Title: The effect of audible sound at specific frequencies (70 Hz, 300 Hz, and 2000 Hz) on the germination time of *Raphanus sativus*.

Research Question: How does audible sound at specific frequencies (70 Hz, 300 Hz, and 2000 Hz) affect the germination time of *Raphanus sativus* seeds?

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Introduction

Seeds are all around us, serving as the precedents of the plants that become fruits and vegetables necessary to our survival. Plants provide food for us to eat, oxygen to breathe, and shelter from the sun and weather. All of those impressive functions begin with one simple item, the seed.

Germination is the process that begins a plant's life, and can be accomplished through various methods. A complex method is used in the germination of *Arabadopsis thaliana*. The seeds are placed in a refrigerator and then taken out, which mimics the transition from winter to spring. Other, simpler methods involve placing the seeds between moist paper towels, or simply into soil. The commonality between each of these methods is that they involve the use of an external stimulus.

Some external stimuli include the traditional factors such as light, water, temperature, and soil quality. These stimuli are all directly related to the way plants function. Enzymes function differently at different temperatures, which leads to molecules being metabolized at different rates. Light and water are used in photosynthesis, which is the process that plants use to generate glucose for energy. Soil provides the growing seed with chemicals and nutrients (Martin, 2022).

Each of these stimuli is commonly found in nature. Another, less studied stimulus, also commonly found in nature, is sound. Sound is a mechanical wave that travels through a medium (Berg, 2024). At high intensities, sound waves can exert physical effects on their surroundings. Given that seeds rely on external stimuli for germination, the intriguing question emerges: can sound, as an external stimulus affect the rate of seed germination?

Studying this topic could be beneficial in a variety of ways. First, to find methods to speed up the process of germination, which could potentially make agriculture more efficient.

Traditional seed germination can be very time consuming. I enjoy gardening and planting, and often grow my plants from a seed. From my experience, germinating seeds for longer periods of time involves a lot of patience and certain risks. For instance, when I germinated an avocado pit, it took about 2 months, and became moldy in the process. If a stimulus can accelerate germination, plants can be grown faster and have a lessened risk of contamination. In a commercial context, the simple influence of sound could allow for more accelerated crop growth, allowing the ever-growing population more food to eat.

On the other hand, an external stimulus could inhibit seed germination. Seed repositories, which aim to store seeds for extended periods, could benefit from this potential inhibitory effect. Utilizing sound, a simple and cost effective intervention, the shelf life of stored seeds could be extended.

So far, this topic has been minimally investigated. Researchers have established that the mung bean's germination time was reduced significantly by sounds in the 2.0-2.5 kHz range at 80, 90 and 100 decibels, and by sounds in the 1.5-2.0 kHz range at 90 decibels (Cai et al., 2014). They also determined that the stem and root growth in the mung bean was improved with sounds in the 2.0-2.5 kHz range at 80, 90, and 100 decibels (Cai et al., 2014). They provided several potential reasons for why this occurred. Some of these reasons include the plants' stomatas being opened by stimulation from the sound, allowing for more nutrients to be taken in, and that plants may have adapted to the sounds of their environment, so frequencies mimicking the sounds of wild bird can help them to "feel at home" (Cai et al., 2014).

Other researchers found similar results by testing much lower frequencies, 40-120 Hz. They described frequencies higher than 70 Hz to be beneficial to *Arabidopsis thaliana* germination (Uchida & Yamamoto, 2002). The 70 Hz treated seeds germinated faster than the

other frequencies tested. They also researched the effects of altering the amplitude of the sound waves. For frequencies higher than 70 Hz, an amplitude of 0.42 mm increased the rate of seed germination. For frequencies higher than 100 Hz, an amplitude of 0.33 mm also increased the rate of germination (Uchida & Yamamoto, 2002). These researchers emphasize that the main focus of the audible sound is not only on the frequency, but also the amplitude.

Last, a researcher determined that white noise and bass sounds at 300 Hz had a positive effect on maize seed germination (Vicient, 2017). This researcher aimed to determine if sound was a viable option for seed priming. Priming is used to promote germination in seeds that have been stored for a long period of time. Vicient sought to determine a new option of priming methods, sound. He determined a relevant cause for the differences in germination rate at 300 Hz versus silence. This cause, found by staining parts of the tested seeds, was damage to the pericarp (Vicient, 2017).

Based on these researchers' findings, I wondered about the range of sound allegedly improving upon seed germination rate. The first researchers tested bands of frequencies, such as 2.0-2.5 kHz. The second researchers tested lower frequencies, with successes at 70 Hz and above. The third researcher found 300 Hz to be effective at decreasing germination time. The investigation of this extended essay seeks to compare these results to one another, to find which of the allegedly successful frequencies contribute to decreased seed germination times.

The experiment that I conducted aimed to determine the differences between low, medium, and high frequency sound signals. The different researchers had successes with each of the frequencies chosen. However, they all used different plant species in their research. I utilized their research to determine viable variables, which applied to only one species of seed, *Raphanus sativus*, the radish.

Literature Analysis and Relevance to Methods

Cai et al., studied not only the effects of different frequency bands, but also of different volumes. They discovered that 2000 Hz at 90 decibels was an effective treatment to reduce Mung bean germination time (Cai et al., 2014). Their results are relevant because they showcase that different frequencies and volumes affect the seeds to different extents. This experiment considers volume as a constant, focusing on the sound's frequency as a variable. 2000 Hz was chosen as the high range frequency for the experiment due to the success of these researchers.

Uchida and Yamamoto studied the effects of monotonal sinusoidal vibration on *Arabidopsis thaliana*. This article gave insight into the relevance of amplitude of the sound waves. The researchers showed success with an amplitude of 0.42 mm , so this measurement was used as the amplitude for each variable (Uchida & Yamamoto, 2002). The lowest frequency that significantly decreased germination time was 70 Hz. Therefore, 70 Hz was used as the low frequency to test on *Raphanus sativus* seeds.

Finally, Vicient studied the effects of frequency specific sound signals on maize seed germination. He discovered that a frequency of 300 Hz decreased the rate of germination (Vicient, 2017). Therefore, 300 Hz was used as the mid range frequency to test. This researcher also studied the effect of duration of sound treatment on germination time. He found an 8% increase with the 3 hour treatment, and a 10% increase with the 5 hour treatment (Vicient, 2017). A 6 hour treatment was chosen, as the researchers described a decrease in germination time with an increase in time. It also made it very simple to check the seeds four times a day. He utilized the software Audacity was used to generate the sound in this experiment. It is simple to use, can generate many hours of sound at once, and requires only a computer and output device to use.

Each of these research articles deal with different seeds. Maize is a large seed that sprouts into a monocot (Sapkota & Abdela 2023). *Arabidopsis thaliana* is a tiny seed that grows to become a dicot (Wixon 2001). Finally, mung beans are a medium sized seed that becomes a dicot (Carter 2022). Despite all of these differences, they are all affected by audible sound. More specifically than only wondering about the effects of audible sound on seed germination time, the differences in beneficial audible sound on some seeds versus others were also considered.

Background Information on *Raphanus sativus*

Raphanus sativus, colloquially known as the cultivated form of the radish, is an edible root vegetable commonly used in salads. It is known as easy to grow and harvest, making it appealing to beginner gardeners. *R. sativus* was chosen because of its ease of access and relatively quick germination rate. The average germination time in soil is 3 to 4 days (Drost 2020).

Seed Structure

R. sativus is a dicotyledon, so it has 2 cotyledons. The cotyledons form the embyonic leaves of the plant and store high amounts of starch as food. Some seeds have an endosperm, which also provides energy to the growing seed. *R. sativus* does not have an endosperm, instead, the cotyledons are the only food storage organs (Leubner, 2017). The seed is further protected by a seed coat, which is strong enough to protect the embryo, but permeable to water. The radicle is the first part of the seed to start emerging, and eventually becomes the root. The hypocotyl emerges above the radicle to become the stem and supports the cotyledons. The epicotyl emerges just above the cotyledon, and it houses the first true leaves and shoot (Leubner, 2017).

Hypothesis

Based on the knowledge gained from the sources, audible sound does seem to have a correlation to seed germination rate. A variety of frequencies are effective in reducing the time needed to germinate both monocots and dicots, in the form of maize, mung bean and *Arabidopsis thaliana* seeds. The hypothesis is that if sound at 70 Hz, 300 Hz, and 2000 Hz with an amplitude of 0.42 mm is played, germination time of *R. sativus* seeds will be reduced.

Materials and Methods

Variables

Controlled Variables	Brand of seeds, species of seeds, headphones used, software used, start time, temperature, volume, light level, 0.42 mm amplitude, 6 hours of silence and 6 hours of sound
Independent Variables	Frequency of sound treatment (70 Hz, 300 Hz, 2000 Hz, silence (control))
Dependent Variable	Germination Time (hours)

Materials

The materials used to conduct the experiment included: Audacity Version 3.3.3, Burpee Organics Cherry Belle Radish seeds, non sterile petri dishes, tap water, spray bottle, paper towels, scissors, HyperX Cloud II Headset, a Sharpie, paper, a ruler, and a computer.

Methods

To generate the tone tracks, the Audacity software version 3.3.3 was used. The “Generate” tab was clicked, then the “Tone” tab was clicked. Each variable had the same settings, except for the Frequency (Hz) setting. The settings selected are as follows: sine for waveform, 0.42 mm for amplitude, and 6 hours for duration. Generate was clicked. A 6 hour duration of silence was then generated. The loop function was activated.

To make the germination dish, a non sterile petri dish was obtained. The dish was labeled with the date and variable to be tested (70 Hz, 300 Hz, 2000 Hz, silence). A paper towel without any dye was folded in half. The petri dish was placed on top of the paper towel. The pen was

used to trace a circle the size of the petri dish onto the paper towel. The circle was cut out slightly smaller than the traced circle. The two paper towels were placed into the petri dish. The spray bottle with tap water was used to slightly dampen the paper towel. Ten radish seeds of similar size and color were placed in two rows, as shown in *Figure 1.1* below.

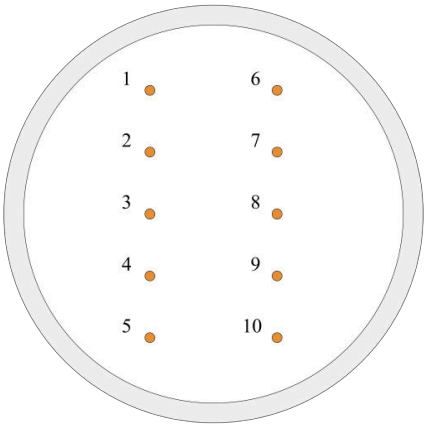


Figure 1.1: Germination Plate Layout (orange circles: R. sativus seeds; white circle: paper towel; grey circumference: petri dish)

For each trial, the prepared petri dish was obtained. It was placed between the earpieces of the headset, as shown in *Figure 1.2*. The top of the headset was covered to keep the environment dark. The Audacity track was retrieved. The volume of the headset, according to Windows 10, was set to 30. All other applications on the computer were muted. At 11:00 AM, the play button was pressed.



Figure 1.2: Experimental Setup (petri dish between earpieces of HyperX Cloud II headset)

Data was collected after each 6 hour time period by counting all of the germinated seeds, starting at 11:00 AM until all seeds were germinated. Germination was determined by the emergence of the radicle.

Statistical analysis was performed. First, the means of each sample set were determined. This was done to be able to better compare the data sets to one another. Then, the standard deviation was calculated. This was done to be able to determine how the germination times were spread apart. Finally, t-tests were performed. As the aim of the investigation is to determine if there is a significant difference in relation to the control group, silence, the following pairings were t-tested: control and 70 Hz, control and 300 Hz, and control and 2000 Hz.

Results:

Figure 2.1: Control Germination Times

Control Sample	Germination Time (hours)
1	24
2	24
3	24
4	30
5	24
6	30
7	30
8	24
9	30
10	30

Figure 2.2: 70 Hz Germination Times

70 Hz Sample	Germination Time (hours)
1	18
2	24
3	18
4	24
5	30
6	18
7	30
8	18
9	18
10	18

Figure 2.3: 300 Hz Germination Times

300 Hz Sample	Germination Time (hours)
1	24
2	24
3	24
4	24
5	24
6	30
7	30
8	30
9	30
10	30

Figure 2.4: 2000 Hz Germination Times

2000 Hz Sample	Germination Time (hours)
1	30
2	36
3	30
4	30
5	30
6	30
7	30
8	30
9	30
10	30

Figure 2.5: Control Plate at 24 Hours

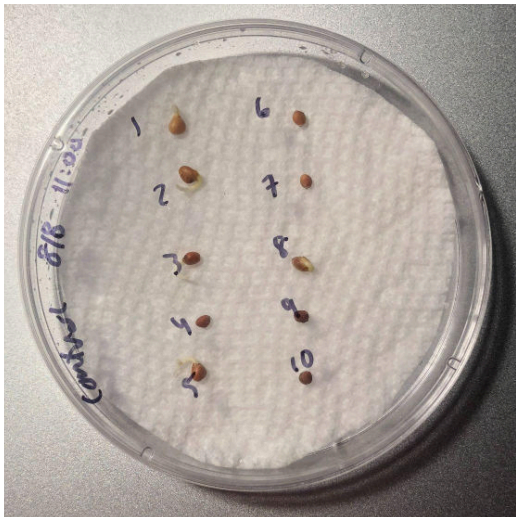


Figure 2.6: 70 Hz Plate at 24 Hours

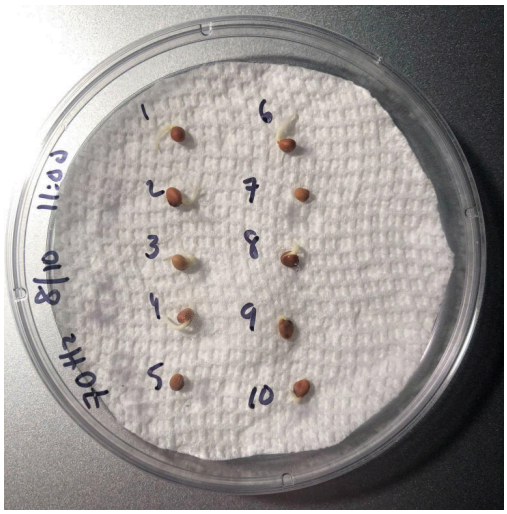


Figure 2.7: 300 Hz Plate at 24 Hours

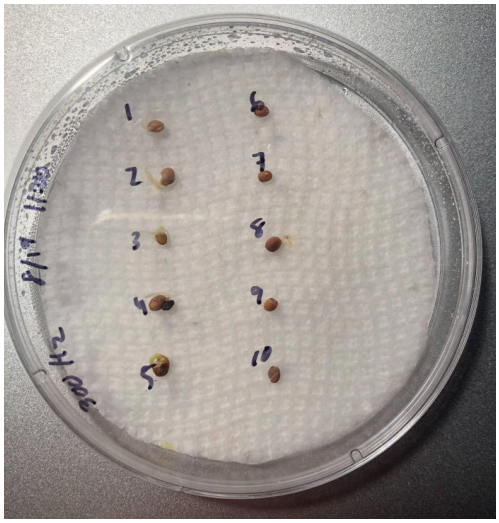


Figure 2.8: 2000 Hz Plate at 24 Hours

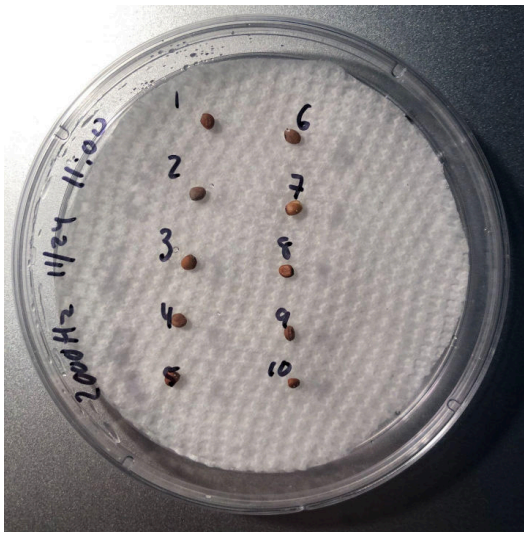


Figure 2.9: Mean Times to Germinate, Standard Error of the Mean, and Standard Deviations of Each Variable

	Control	70 Hz	300 Hz	2000 Hz
Mean Time to Germinate (Hours)	27	21.6	27	30.6
SEM	1	1.6	1	0.6
Standard Deviation	3.16	5.06	3.16	1.9

Figure 2.10: Mean Time to Germinate R. Sativus at Various Frequencies with Standard Error of the Mean Error Bars

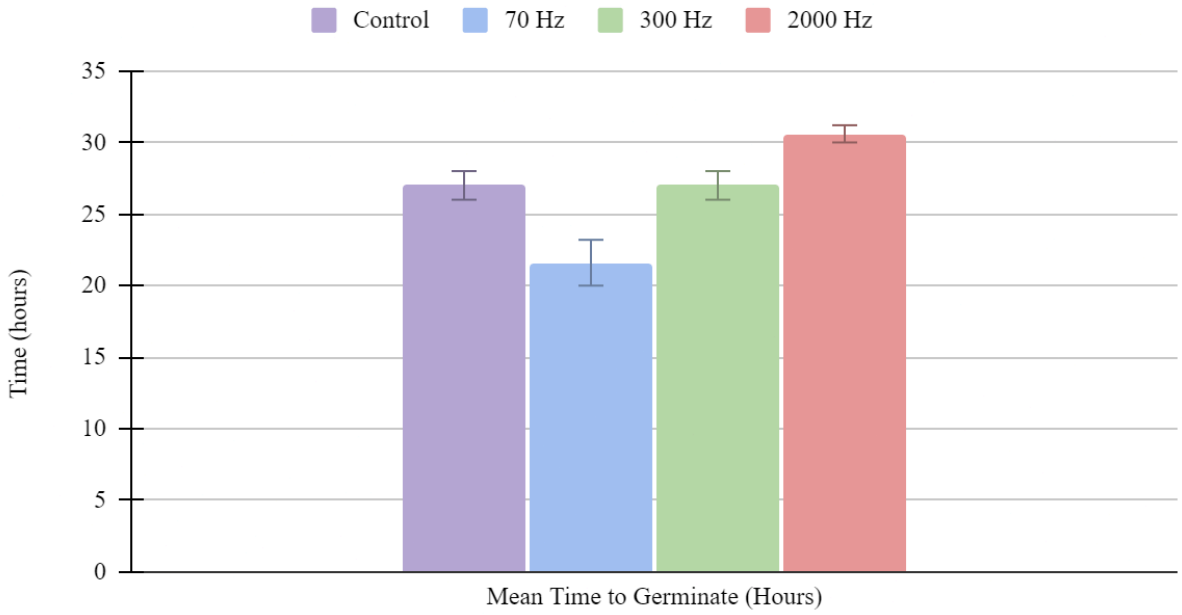


Figure 2.11: P-values between Control and Variables

	70 Hz	300 Hz	2000 Hz
Control	0.010	1.00	0.006

Analysis and Evaluation:

Upon analysis of the data collected, a trend appeared, which is more obvious when viewed in *Figure 2.10*. The control variable had a mean germination time of 27 hours. The lowest frequency tested, 70 Hz, had a mean germination time of 21.6 hours. The mid range frequency, 300 Hz, had a mean germination time of 27 hours, just like the control. Finally, the highest frequency tested, 2000 Hz, had a mean germination time of 30.6 hours. Based on the data collected in the experiment, there is an apparent trend linking increasing frequencies of sound to increasing germination time.

There was a significant difference between the control and the 70 Hz treatments, as shown in *Figure 2.11*. This is because the p value calculated between the control and 70 Hz was 0.010, which is below the alpha value of 0.05. The results obtained from the 70 Hz sample correspond with those expected, according to the researchers from the second article (Uchida & Yamamoto, 2002). The 70 Hz treatment significantly decreased the germination time by 20%.

The p value between the control and 300 Hz treatments was 1.000. The results obtained between these two variables indicate that they are completely the same, with no statistical difference. Both had a mean germination time of 27 hours and a standard deviation of 3.16.

The p value between the control and 2000 Hz treatments was 0.006, indicating a statistical significant difference. The 2000 Hz treatment increased the germination time by 13%.

The results depict a trend, that the germination time increases as the frequency of sound signals increases in *R. sativus.* This could be the result of a variety of causes. The first plausible cause is that the sound alters the rate at which cells grow and divide. During prophase in mitosis, spindle fibers start forming. They help to move chromosomes during mitosis. In metaphase, they attach to the chromosomes, and then pull them apart during anaphase (Rye et al. 2016). Perhaps

sound signals help the spindle fibers move to the right positions faster, therefore pushing and pulling chromosomes apart faster, accelerating the speed of mitosis. With the higher frequencies tested, it took a longer amount of time to germinate. Applying this theory, the high frequencies would move the spindle fibers faster. However, this does not allow for cells to grow adequately. Thus, they would divide faster, and at smaller sizes.

Conversely, the spindle fibers would move less at lower frequencies. This would allow the cells to grow to an optimal size and divide, but faster. An optimal frequency for cell germination would factor in allowing the cell to grow to an adequate size as well as divide slightly faster than normal. To analyze this potential explanation of why altered rates of germination occurred, a study could be conducted that measures the rate of cell division and cell sizes when different frequencies are played.

Another plausible cause for the results observed is cell damage. Vicient, who analyzed pericarp damage as a potential reason for the change in germination time that he observed, saw that the pericarp was damaged by using fast green staining. The pericarp affects seed germination by preventing water and oxygen absorption (Vicient, 2017). The researcher only performed this analysis with respect to 300 Hz on maize seeds. Based on the fact that the 300 Hz treatment was able to physically alter the pericarp, perhaps different frequencies could affect the pericarp differently. As germination time was increased as a result of the 2000 Hz treatment, perhaps other parts of the seeds were compromised, leading to reduced growth. This reason should be researched further in depth with a larger variety of frequencies in order to determine how different frequencies of sound affect the pericarp and rest of the seed's integrity differently. This researcher also found that 300 Hz was an effective treatment for maize seeds, whereas *R. sativus* seeds showed no significant difference from the 300 Hz trial. This could indicate that size of the

seeds plays a role in the way sound interacts with the seed. Rather than a one size fits all approach to the effect of sound on plants, one must consider the plant or seed's anatomy. Each type of plant could have its own range of optimal frequencies to be able to stimulate germination, determined by its size and layers of seed coat and endosperm.

An optimal frequency could potentially stimulate processes within the cells of the seed. Different hormones are known to cause growth and inhibition in plants (Gutiérrez & Perez, 2004). Perhaps different frequencies could stimulate the presence of different chemicals. *R. sativus* produces growth inhibitors, such as cis- and trans-raphanusanins and raphanusamide (Gutiérrez & Perez, 2004). A future study could be done to analyze the presence of these compounds in coordination with different frequencies.

Conclusion

In this investigation, *R. sativus* germination time was found to change when different frequencies of audible sound were played. Altering between 6 hours of 70 Hz sound and 6 hours of complete silence decreased germination time by 20%. Altering between 6 hours of 300 Hz sound and 6 hours of complete silence did not affect germination time. Altering between 6 hours of 2000 Hz sound and 6 hours of complete silence increased germination time by 13%. As a minimally studied topic, there are a variety of plausible reasons for the results observed, but no confirmed ones. These include, but are not limited to, alterations in the movement of spindle fibers to cause changes in the rate of mitosis, cell damage, and presence of different chemicals.

Modifications could be made to improve this experiment. A greater number of seeds could be germinated to obtain more accurate results. A better germination setup could be made to reduce the amount of vibrations due to external sources. Without the limitations of the lack of equipment and experience, this topic could be studied in much greater detail.

A reason as to why germination time was increased and decreased should also be considered. Further studies could be conducted on a higher level to determine an more supported reason for the results, such as cell size, rate of mitosis, cell damage, and the presence of growth factors or inhibitors at different frequencies. Another study to be done could be about differing seed sizes in relation to sound and germination. Determining the way plants are affected by sound would offer an interesting insight into how plants, which are organisms without any auditory organs, are still impacted by it as a part of their environment. Depending on the promotional or inhibitory affects to growth, different aspects of the plant industry could be impacted. Commercial agriculture could be positively affected, allowing for crops to be grown

and distributed at a higher rate. Seed repositories could employ sound as an inhibitory measure to increase shelf life of seeds.

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