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PURSUIT

Effects of Mycorrhizal Fungi on *Vigna Radiata* Growth in Soil Differing in Fertilizer Concentration

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Mycorrhizal fungi form mutualistic relationships with the roots of some plants, allowing the plant access to nutrients and minerals while the fungi obtain food from the plant. Given that this relationship is beneficial to the plant, this paper investigates the nature of the impact of presence of mycorrhizal fungi on the growth of *Vigna radiata* (mung beans) in soil of differing chemical environments. Through comparing the stem lengths of plants seven days after germination, it is found that in soil with 0.0% fertilizer, the presence of locally collected, unclassified mycorrhizal fungi impacts the growth of *Vigna radiata* negatively; in intermediate fertilizer concentrations (1.0%, 1.1%, 1.2%) there is no significant effect; at higher fertilizer concentrations, the mycorrhizal fungi aid in the plants' survival and growth. This paper concludes that in nutrient-deficient environments, the mycorrhizal fungi compete for nutrients with the plant, yet benefits the plant by stabilizing its growth when nutrients are available. Due to the unclassified nature of the mycorrhizal fungi used in this experiment, this investigation is very preliminary and opens itself to many more topics of research in the future.

1 Introduction

The goal of this paper is to investigate the impacts of mycorrhizal fungi on the growth of plants in soil has been eliminated of microbes, weeds, and parasites. Currently, some commercial farms remove weeds, parasites, and denitrifying bacteria from the soil, killing mycorrhizal fungi originally in the soil in the process of sterilization; this preliminary investigation explores the potential benefits of re-adding mycorrhizal fungi into the soil.

Mycorrhiza is the mutualistic relationship between fungi and the roots of certain plants. The hyphae of the fungus form a mass around the root (ectomycorrhizal fungi) or penetrate the root cells (arbuscular mycorrhizal fungi), and grow into the soil to absorb crucial minerals and supply these minerals to the plant, while the fungus benefits by obtaining sugar from the plant.[1] Mycorrhizal fungi were estimated to be found in 80% to 90% of all plants[2]; given their abundance, mycorrhizal fungi were expected to be found in local soil.

Because *Vigna radiata* seeds (mung beans) were easy to obtain and grow quickly, they were chosen for the experiment to investigate the interaction between plants and fertilizers. The fertilizer concentration of 20% nitrate, 30% phosphate, and 20% potash, which provide nitrogen, phosphorus, and potassium, was chosen because mung beans are "light feeders"[3] that thrive in these nutrient ratios.

2 Methodology

2.1 Obtainment and recognition of mycorrhizal fungi

Roots of dicots were collected locally, on Braemar Hill, Hong Kong, rinsed and placed into an agar medium that encourages fungal growth. Days later, fungi were apparent on some Petri dishes, and the hyphae were scraped off and mixed with water. The mixture was then poured onto ungerminated mung beans. After another day, some germinated mung beans with apparent mold (suggestive of fungal presence) were planted in sterilized soil such that fungi were inoculated into the soil.

After the plants were allowed to grow for two weeks, fungi were extracted from the mung beans by severing the roots of plants. To ensure the presence of mycorrhizal fungi, a small snippet of the roots was dyed following the instructions of Vierheilig et al.[4] The root was boiled for five minutes over a Bunsen flame, then boiled in vinegar and ink for another 10 minutes. Excess ink was washed off gently before the resulting dyed root was observed under a light microscope.

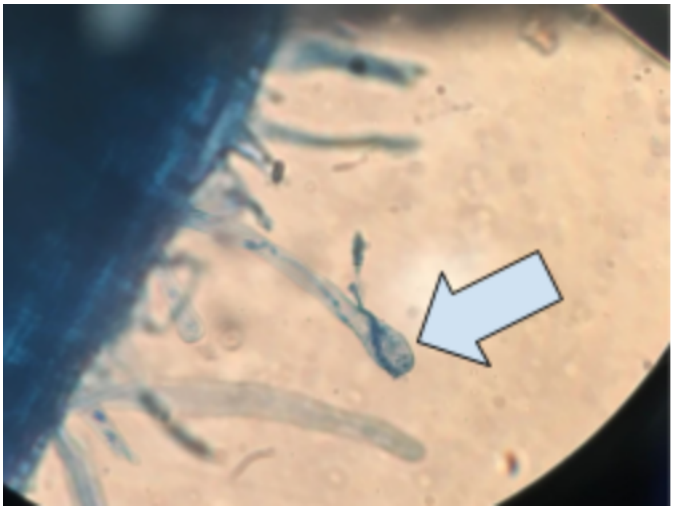


Image 1
Dyed plant root underneath a microscope, 400x magnification

In Image 1, the large dark mass on the left of the image was a root. As indicated by the arrow, an elongated structure grew from it. Closer inspection revealed a dark line separating the structure into two, indicating a multicellular structure that eliminated the possibility of the object being a root hair and thus suggesting the structure to be mycorrhizal fungus.

2.2 Method

400 germinated mung beans were spread evenly in a shallow tray. Sterile deionized water was poured into the tray until the water covered the bottom half of the beans. The tray was then covered with tin foil. A gap was left for ventilation. After two days, 240 germinated beans with root length of approximately 20mm, measured with a ruler, were chosen to be planted. 24 plastic pots were placed in their saucers. Roots of pre-prepared mung beans with mycorrhizal fungi attached were cut into smaller pieces with scissors. These root pieces were mixed well with 2400g of sterilized soil. 100 grams of mycorrhizal fungi-infused soil were put into 12 pots, and 100 grams of sterilized, fungal-absent soil were put in the other 12 empty pots.10 germinated beans from Step 4 were placed in each pot. 70mL of fertilizer was diluted with 630mL of deionized water in a 1L beaker to make a 700mL of 10% fertilizer solution and mixed thoroughly. To make fertilizer solutions of 0, 1.0, 1.1, 1.2, 1.5, 2.0%, the 10% fertilizer solution was then mixed with deionized water (see dilution table below).

Desired fertilizer concentration (%)	Volume of deionized water (mL)	Volume of 10% fertilizer solution (mL)
0.0	200.0	0.0
1.0	180.0	20.0
1.1	178.0	22.0
1.2	176.0	24.0
1.5	170.0	30.0
2.0	160.0	40.0

Table 1
Dilution table for fertilizer solutions

50mL of each solution was poured into two pots with mycorrhizal fungi, and 50mL into two without. Two 20-watt spotlights were attached to a boss and clamp stand, which was fixed at 50cm from the pots (Image 2). After the first day, the plants were watered when necessary. On the seventh day, the stem lengths of each plant were measured with a ruler.

Image 2

Photo of experiment at the end of the 7-day data collection period. Each pot hosted 10 trials. The plants grown in soil with and without mycorrhizal fungi were organized in groups for ease (2 trays each), though that was not strictly necessary for the experiment. The leftmost 2 trays are plants grown in soil without mycorrhizal fungi, while the other 2 trays are plants grown in soil with mycorrhizal fungi. Note that the leaves of plants grown in soil with mycorrhizal fungi had a deeper color than the leaves of plants grown in soil without mycorrhizal fungi.

3 Results: Data Collection and Processing

3.1 Raw Data

Note: some plants did not witness any stem growth. They are denoted by a dash.

Stem Length of Mung Beans Grown in Different Fertilizer Concentrations in Soil without Mycorrhizal Fungi																				
Fertilizer concentration (%)	Stem length (±0.5mm)																			
	Trial																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0.0	151	134	132	154	88	131	157	140	97	88	164	150	160	150	144	142	171	125	145	-
1.0	132	131	97	116	122	126	99	146	141	136	121	88	140	133	125	110	95	120	65	-
1.1	146	100	135	127	120	110	114	81	125	141	151	123	43	141	92	84	90	120	60	-
1.2	146	136	118	126	76	109	127	72	111	131	30	133	88	110	132	125	98	80	-	-
1.5	92	94	72	51	91	41	32	36	99	95	115	52	110	27	100	132	-	-	-	-
2.0	20	103	87	90	77	92	33	56	105	107	25	25	32	-	-	-	-	-	-	-

Table 2

Raw data table showing the stem length of mung beans grown in different fertilizer concentrations in soil without mycorrhizal fungi

Stem Length of Mung Beans Grown in Different Fertilizer Concentrations in Soil with Mycorrhizal Fungi																				
Fertilizer concentration (%)	Stem length (±0.5mm)																			
	Trial																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
0.0	92	128	103	85	120	62	95	47	155	132	104	158	152	116	171	150	144	156	-	-
1.0	55	75	82	80	82	100	108	110	124	173	135	122	113	83	90	146	116	110	-	-
1.1	111	132	93	102	111	94	126	72	72	83	140	84	16	124	96	121	160	141	-	-
1.2	35	70	104	118	118	122	110	128	124	132	59	130	110	132	60	102	148	136	136	125
1.5	87	116	68	91	95	55	34	22	106	146	122	115	135	140	125	156	156	141	-	-
2.0	52	50	92	73	101	138	132	115	136	23	55	45	105	121	98	140	136	-	-	-

Table 3

Raw data table showing the stem length of mung beans grown in different fertilizer concentrations in soil with mycorrhizal fungi

3.2 Observations

Plants grown in soil containing mycorrhizal fungi seemed to require more water, as the soil looked noticeably drier even though all plants received roughly the same amount of water at the same times, suggesting that the fungi were consuming some of the water.

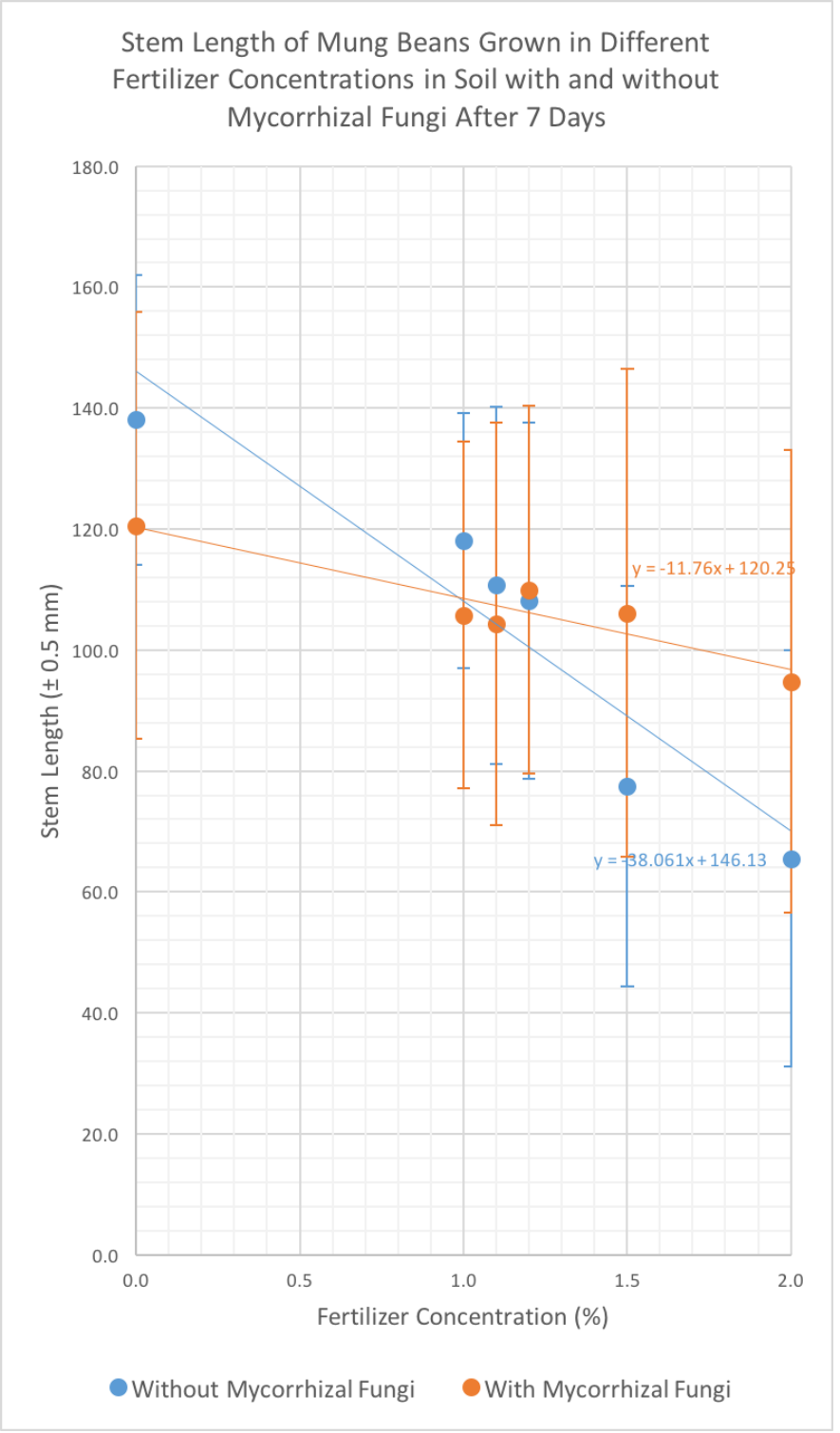
3.3 Results

This Table Shows the Stem Length of Mung Beans Grown in Different Fertilizer Concentrations in Soil with and without Mycorrhizal Fungi				
Fertilizer concentration (%)	Stem length (mm)			
	Without mycorrhizal fungi (±0.5mm)		With mycorrhizal fungi (±0.5mm)	
	Mean	Sandard deviation	Mean	Standard deviation
0.0	138.1	24.0	120.6	35.3
1.0	118.1	21.0	105.8	28.6
1.1	110.7	29.6	104.3	33.2
1.2	108.2	29.4	110.0	30.4
1.5	77.4	33.1	106.1	40.3
2.0	65.5	34.4	94.8	38.3

Table 4

Processed data table comparing the stem length of mung beans grown in different fertilizer concentrations in soil with and without mycorrhizal fungi

A scatter graph comparing the mean stem lengths of mung beans grown in different concentrations of fertilizer in soil with and without mycorrhizal fungi was then plotted.



Graph 1

3.4 Statistical Analysis: T-test

The standard deviations of both data sets are quite large and are reflected in the graph by error bars, which overlap at all data points. A statistical analysis must therefore be performed to ascertain that the groups are significantly different in stem length.

An unpaired t-test was used to determine if the difference in the stem lengths of mung beans grown in soil with and without mycorrhizal fungi was statistically significant and not occurring by chance. This information is shown in Table 5.

Note that each data point has a different number of degrees of freedom because different numbers of plants survived.

Concentration of fertilizer (%)	Mean stem length (mm)		T value	Degrees of freedom
	Without mycorrhizal fungi	With mycorrhizal fungi		
0.0	138.1	120.6	1.75	35
1.0	118.1	105.8	1.48	35
1.1	110.7	104.3	0.61	35
1.2	108.2	110.0	0.18	36
1.5	77.4	106.1	2.28	32
2.0	65.5	94.8	2.20	28

Table 5

Comparison of t values obtained through a t-test

The table indicates that the probability that there was no significant difference between the two groups—and that the difference was created by chance—is less than 0.05, with a confidence level of 95%. By conventional criteria, where the critical value is set at 0.05, this means that the difference is statistically significant.

Comparing the t values obtained from the table of t-values, only data points of 1.5% and 2.0% fertilizer solution have differences between groups that are statistically significant at a confidence level of 95%. However, though the results for 0.0% fertilizer solution are not in the 95% confidence range, they are in the 90% confidence range, indicating some degree of statistical significance. The T value of 1.0% fertilizer solution places significance at 80% confidence, which makes significant difference between the two groups doubtful; the results from 1.1% and 1.2% fertilizer solutions indicate no significant difference. The confidence levels rise again in the 1.5% and 2.0% fertilizer solution groups, which match the trend lines observed in Graph 1, where they intersect around the 1.2% fertilizer concentration before diverging. The statistically significant difference in higher fertilizer concentrations of 1.5% and 2.0% indicate that mycorrhizal fungi is beneficial to the growth of mung beans in those environments; on the other hand, though the data point at 0.0% fertilizer does not meet the critical confidence level of 95%, its T-value does not reject it at a 90% confidence level, suggestive of the fact that the fungi have a negative impact on plant growth in soil that have extremely low nutrient levels.

4 Data analysis

Referencing Graph 1, the trend lines for both mung beans grown in soil without and with mycorrhizal in increasing concentrations of fertilizer are negative: the trend line for plants grown with soil with increasing concentrations of fertilizer and without mycorrhizal fungi is a straight line with a slope of -38.0mm per 1% of fertilizer in soil; the trend line for mung beans grown in soil with increasing concentrations of fertilizer and with mycorrhizal fungi is a straight line with a slope of -11.8mm per 1% of fertilizer in soil.

Interestingly, the trend lines of the two groups cross. As seen in Graph 1, at 0.0% fertilizer concentration, mung beans grown in soil without mycorrhizal fungi had a longer mean stem length than those grown in soil with mycorrhizal fungi; however, as the concentration becomes higher, the

mean stem lengths cross. After crossing, the mean stem length of mung beans grown in soil containing mycorrhizal fungi becomes higher than the mean stem length of those grown in soil without the fungi. The difference between the stem length of plants grown in soil with mycorrhizal fungi and without is greatest at a fertilizer concentration of 2.0%. According to the results in the t-test, it should be noted that the difference between the stem lengths are only significant at fertilizer concentrations 1.5% and 2.0% at a 95% confidence level.

Data points deviated more from the trend line for the group grown in soil without mycorrhizal fungi, suggesting that the presence mycorrhizal fungi resulted in more stable stem lengths.

5 Conclusion

Because of Mycorrhizal fungi, *Vigna radiata* can survive and grow in a range of environments that are not completely or almost completely nutrient deficient. One possible reason for the negative relationship between fertilizer concentration and stem length at an early stage of development could be an overabundance of nutrients in the soil, especially when the plants have just started growing and do not require as many nutrients.

The fungi allowed the plants to be better suited for a wider range of fertilizer concentrations. Twice as many beans planted in soil without fungi were unable to grow at the highest fertilizer; furthermore, whereas the fungi-absent plants' stem lengths decreased dramatically as fertilizer concentration increased, the plants grown in soil with fungi saw a more stable decrease in stem length. From the results of this experiment, it is suggested that, in nature, as nutrient availability changes due to season and weather, mycorrhizal fungi should help the bean plants survive and maintain stem length.

The results also suggest that the fungi help the plants utilize the nutrients in the soil, helping them grow taller within the data collection period. This is witnessed in higher fertilizer concentrations of 1.5% and 2.0%, where the stem length of mung beans grown in soil with fungi are statistically significantly higher than those grown in soil without.

However, at extremely low nutrient levels, the mycorrhizal fungi have a negative impact on plant growth. This result could arise from the fact that the fungi may compete for the nutrients in the soil[5], making less nutrients available to the plant itself, hindering its growth. Research suggests that mycorrhizal fungi are still able to delegate the nutrients in a way that is ultimately beneficial[6]; however, limited time frame of this experiment meant that such benefit is not witnessed here.

The data collected from this experiment is not entirely conclusive, and thus more research must be conducted; nonetheless, the findings of this experiment have important implications because if mycorrhizal fungi are shown to be beneficial for the growth of mung beans in the long run, then the fungi may aid other plants as well. If so, if used in agriculture, mycorrhizal fungi may better crop yield. Many commercial farms sterilize their soil to ensure that pathogens do not affect crop yield negatively and to destroy any denitrifying bacteria (which removes nitrates and nitrites in the soil, leading to increased fertilizer use[7]); however, doing so also kills other, potentially beneficial, organisms in the soil, such as fungi. These commercial farms may wish to add back mycorrhizal fungi into the soil if there is proven benefit.

6 Discussion

It is stressed that the results of this experiment are very preliminary. The fungi used were not identified. Though the fungi were confirmed to form an endosymbiotic relationship with the mung beans through microscopic observation, its origins and species remain unknown. Furthermore, though there is an equal amount of fungi in the soil of the fungi group, its concentration is unknown.

Another experimental variable that can be changed is the timing of plant fertilization: perhaps watering plants with a little fertilizer over many days may yield different results.

The growth of plants and their ability to gain nutrients is crucial to crop yield. Given the implications of mycorrhizal fungal presence for the growth of mung beans, many experiments that can further the understanding of the effect of mycorrhizal fungi on plant growth can be conducted. To

further investigate the long-term impacts, a similar experiment with a longer experimental period could be conducted. An experiment where the number of seeds produced by plants is measured instead of height is also useful for investigating the effects of mycorrhizal fungi on fruit or crop yield. Furthermore, this experiment can be performed on other plants. If other plants are also shown to be able to produce more crops with the presence of mycorrhizal fungi in large-scale trials, then mycorrhizal fungi may be used, in regions where they are less abundant or are killed in the process of soil sterilization in commercial farming.

Acknowledgements

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Endnotes

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