The Spice Showdown

The Spice Showdown: A Seed-Sational Showdown of Nutmeg vs. Lettuce

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1. Introduction

Allelopathy. A mouthful, right? But what does it really mean? Well, Allelopathy is the phenomenon of an organism releasing biochemicals that can negatively or positively affect other organisms in its environment. Whether through increased growth and germination, or the detriment of these things through the release of phytotoxic volatiles. Ferguson et al, (2003) says that there are four main ways that plant organisms release these biochemicals: leaching, root exudation, residue decomposition, and lastly, the one we will be talking about today, volatilization. Plants aren't just releasing these volatiles for no reason though. They are doing it as a defense mechanism. Rice (1977) helps explain that plants release these chemicals not only to protect themselves from disease but to also create a larger and safer space for themselves by reducing the competition of neighboring plants. So, if allelopathy has both beneficial and detrimental effects, how can we use it to our benefit?

That is what we'll be testing and researching today. Through six different bioassay trials, we conducted an experiment that measured the germination and root length of lettuce seeds when put in an allelopathic environment (Petri dish). To ensure the results of this experiment we chose to test our dependent variable (lettuce seeds) against two different allelopathic plants; 3 dishes with the control group (carrot) and 3 dishes with the experimental group (nutmeg). We chose nutmeg as our experimental group due to past researchers' findings. These show that nutmeg, although a good seed protectant from pests, has high allelochemical content, so too much of it can hurt germination. (Purwanto et al., 2024). And, in a study comparing the allelopathic effects of leaves from cloves and nutmeg, against the germination percentage of red amaranthus; it showed that while cloves had a lower germination % on the red amaranthus, nutmeg also had a very low germination % against the control group (Jose et al., 2020). Showing, again, that

nutmeg has very high allelochemical content and too much will negatively affect germination and growth.

So, now that we know this information, what can we do with it? We can not only question it but create our own questions to test. If the nutmeg has higher volatile excretion than the carrots, and the lettuce seeds are affected by these different allelopathic effects, then the lettuce seeds will have less germination and root growth. But that brings us to our big question. Do nutmeg's phytotoxic volatiles have a negative allelopathic effect on the growth and germination of lettuce seeds?

2. Methods

2.1 Materials and Setup

To answer our big question, we first must know what we used to conduct our experiments. This list includes 6 Petri dishes, 60 lettuce seeds (10 in each dish), 3.9 grams of carrot (1.3g in each of 3 dishes), 3.9 grams of nutmeg (1.3g in each of 3 dishes), 12 filter papers (2 in each dish), a couple of drops of water (to dampen each filter paper), 6 aluminum "boats" (3 for carrots, 3 for nutmeg), and lastly 6 strips of parafilm (to secure the petri dish). With these materials, we conducted three replicates for the experimental group (nutmeg), and three replicates for the control group (carrot). For each of these replicates, we began by taking one petri dish (control variable) and placing 2 pieces of filter paper (control variable) at the bottom of it. We then dampened the paper, set either 1.3g of nutmeg or carrot (depending on whether it was the control (carrot) or experimental group (nutmeg); both independent variables) in an aluminum boat (control variable), and placed that boat in the center of the same petri dish. Lastly, we placed 10 lettuce

Image 1



The 6 Petri dishes (3 control top row, 3 experimental bottom row) completely setup before allelopathic effects begin

seeds (dependent variable) around the boat, on the filter paper, and then sealed the petri dish with parafilm (control variable). These steps were completed for each of the 6 Petri dishes, in respect to 3 control groups and 3 experimental groups. See “Image 1” above.

2.2 Pre-Treatment Method

Once all Petri dishes were complete with their respective group, the treatment process began. The damp filter paper works as a growing medium allowing the 10 lettuce seeds in each dish to be affected by the allelopathic compound (whether 1.3g of carrot or 1.3g of nutmeg) in the boat, either increasing or decreasing germination based on the volatiles released from each plant. The carrot dishes act as a control group to determine a baseline of germination on the lettuce seeds to then be compared against our experimental group of nutmeg. After one week of exposure, we will take out the lettuce seeds, measure the total germination amount in each dish, and measure the length of the root in millimeters to determine the allelopathic effects of nutmeg (experimental) vs carrot (control).

3. Results

Through these experiments, we found a multitude of data. First, germination was measured by the total number of seeds that showed any germination/growth. When lettuce seeds were grown with carrots (the control group), 13 of 30 seeds germinated; when grown with nutmeg (the experimental group), 0 of 30 seeds germinated (t-test, p = n/a). See “Figure 1” below for this represented in graph form. Secondly, root length was measured through millimeters from the start of the root to the end of it (only germinated seeds were measured). When lettuce seeds were grown with carrots (the control group), the average mean root length was 2.41mm, with the longest being 9mm and the lowest 0mm. When lettuce seeds were grown with nutmeg (the

experimental group), the average mean root length was 0mm (t-test, p = 0.0128), with the longest being 0mm and the shortest 0mm. See "Figure 2" below for this information represented in a graph. Lastly, "Image 2" shows the same Petri dishes from "Image 1" after the germination process has been completed (one week later). Confirming the data represented in "Figure 1" and "Figure 2."

Figure 2

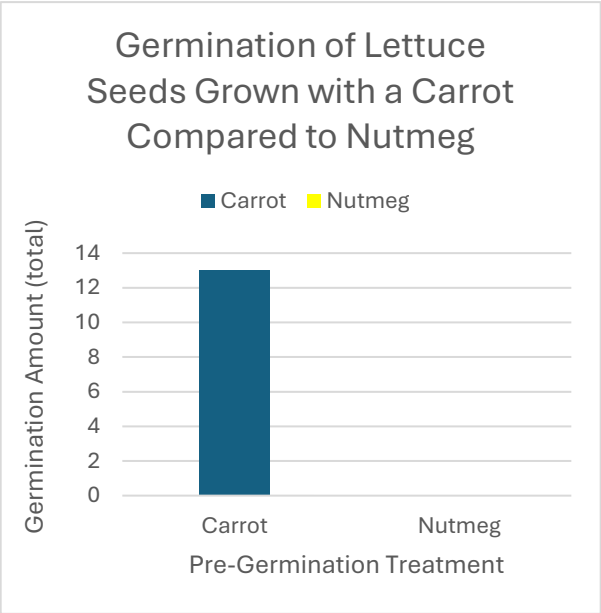


Figure 1

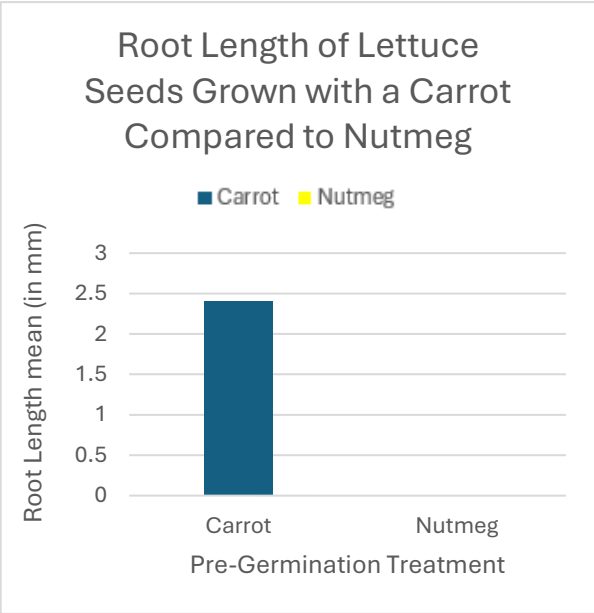
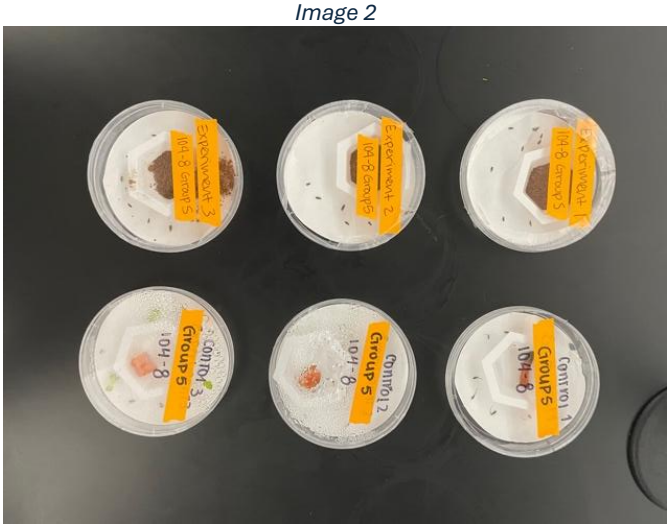


Image 2



The 6 Petri dishes (3 experimental top row, 3 control bottom row) a week after allelopathic effects occurred

4. Discussion

4.1 Data Interpretation

Our results show that both germination and root length have a probability less than 0.05 ($p \leq 0.05$), which rejects our null hypothesis of nutmeg's volatiles having no allelopathic effect on lettuce seeds. It also reinforces our hypothesis, that there is a statistically significant difference in the volatiles excreted from nutmeg causing negative allelopathic effects, such as less germination and root length. So, nutmeg is an allelopathic plant that releases volatiles that are toxic to other plants. Both statements can be reinforced by looking at the "Figures" and "Images" above. "Image 1" represents the experiment before the allelopathic effects occurred, and "Image 2" shows how the volatiles from each plant affected the lettuce seeds. Looking closely at the control group, you can see stem growth and germination. However, looking at the experimental group, you can tell that no germination occurred on the lettuce seeds. Thus, proving our hypothesis that the volatiles from nutmeg cause negative allelopathic effects and inhibit plant growth. "Figure 1" and "Figure 2" also both prove this hypothesis and reject our null hypothesis through the fact that the graph shows significant germination and root growth for the control group (carrot) compared to the experiment group (nutmeg). In the end, not only can you see the difference in allelopathic effects, but the data proves it.

4.2 Claim Support

In a study where nutmeg and citronella oils were used, at different concentration levels, it was found that too high of a concentration of nutmeg directly affects germination due to its high allelochemical content (Purwanto et al., 2024). These results directly back up our claim and reinforce the data we gathered by showing once again that nutmeg is an allelopathic plant that

releases phytotoxic volatiles that inhibit plant growth. These phytotoxic effects can also be seen in a study testing the essential oil, from African nutmeg seeds, effect on the growth of tomatoes, maize, and cowpeas. In this, the nutmeg's oil reduced the shoot and root of all three dependent variables (Awojide et al., 2023). Reinforcing that nutmeg has negative germination effects.

4.3 Claim Refutation

The results show again and again that nutmeg is an allelopathic plant with phytotoxin volatiles harmful to other plants. However, some confounding variables and circumstances could've hindered different results. These are the amount of sunlight that each dish received, the seed quality of each lettuce seed, the amount of moisture the seeds absorbed from the paper, and even the amount of nutmeg and carrot we used for each dish could've made a difference (lower or higher grams). While we tried to make sure each of these things was controlled; sometimes the paper would take more drops to dampen, you never know the quality of a seed until it sprouts, and as previous research showed, nutmeg can have positive effects when yielded at a lower dose. So, if we had done 1 gram or even less, the possibility of germination increases as the amount of the allelopathic compound decreases.

A study measuring the growth parameters of pepper and nutmeg against sterile and unsterilized soil from a coconut garden showed that nutmeg can grow in unsterile soil with coconut leachate (Gopal & Gupta, 2015). This refutes our claim by proving that plants (coconut leachate in this example) can grow in an environment with nutmeg through the fact that nutmeg was able to grow in this experiment around other plants. Our claim is also refuted by the fact that carrots (our control group) and nutmeg (our experimental group) share a similar compound of myristicin (Ingold, 2020). This refutes our claim by stating that carrots and nutmeg have similar properties,

so them being our control group (carrot) and our experimental group (nutmeg) should have made no difference in the phytotoxin volatiles released and germination effects.

4.4 Conclusion

Allelopathy is the release of bio-chemicals that can help or hurt other organisms. This study showed how nutmeg is an allelopathic plant that releases phytotoxic volatiles that inhibit germination through the 3 replicates of the experimental group (nutmeg) compared to the 3 replicates of the control group (carrot). While some studies show that the concentration of nutmeg can affect the allelopathic effects, our study still proves the fact that nutmeg is an allelopathic plant. “The Spice Showdown” can only have one winner. And nutmeg never even let lettuce get started.

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