$$

$$=\sqrt{\frac{0.004}{6}}$$

$$=\sqrt{0.00066}$$

$$= 0.025$$

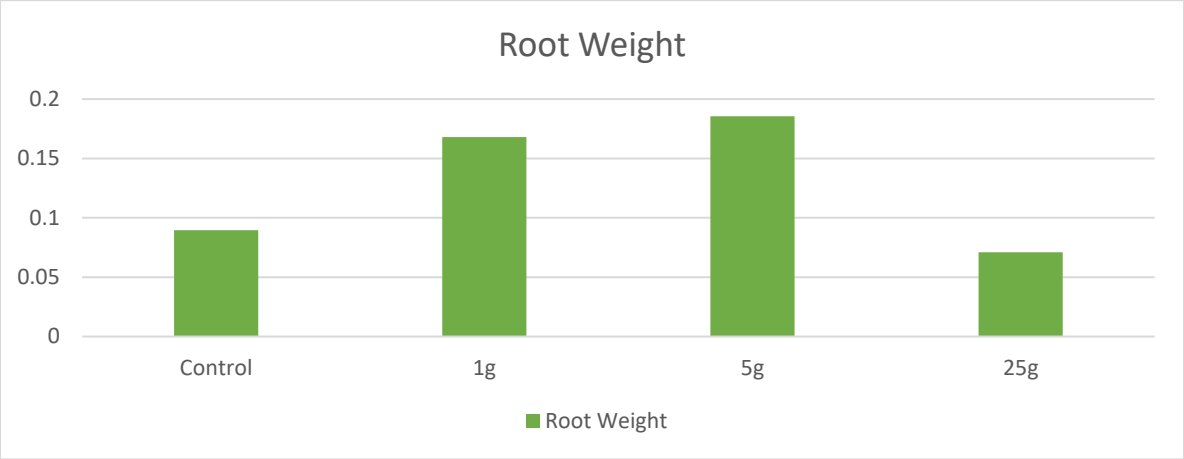
Means

C 0.08967 (2)

1g 0.16800 (3)

5g 0.18550 (4)

25g 0.07100 (1)



APPENDIX 7, LSD test on combined weight

Combined weight

$$\sqrt{\frac{2\ MSerror}{n}}$$

$$=\sqrt{\frac{0.006*2}{6}}$$

$$=\sqrt{\frac{0.012}{6}}$$

$$=\sqrt{0.002}$$

$$= 0.044$$

LSD= + 0.05, 20 * 0.044

= 2.086 * 0.044

= 0.091cm

Means

C 0.12917 (1)

1g 0.20367 (3)

5g 0.26817 (4)

25g 0.13050 (2)