

## The effects of alcohol toxicity on seed germination

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### ABSTRACT

This experiment discovers the effects of three different alcohols at three different concentrations (methanol, ethanol, and isopropanol/ 0.6%, 1.2%, and 2.4%, respectively) on seed germination rates. Studies have shown that these three alcohols have been effective in-home gardening to fight off pests and increase germination rates. To find greener alternatives to commercial pesticides and insecticides that most farmers use, the idea of using small amounts of these alcohols comes to mind. This experiment found that ethanol had the best effect on germination rates, which was as to be expected because it is the most commonly found alcohol in nature as a byproduct of fermentation. However, methanol had a surprising positive effect on seed germination, meaning that further research on the effects of methanol on seeds needs to be done. Isopropanol was lacking in all concentrations of seed germination, which means that the environment isopropanol created was too toxic for the germination process.

Keywords: *Germination, toxicity, seed germination, methanol, ethanol, isopropanol, germination rates.*

### INTRODUCTION

Agriculture is a very important part of our lives. Pesticides are increasingly being applied to crop fields in order to control bugs and animals that try to eat these crops. These applications can have many negative effects on the environment and human health, such as headaches, nausea, cancer, reproductive harm, and endocrine disruption (Polyxeni Nicolopoulou-Stamati, Et. al 2016). Because of this, farmers and gardeners alike are searching for greener alternatives in the form of natural pesticides like isopropyl alcohol.

Alcohols such as methanol, ethanol, and isopropanol are used in the field of agriculture as a "greener" alternative to pesticides. (DG Davis et al, 1978) Pesticides and nutrients are sometimes put on the plants at the same time, causing harmful water runoffs and potential sites for eutrophication. Although most nutrients are easier to break down over time, pesticides are harder to, causing a lot of problems for local wildlife of any kind. Methanol, ethanol, and isopropanol are either one, two, or three chained carbon chains with an oxygen attached, respectively. Their composition is relatively simple, and easier to break down because they are miscible in water (Gbashi et al. 2016).

Methanol, ethanol, and isopropanol are in many different household and commercial items, but one thing that ties them to research in agricultural laboratories is the fact that they are all used in pesticides and herbicides such as: Safer Brand Fast Acting Weed and Grass Killer, 5055, Pump Spray-Old Product (methanol), Forbid 4F Ornamental Insecticide Miticide (ethanol), and Casoron 4G Herbicide (isopropanol). These herbicides and pesticides are used in a lot of farms and helps procure masses of produce over the seasons, containing residues of these things that we then put in our bodies.

Farmers and home growers alike understand the sometimes-irritating task of seed germination. In regular conditions (water, sunlight, dirt) seed germination rates differ amongst different types of plants. To make a seed germinate, farmers and home growers alike swear by the rule of soaking seeds in a shallow dish for at least twenty-four hours. This helps water penetrate the sheath of the seed (Bewley 1997) to help it maintain some of it before it gets put in the ground. In a lot of cases though, using a growth medium for germination, such as rockwool grow cubes or anything similar, helps maintain what a seed needs to survive.

Research shows that methanol and ethanol in different concentrations had slowed down the germination process (Idu M. Et. al 2006), yet still had germination. It is unclear whether these alcohols have long term effects on future germination rates, but doing research about germination rates under alcoholic conditions have the potential to help farmers and those who like to garden at home understand how these common compounds affect their crop yield and overall production value.

Ethanol usually has the title of "the ripener" in home gardening. Under anaerobic conditions, ethanol has proved to decrease ADH (Alcohol Dehydrogenase) activity. Usually, under anaerobic conditions without ethanol, ADH levels increase. (Maricle et. Al 2014) ADH is plays a very important part in fermentation, making an acetaldehyde, which gets reduced into ethanol by ADH1. This step is important because it makes sure that there is a constant supply of NAD<sup>+</sup>. NAD<sup>+</sup> can be converted to ATP through the electron transport chain and is very critical to photosynthesis. Ethanol has a toxic effect on germination if the plant is not tolerant to flooding (Maricle et. Al 2014). Ethanol and methanol have also shown the potential to start a

stress response from plants, making them go into a defense mode, but when re-harvested and re-germinated, the seeds could not turn on their defense genes- PR-1 and PI-1 (Hann et. Al 2014). This is interesting because if the plants cannot go into defense mode, they will not be able to survive, making the study of the effects of alcohols on plant germination essential to the future of agricultural science. To better understand these studies, a question of testing these alcohols and their effects of their toxicity needs to be studied so that their effects can be understood. This provides the potential for future agriculture practices to become more green and sustainable.

**MATERIALS AND METHODS**

This experiment as a whole is how lettuce, radish, and watermelon seeds germinate under different concentrations of methanol, ethanol, and isopropanol mixed with water.

To begin, three different seed types need to be purchased. These seed types were picked because they are inexpensive, easily found, different species, and germinate at a fast rate. Once those are acquired, three separate petri dishes were made per seed per alcohol concentration along with three control groups that have only water as a baseline. This will end up being about 84 petri dishes, which includes some extras. This will require about 40.7 g of nutrient agar, distilled water, a hot plate, and plastic petri dishes. Each petri dish will have about 6 seeds of either lettuce, radish, or melon. For the alcohol concentrations, methanol, ethanol, and isopropanol were used. They were combined with water to create a diluted mix. The following concentrations per each alcohol type were made: 0.6%, 1.2% and 2.4%.

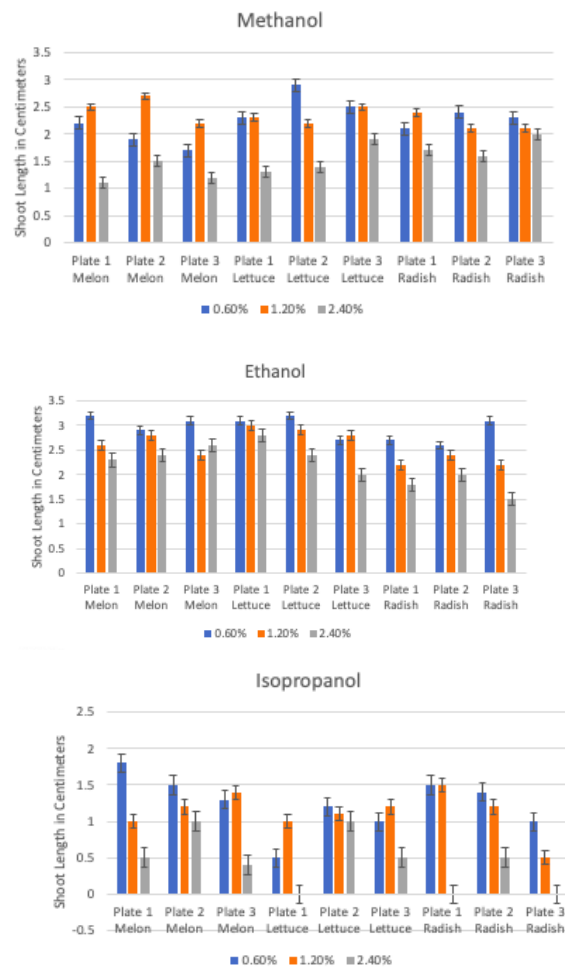
The following were needed for the experiment: petri dishes with agar, seeds, and then the different concentrations in graduated cylinders that can go up to 20 mL. Once the amounts of the diluted mixes are in the petri dish with the seeds, they were placed in the window so that they get eastern rising light. These will germinate at similar rates because they will be in ideal conditions. The petri dishes should be kept in a room temperature environment, which is an optimum temperature to germinate seeds. To keep things equal they were checked at the five-day mark. To measure their germination rates, a ruler was used to get the length of the root and make sure to only measure the root length, not the shoot or the seed itself. Seeds that have broken roots, did not germinate, or dried out will not be part of the study. This will be done twice to get the most amount of data for my experiment.

Once the experiment was complete, the measured data that was included in data collection included: seed root length individually, and the time that it took to germinate. Broken, un-germinated, or dried out

seeds will not be included because unless the entire group is un-germinated, there is no reason to include that in the data. In every situation, there should be some type of germination because of the small amount of alcohol concentration that is included.

**RESULTS**

By referring to images one, two, and three, it can be inferred that the seeds with the most consistent germination rates are the seeds under ethanol concentration. This is probably because ethanol is found more often naturally than either methanol or isopropanol. The isopropanol graph shows the lack of germination across all seed types and concentrations. This is probably due to the fact that isopropanol has proved to be more toxic to plants than methanol and ethanol. The methanol graphs have a decent amount of germination rates, and that is due to the fact that plants can show the use of methanol and carbon dioxide as energy sources (Aktar et. Al. 2009).



An ANOVA was also ran to decipher is seed

germination was statistically significant for each seed type. For the lettuce ANOVA alcohol x concentration value was .169 which is not statistically significant. This means that the alcohol and the different concentrations did not have an effect on the lettuce seeds. For the radish seeds, the ANOVA alcohol x concentration value was  $<.001$ . This was statistically significant. The watermelon alcohol x concentration p value was also  $<.001$ . This is also statistically significant. So, that means that the null hypothesis- that there would be no change in growth- would be rejected for the watermelon and radish seeds. However, for the lettuce seeds, it would be accepted because the change was not statistically significant.

## DISCUSSION

The first time the experiment was ran the seeds did not germinate. This is because there was not enough water in the petri dish. Learning from this, the secondary trials got "spritizes" of water on every other day.

Ethanol is a two-chain carbon atom with an "-OH" group on one of the terminal ends of the molecule. Ethanol is also more commonly known as the alcoholic beverage. The purest form of ethanol in consumer form is 95% proof vodka. This is what was used for the experiment. Quite commonly, home gardeners use vodka as a pesticide, but this research suggests that it also could be used to increase germination yield.

Surprisingly, methanol proved to also help with seed germination. Although it was not as successful as methanol, all of the concentrations of methanol had a successful amount of yield to be significant. This is probably due to the simplicity of the chemical makeup and the plant utilizing it as an energy source in place of carbon dioxide.

Isopropanol had little to no germination, meaning that it did not aide the germination process. This is probably due to the fact that isopropanol is more toxic to plants.

To redesign this experiment, germinating the seeds in a soil environment, rather than on a nutrient agar dish would seem more beneficial. Another redesign would be to add more seeds at different germination rates and soak them in the water/alcohol mix to see if the alcohols really helped the germination.

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This is for you grandpa.

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