TED ANKARA COLLEGE FOUNDATION

PRIVATE HIGH SCHOOL

Effect of duration of applied cold storage on the germination behavior of scallion seeds

BIOLOGY EXTENDED ESSAY

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ABSTRACT

Cold storage is a process applied to seeds to enable them to germinate more efficiently. The purpose of this extended essay is to determine whether the duration of cold storage applied affects the germination behavior of a certain type of seed.

The hypothesis formed for the claimed research question "How does the duration of cold storage applied for 0, 2, 4, 6 and 8 weeks to five groups of scallion seeds affect their behavior of germination under same conditions?" is that there would be a significant increase in the number of germinated seeds and affect its efficiency as the duration of exposure increases.

So as to support the hypothesis, scallion seeds were exposed to cold storage for 0, 2, 4, 6 and 8 weeks for five different test groups with the exact same conditions. The duration of cold storage was the independent variable. In order to observe the effect more easily, the seeds were let to germinate in a water bathe then stratified on sterilized soil.

The results have showed that there was a significant mean difference between the number of germinated seeds in each test group when the duration of applied cold storage is changed. Therefore, the percentage of germinated seeds can be altered by changing the time interval of cold storage.

Word count: 214

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INTRODUCTION

My mother is keen on gardening. She prefers to grow her own products than to buy them. Because nowadays, almost all of the greengrocery products are genetically modified. Since she is a pediatrist, she wants me to have healthy nourishment. However, growing our own food has some disadvantages. Gardening is a time and care taking process that has a low product outcome. In addition, plants attract insects. Some of these eat the plants' leaves and some may cause the plant to get ill or die. As a result, we are forced to use insecticides which are insalubrius.

Additionally, temperature is one of the disadvantages that can't be controlled. Some plants are resistant to extreme cold or hot weathers where some of them are not. Another problem is that the watering of the plants is not scheduled. It is possible that both the gardener and my mother can water the same plant the same day. It may also rain unexpectedly where the plant gets extreme amount of water. What is more, as my mother is very busy with her work, she usually misses the planting seasons.

Therefore in order to minimize these extreme conditions and stabilize the outcome and store seeds in a way that they can be used next year, I did a research about different methods for germinating seeds where one of them caught my attention.

To begin with I found the three fundemental things for a seed to germinate;

1) Seed viability is the ability of the embryo to germinate and is affected by a number of different conditions. Environmental conditions like flooding or heat can kill the seed before or during germination. The age of the seed affects its health and germination ability since the seed has a living embryo. [1][2]

2) Any dormancy requirements that prevent germination must be overcome. The dormant seeds respond to more than one condition and this process of breaking the seed dormancy is called afterrippening. If the seed becomes too dry (5% water content or less), the effectiveness of afterripening is diminished. Another factor that the seed responds is low temperature, or chilling. Many seeds require a period of cold (0 to 10°C) while in a fully hydrated (imbibed) state in order to germinate.[1][2]

3) Proper environmental conditions must exist for germination which include oxygen, temperature and water. Water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present. [3]

While doing my research, I have found a procedure that is applied to the seeds before they germinate. Chilling seeds to break their dormancy is a time-honored practice in horticulture and forestry and traditionally has been referred to as ***stratification***.[4]

In gardening, stratification is the process where the ungerminated seeds undergo cold storage for a period of time. The time taken to stratify seeds depends on species and conditions; though in many cases two months is sufficient. Typically, temperatures must be between 1°C and 5°C where seeds were layered (stratified) between layers of moist soil and exposing these strata to winter conditions. [4]

After finding this method, I have done a comprehensive research about it.[5][6][7][8] I visited the Ministry of Forestry to learn more. I was lucky that they were carrying out exactly the same procedure for a long time so I had a chance to talk about it with experts. They also have given me some of their materials and walked me through the procedure so that I could carry it out at home.

The next thing I have researched about was a specific plant which is able to endure a various range of temperature, easy to germinate and has a high outcome. I have found out that green leaves of onion -the top part of onion after germination is capable to germinate in a short period of time like 8 to 14 days easily and effectively in between 5^{o}C and 15^{o}C. It also has many plentiful seeds and easily found. Another reason that I have chosen green onion is that it is used highly in our house and the ones we buy from a supermarket go bad very easily.

When it comes to the importance of stratification for the future, healthy nutrition is very important especially for the new generations as almost every nutrient is genetically modified. This method provides high efficiency with low intake. Moreover, the energy needed to grow plants with the way we do now consumes high amount of energy. Stratification is a promising method to enable the growth of plants more efficient with less energy consumation. Speaking for the years ahead, the global warming will increase creating an environment that is too hot for the seeds to germinate to grow into plants. In case there would be a shortage of food, with this method, every house would be able to stratificate any desired seed and plant them any time they want. Also with this facility to hibernate seeds, seed banks will gain importance. That is why I will determine the duration of time period that the seeds are stratified for to find the most efficient time interval for the seeds to germinate.

HYPOTHESIS

The fundemental factors that affect the germination of a seed are mainly temperature of the environmentfor metabolism and growth, presence of water to be absorbed and oxygen for respiration. Germination of a seed is a process which requires specific conditions that change dependingly on the type of the plant. Cold storage is a method applied on seeds which are to be germinated. Since it is neccesary to keep the seeds under cold temperature, it will affect the seeds' ability to germinate by augmentating its activity. As the duration of cold storage is extended the efficiency of the germination of scallion seeds which will grow into the green leaves of onion, will increase respectively. Because as the seeds get exposed to cold extendedly, their endosperm will freeze and will preserve the nutrients for the seed to germinate until the process. A frozen endosperm would last a longer time and won't lose its nutrition. In the light of this information, I hypothesize that the longer the seeds are exposed to cold, the more effectively they will germinate. Because there must be a significant mean difference between the average number of germinated scallion seeds during a period of seven days which are previously exposed to cold for zero, two, four, six and eight weeks respectively.

METHOD

DEVELOPMENT & PLANNING

In the wild, seed dormancy is usually overcome by the seed spending time in the ground through a winter period and having its hard seed coat softened up by frost and weathering action. By doing so the seed is undergoing a natural form of "stratification" or pretreatment. This cold moist period triggers the seed's embryo, its growth and subsequent expansion eventually break through the softened seed coat in its search for sun and nutrients.[9]

Thus, stratification is basically the process of artificially exposing seeds to both cold and moist conditions. Typically, temperatures must be between 1°C and 5°C.[10]

The employees ofMinistry of Forests have been very helpful with the questions I had, briefed me about their work and suggested alternative methods for my study. I learned that germinating seed on filtration paper in a water bathe after cold storage then stratifying had higher outcomes than stratification only. *(The process is explained with details in "Method: Procedure" (see page 9) (For the pictures of the experiment see Appendix))*

The reason I have combined these two methods is that they would save me a significant amount of time and it would be easy to collect data to evaluate. Also this method is frequently used by botany researchers because it has a high accuracy. [5][6]

I started the experiment with the first group which was not exposed to cold to obtain some data to shape the experiment around it. The experiment was carried out with a 7L container filled with 1.5 L of tap water at 20^{o}C. My father measured and carved the wooden plates to fit the container. I preferred to use wooden plates instead of glass because there would be a mess if the glasses broke.I got access to plastic circular plates and the funnels from the Ministry of Forestry.

In addition, the commissioners have taught me about the bathing method and suggested filtration papers. I cut the filtration papers according to the measurements explained in the method. After finishing the installation I have counted the seeds that weren't exposed to cold which I had divided into five before and placed them onto the wet filtration papers. The reason that I have used filtration paper is that fewer bacteria can generate on them also it is a special paper that holds the humidity on itself and stay wet as it takes only the amount of water it needs.

Mostly, seeds germinate in less than 10 to 20 days in appropriate conditions. But as scallions are a part of onion family, they not only grow fast but also germinate faster than most other seeds. There isn't any literature value for its germination; however they usually germinate in 8 to 14 days almost everywhere above 10^{o}C. The reason I chose scallions is that they can adapt to nearly every condition.

In the light of the information that I have learned both from my research and Ministry of Forestry, I expect that there would be a significant increase in the number of seeds that are germinated as the duration of exposure to cold increases respectively.

MATERIALS USED IN THE EXPERIMENT

- Thermometer (uncertainty: ± 0.5°C)
- pH meter
- Millimetric ruler (uncertainty: ± 0.5 mm)
- Graduated cylinder of 500 ml
- A container of minimum 7 L
- A pair of plastic tweezers
- A pair of scissors
- Filtration paper
- 5 plastic circular plates with a breach in the middle
- 5 funnels with open tops
- 4 pieces of 40x8x1 wood plates
- A cutting implement

METHOD

PROCEDURE

A. Labeling the Seeds

1. Prepare four small containers/plastic bags to fill them with seeds for cold storage
2. In order to have a realistic and reliable data, a larger number of seeds for each trial would be better so approximately 5000 scallion seeds are required. Assort the seeds into 1000 groups and put 4 groups into the bags.
3. Label the first bag as "2 weeks" and write down today's date.
4. Label the other bags as 4, 6 and 8 weeks and write down the date exactly two weeks after one another respectively.
5. Place the four bags into the freezer.
6. Sort the last group of seeds into 5 groups that have approximately 200 seeds each.

B. Preparing the Water Bathe& Setting Up the Environment

1. Measure the wooden plates according to the container and carve them with the cutting implement until they fit the container. (*See Appendix 1)*
2. Cut the filtration papers in circle shapes with same radius with the circular plates.(*See Appendix 2)*
3. Mark another sheet of filtration paper with the ruler and cut it as 2.5x30.(*See Appendix 2)*
4. Use the graduated cylinder for 1.5 L of water for the water bathe.
5. Measure the pH value of tap water as 7.1
6. Measure the temperature of water as 20^{o}C
7. Place the wooden plates with slight spaces between them.(*See Appendix 3)*
8. Place the long filtration papers after folding them into half.(*See Appendix 4)*
9. Put two sheets of paper that was cut circular on each plastic plate after damping them in water.
10. Put the circular plates on the wooden plates. *(See Appendix 5)*
11. Bed the seeds that you have counted before as 200 ± 5 on one filtration paper. *These are the seeds that weren't exposed to any cold storage.*(*See Appendix 6)*
12. Use the funnel to cover the plastic plates.(*See Appendix 7)*
13. Place the water bathe approximately 50 cm away from a heat source to be able to control the temperature.

14. Measure the room temperature as 25°C
15. After 1 week, count the seeds that have germinated with the help of a pair of plastic tweezers then the total number of seeds.
16. Note down the number of seeds that have germinated in each 200 group.

C. Stratification

1. Many sources recommend using peat, a combination of peat and sand, or vermiculite as the medium for cold stratifying seeds. However, any type of sterile soil mixture may be used as well. Fill the half of the metal container with peat but do not use force to flatten out the surface.*(See Appendix 8)*
2. Place some paper, useless pens or any desired long object to divide the container into five.
3. Place gently the germinated seeds only of every group into the divisions.*(See Appendix 9)*
4. Asperse some peat over the seeds verygently to cover them.
5. A spray can be used to water the seeds in every 12 hours. Otherwise, the water should be given in small and equal amounts over the container.
6. After one week, take a photograph of the container to compare it with the other trials since it would be hard to count all of the buds.

See Appendix 10 for detailed view of germinated seeds and differences between different groups.

DATA COLLECTION AND PROCESSING

Duration of applied cold storage(weeks)	Number of trials	Number of seeds germinated in each trial (n)($\pm\sqrt{n}$)	Total number of seeds in each trial (n)($\pm\sqrt{n}$)	Duration of germination allowed for the seeds (weeks)	Volume of water for the water bathe L(±1)
0	1	112 ($\pm\sqrt{112}$)	206 ($\pm\sqrt{206}$)	1	1.5
	2	98 ($\pm\sqrt{98}$)	217 ($\pm\sqrt{217}$)		
	3	110 ($\pm\sqrt{110}$)	200 ($\pm\sqrt{200}$)		
	4	131($\pm\sqrt{131}$)	206 ($\pm\sqrt{206}$)		
	5	122 ($\pm\sqrt{122}$)	207 ($\pm\sqrt{207}$)		
2	1	116 ($\pm\sqrt{116}$)	198 ($\pm\sqrt{198}$)	1	1.5
	2	91 ($\pm\sqrt{91}$)	170 ($\pm\sqrt{170}$)		
	3	101 ($\pm\sqrt{101}$)	171 ($\pm\sqrt{171}$)		
	4	92 ($\pm\sqrt{92}$)	185 ($\pm\sqrt{185}$)		
	5	92 ($\pm\sqrt{92}$)	169 ($\pm\sqrt{169}$)		
4	1	123 ($\pm\sqrt{123}$)	201 ($\pm\sqrt{201}$)	1	1.5
	2	138 ($\pm\sqrt{138}$)	209 ($\pm\sqrt{209}$)		
	3	119 ($\pm\sqrt{119}$)	197 ($\pm\sqrt{197}$)		
	4	135 ($\pm\sqrt{135}$)	216 ($\pm\sqrt{216}$)		
	5	129 ($\pm\sqrt{129}$)	200 ($\pm\sqrt{200}$)		
6	1	133 ($\pm\sqrt{133}$)	200 ($\pm\sqrt{200}$)	1	1.5
	2	140 ($\pm\sqrt{140}$)	211 ($\pm\sqrt{211}$)		
	3	124 ($\pm\sqrt{124}$)	197 ($\pm\sqrt{197}$)		
	4	125 ($\pm\sqrt{125}$)	195 ($\pm\sqrt{195}$)		
	5	134 ($\pm\sqrt{134}$)	213 ($\pm\sqrt{213}$)		
8	1	148 ($\pm\sqrt{148}$)	203 ($\pm\sqrt{203}$)	1	1.5
	2	151 ($\pm\sqrt{151}$)	208 ($\pm\sqrt{208}$)		
	3	153 ($\pm\sqrt{153}$)	215 ($\pm\sqrt{215}$)		
	4	139 ($\pm\sqrt{139}$)	196 ($\pm\sqrt{196}$)		
	5	142 ($\pm\sqrt{142}$)	202 ($\pm\sqrt{202}$)		

Table 1: Table 1 is the raw data table.It shows the number of germinated seeds out of total number that were allowed to germinate on the water bathe for one week with respect to the time of being exposed to cold for zero, two, four, six and eight weeks. The uncertainty values are various through the data because since the number of total seeds differ in each trial, the odds of the seeds to germinate differ accordingly as well.

Duration of cold storage (weeks)	Number of trials	The ratio of germinated seeds to the total number in each trial	Percentage of germination(%)
0	1	112/206	54.4
	2	98/217	45.2
	3	110/200	55.0
	4	131/206	63.6
	5	122/207	58.9
2	1	116/198	58.6
	2	96/170	56.5
	3	101/171	59.1
	4	97/185	52.7
	5	92/169	54.4
4	1	123/201	61.2
	2	138/209	66.1
	3	119/197	60.4
	4	135/216	62.5
	5	129/200	64.5
6	1	133/200	66.5
	2	140/211	66.3
	3	124/197	62.9
	4	125/195	64.1
	5	134/213	62.9
8	1	148/203	72.9
	2	151/208	72.6
	3	153/215	71.2
	4	139/196	70.9
	5	142/202	70.3

Table2: Table 2 shows the ratio of the germinated seeds to the total number of seeds in each trial with their percent efficiencies of germination.

EXAMPLE OF CALCULATIONS

$$\frac{112}{206} = \frac{x}{100}$$

x: percent value of the number of germinated seeds in related trial

$$\frac{112 \times 100}{206} = x \ = 54.4\%$$

Trial					
	0.0	**2.0**	**4.0**	**6.0**	**8.0**
1	54,4	58,6	61,2	66,5	72,9
2	45,2	56,5	66,1	66,3	72,6
3	55,0	59,1	60,4	62,9	71,2
4	63,6	52,7	62,5	64,1	70,9
5	58,9	54,4	64,5	62,9	70,3
MEAN	55,520	58,760	62,940	64,540	71,680
MODE	-	-	-	62,900	-
MEDIAN	55,000	54,400	62,500	64,100	71,200
RANGE	18,400	9,400	5,700	3,600	2,600
VARIANCE	46,172	15,113	5,523	3,128	1,257
SD	6,795	3,888	2,350	1,769	1,121
SE	3,039	1,739	1,051	0,791	0,501
T	2,776	2,776	2,776	2,776	2,776
%95 CI (SE * T)	8,437	4,827	2,918	2,196	1,392
%95 CI (EXCEL)	5,956	3,408	2,060	1,550	0,983

Table 3: Table 3 shows the efficiency percentage values of the germinated seeds in each trial with the mean, mode, median, range, variance, standard deviation, standard error, t and 95% confidence values of the collected data.

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	952,6464	4	238,1616	16,72647592	3,6246E-06	2,866081
Within Groups	284,772	20	14,2386			
Total	1237,4184	24				

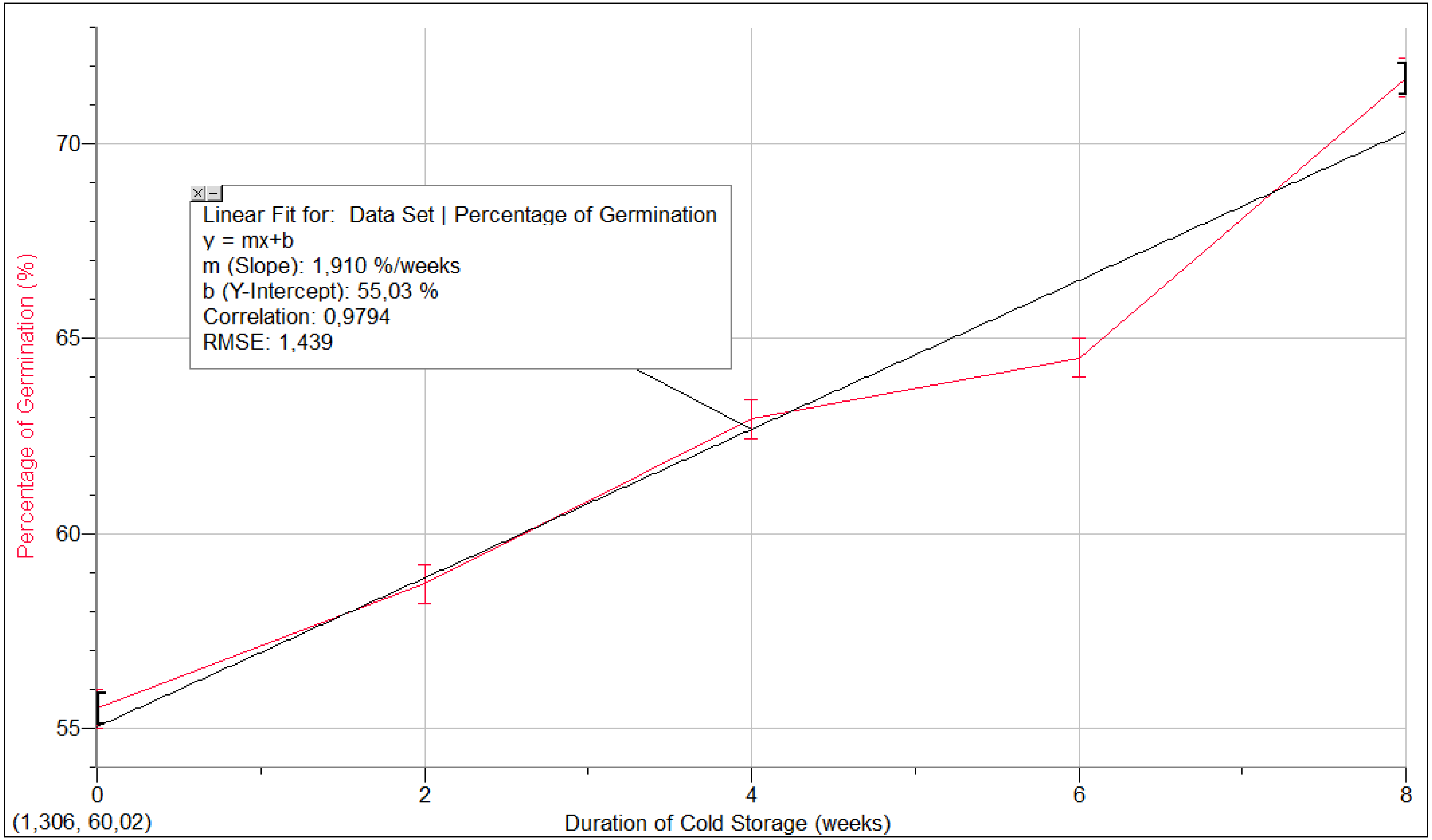
Table 5: Table 5 shows the variation sources; SS, df, MS and F values, P-value and F-crit.

H_0 = There is not a significant mean difference between germination percentage of scallion seeds that are exposed to varying durations of cold storage.

H_1= There is a significant mean difference between germination percentage of scallion seeds that are exposed to varying durations of cold storage.

P= $3,62*10^{-6} < 0,05$

Reject H_0, accept H_1



Graph 1: Graph 1 shows the change in the percent efficiency of germination of scallion seeds that are exposed to cold storage which affects the germination behavior due to the duration of cold storage for 0, 2, 4, 6 and 8 weeks respectively. This graph includes the mean of five percent germination values from each test group that is shown with the red line.

CONLUSION AND EVALUATION

Stratification is simply the process of pretreating seeds to simulate natural winter conditions that a seed must endure before germination. This process enables almost any seed to germinate artificially if the environment is not sufficient. It can also be executed almost everywhere. There are alternative methods however this method is both easy and less energy consuming.

In this experiment the aim was to determine the effect of duration of cold storage on the efficiency of germination of the green onion *(scallion)* seeds. To determine this, an experiment was carried out with five different test groups that were exposed to cold for zero, two, four, six and eight weeks. After ending their storage, a water bathe was set up and filtration paper was used to let the seeds germinate without giving them too much water. The hypothesis that was suggested was that there would be a significant rise in the mean value of the number of germinated seeds due to the duration of cold application.

It was seen that the highest number of germinated scallion seeds belonged to the fifth group in which the scallion seeds were exposed to cold for 8 weeks straight while the value of the number of germinated seeds in the other four groups that were exposed to cold for 6, 4, 2 and 0 weeks followed decreasingly. The percent efficiency values which shows the efficiency of germination of each group were found as 55.42%, 55.06%, 62.94%, 64.54% and 71.58% for the duration of applied cold storage as 0, 2, 4, 6 and 8 weeks respectively. The highest germination percentage is in the group of seeds that were exposed to cold for 8 weeks and the lowest is in the group that were not exposed to cold.

Graph 1 shows the relationship between the duration of cold storage and the efficiency percent of cold storage. As it is seen, the efficiency percent increases respectively as the duration of cold storage is extended. For the first three groups (0, 2 and 4 weeks) the mean values of the five trials within each group follow a linearly increasing trend. The fourth group in which the seeds were exposed to cold for 6 weeks, however, has a slightly smaller mean value than that of the seeds that were exposed to cold for a duration of 4 weeks. The fifth group was exposed to cold for 8 weeks and has a value more than expected as it is marked over the linear fit of the data. Also the mean percent values for each group can be seen easily. The fourth mean value is close to the mean value of the third group yet it is still higher than that of the third. Since the trend of the graph is increasing, it can be said that there is a direct relation between the duration of cold storage and the mean value of efficiently germinated seeds.

As a literature value to base the experiment on, a project on germination of Dwarf Huckleberry was analyzed. Even though the method used is not the same but similar, the result of the experiment is supporting. It is found out in the experiment on dwarf huckleberry that the stratification of seeds that were not exposed to cold did not significantly affect germination. On the other hand, the germination percentages for the seeds that were stored for 6 months were found out to be nearly 90%.[11] Thus it can be concluded from the experiment that seeds that were exposed to cold storage for a period of time had a significantly higher percentage of germination than that of the seeds that were not exposed to cold.

The experiment was carried out professionally and extendedly. Thus the results were accurate to be used as a literature value. Although the experiment I have carried out was not as long as the other one, the results are related. Therefore the trend in graphs, the collected data and a related literature value supports the hypothesis "As the duration of cold storage is extended up to 8 weeks the efficiency of germination of scallion seeds will increase respectively."

The standard deviation values of the groups are 6.795, 3.888, 2.350, 1.769 and 1.121 where the standard error values are 3.039, 1.739, 1.051, 0.791 and 0.501. Both values decreasing from first group to fifth show that the data gets more precise and coherent. The reason that the deviation and error is higher at the beginning and decreases through the end may be that trials after the first group got more experienced and handled more professionally.

The initial hypothesis (H_o) suggests that there is not a significant mean difference between germination percentages of scallion seeds that are exposed to varying durations of cold. However, the calculations from ANOVA showed that the probability of the hypothesis (P-value) which is found as $3,62*10^{-6}$ is smaller than $P = 0,05$. These calculations show that there is a significant mean difference between germination percentages of scallion seeds that are exposed to varying durations of cold storage. Additionally, it increases as the duration of cold storage is extended up to 8 weeks.

The experiment was carried out in the best conditions of a house environment. The temperature of the room was measured at intervals, however the set-up was next to the radiator therefore the seeds could have been affected by the radiator. Because the temperature of the room was approximately 25°C and the radiator's was more than 25°C. Also the container for the water bathe was plastic that there could have been a heat exchange from the radiator which may have made the water warmer.

For stratification after the bathe, a soil mixture from the garden was used. It was sterile however it probably contained pesticide residues and even slaked lime that was used by the gardener. These toxic residues might have affected the germination behavior of the seeds in a negative way. In addition, the container that was used to stratify the seeds were relatively small and the seeds had to grow over each other. Thus some of the seeds that were going to germinate could not be successful as they are not as resistant to these chemicals as others. This may mean that the genetic structure might differ among the seeds. This might have caused a difference in percent efficiencies of germination within the same group by decreasing the number of germinated seeds.

Watering procedure was also not scheduled, the seeds were watered twice or three times per day. The seeds were not watered evenly so this might also have affected the efficiency negatively. Lastly counting the germinated seeds out of each group was the hardest part since there were a big number in each. A small number might have eluded observation during counting and this may also have decreased the overall efficiency.

If the experiment is to be performed again, laboratory conditions would provide more accurate results. Firstly, a heat-proof container would be better than a plastic or a metal container since it wouldn't let any heat exchange with the outer environment which would affect the temperature of water for the bathe.

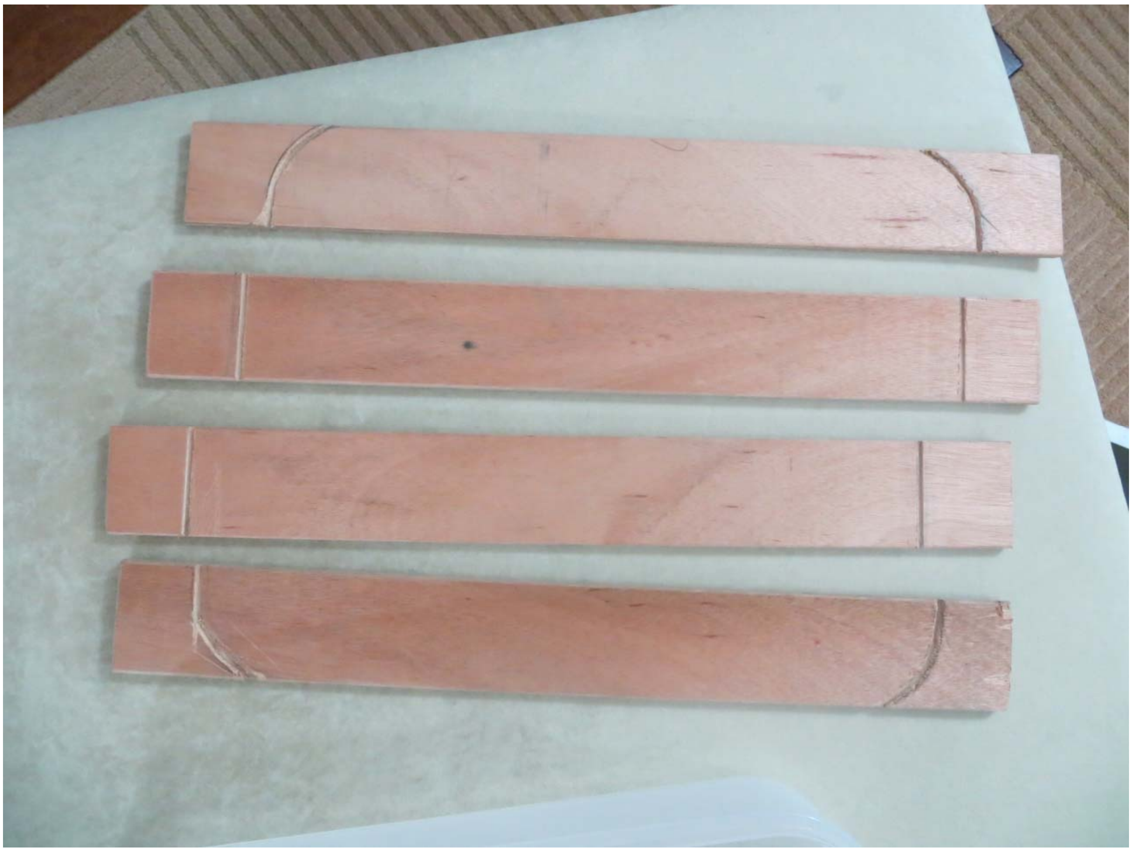
Additionally, peat would be the best option for stratification since it is a mixture of different types of soil. It does not contain any toxic residues that may affect the process negatively however it is not common, thus not easy to find. What is more, the container for stratification should be big enough for the seeds to be next to each other, not on top.

Watering procedure is also important and needs to be scheduled to help the seeds to germinate better and effectively. For example, they can be watered in every 8 hours with certain amount of water depending on the seed type.

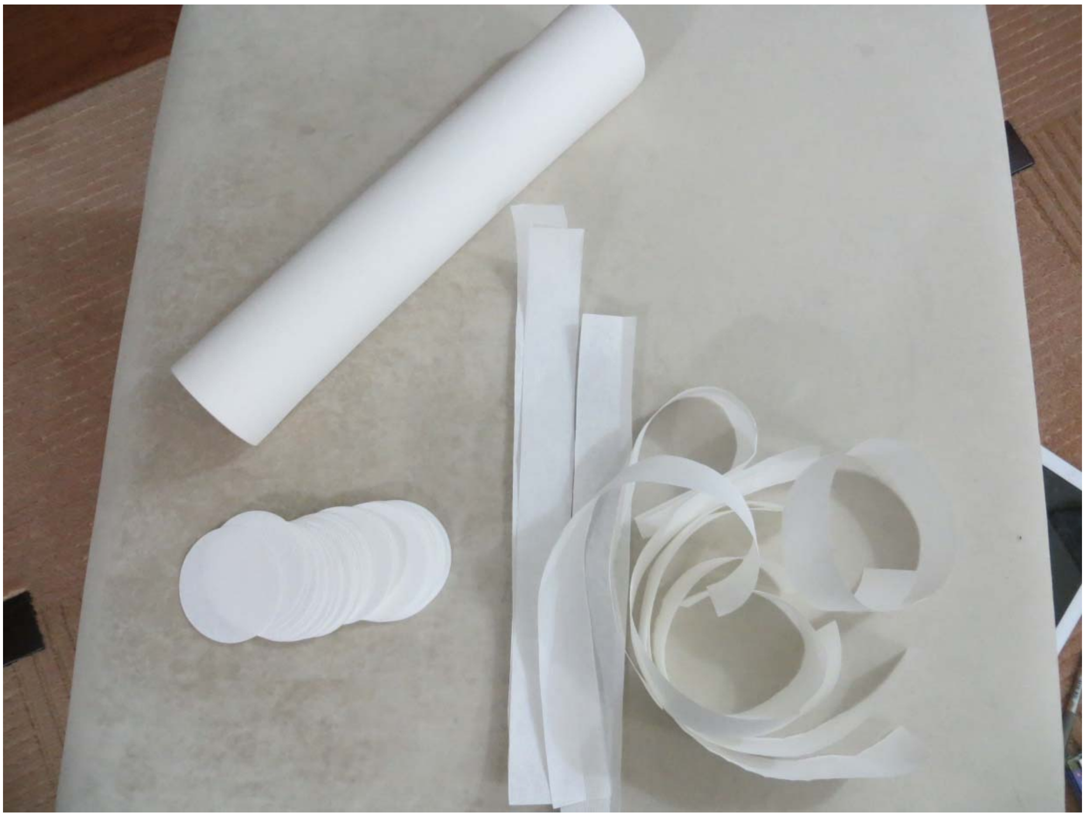
Finally, even though counting is hard and time consuming, it must be done with great attention and care for more accurate results. Also more trials on smaller amounts of seeds can be executed because as the number of seeds gets smaller within groups counting gets easier and any results that may occur caused by inattention may be prevented. This way, the calculations would be more precise and the reliability of the data and the experiment would increase. Additionally, the duration of cold storage can be extended to observe whether there is a significant difference as the seeds get exposed to cold for a longer period of time.

APPENDICES

Appendix 1



Appendix 2



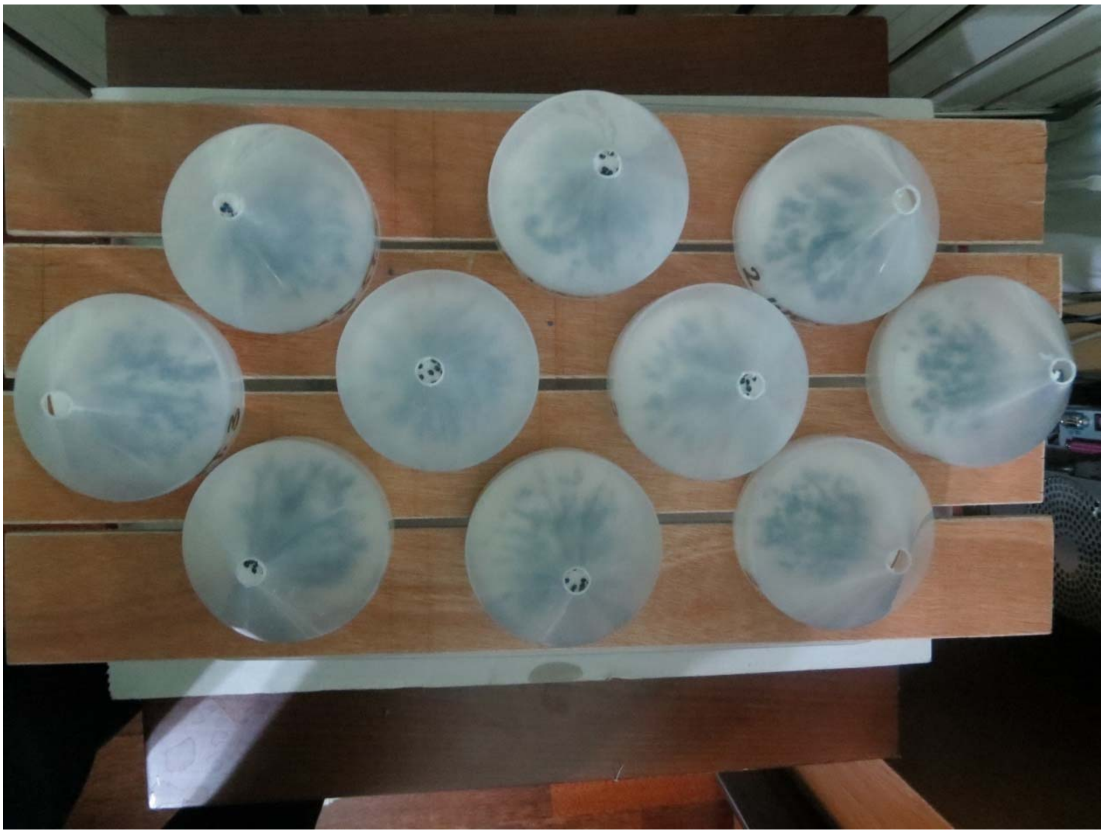
Appendix 3

Appendix 4

Appendix 5

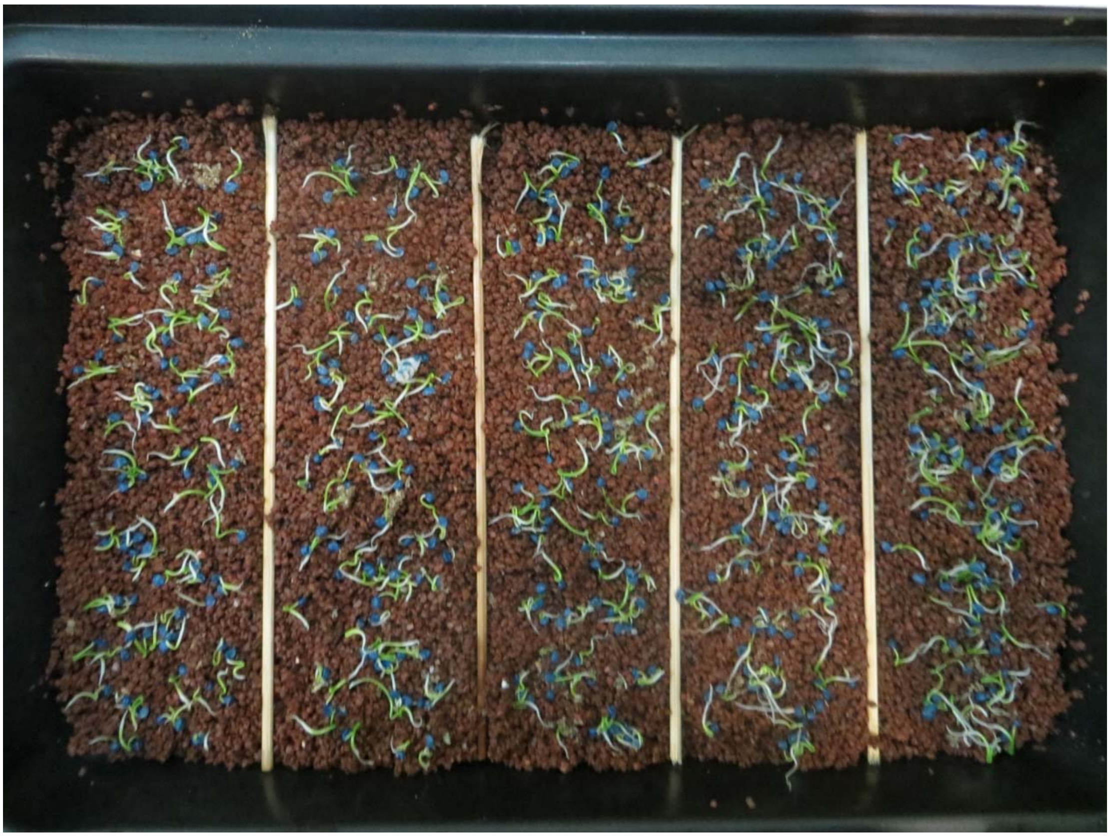
Appendix 6

Appendix 7



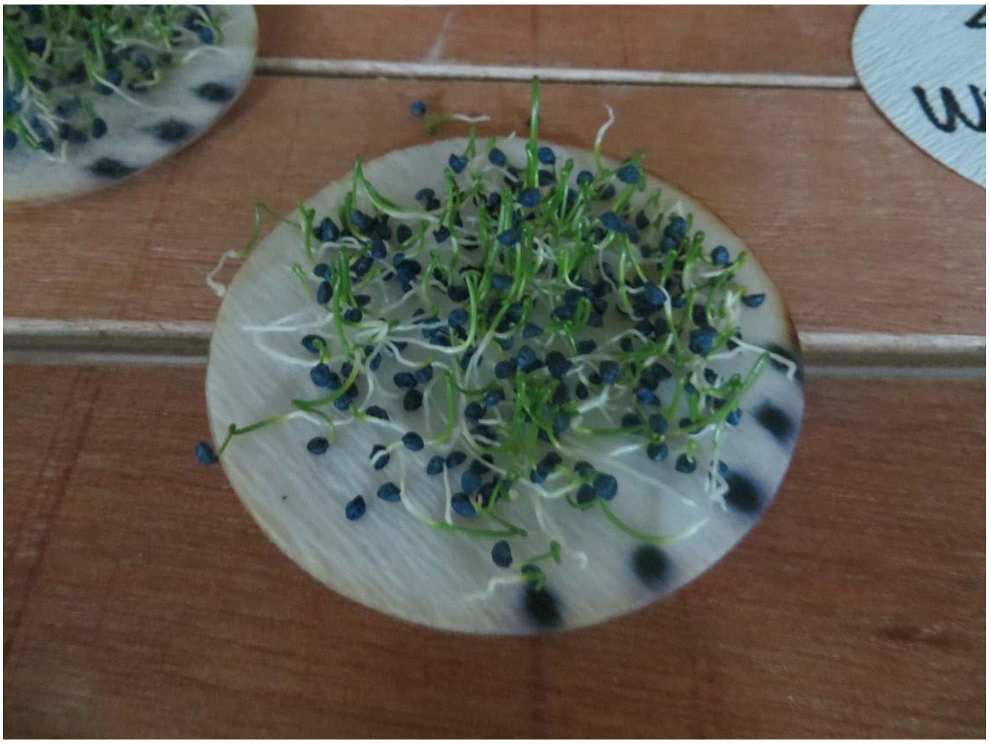
Appendix 8

Appendix 9



Appendix 10

One week after the germination of the 0-week group



One week after the germination of the 8-week group

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