Testing if Liquid Ashwagandha Supplement Will Increase the Rate of Growth for Cilantro

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Abstract

Ashwagandha is a plant supplement trending in recent years due to its immense health benefits for humans. Ashwagandha also has many proposed allelopathic properties that impact the growth of its surrounding plants and environment. This experiment tested ashwagandha's allelopathic properties by directly adding ashwagandha extract to cilantro seeds upon planting to observe its effect on plant height. The goal of the experiment was to determine if a liquid ashwagandha supplement impacted the rate of growth of cilantro. There were eight groups, totaling 320 samples of cilantro. Group one was the control group, while the other seven groups received treatments of fertilizer and ashwagandha in various combinations and amounts. Plants were grown under growth lights for four weeks to ensure consistency in light and temperature. All groups received the same amount of water and had their height recorded weekly for (n = 40) per group. The two-way ANOVA test resulted in p-values of 0.0001 for 0.25 mL, 0.50 mL, and 1.00 mL of ashwagandha; fertilizer; and 0.25 mL, 0.50 mL, and 1.00 mL of ashwagandha and fertilizer together. These significant results indicated that the various treatments reduced the plant growth rate. Cilantro plants that received 0.25 mL ashwagandha treatment had a mean height in cm (1.7 +/- 0.59) that was significantly lower (p = < .0001) than the control plants (5.27 +/- 0.78). The mean height of the control plants (5.27 +/- 0.78) was significantly higher (p =<.0001) than the mean height of the plants that received fertilizer and 0.25 mL of ashwagandha (0.47 +/- 0.27). Many samples that received ashwagandha supplements did not make it past germination, which aligned with previous studies on other plants. The results of this experiment could be generalized to other plants due to past studies having similar results. Additional experiments could be done focusing on the effect of fertilizer on cilantro due to the unexpected reduction of germination and growth in some groups treated with fertilizer.

Introduction

Withania somnifera, commonly known as ashwagandha or winter cherry, has been rising in popularity recently due to its immense popularity and physical effects. Ashwagandha has been trending recently in the fitness community on social media due to the benefits it can have on one's body. However, the supplement may have been excessively overhyped due to the misinformation spread on the internet and social media. Ashwagandha has been around for generations, dating back to 6000 BC in ancient India. The typical environmental conditions in which ashwagandha grows are between 68°F and 95°F in a sunny location. Ashwagandha was commonly used in the traditional medicine of India for healing. Ashwagandha is very calorie-dense and composed entirely of carbohydrates (Mikulska et al., 2023). The main compounds present in ashwagandha include Withaferin A, Withanolide A, and Withanoside IV (Mikulska et al., 2023). Ashwagandha is an adaptogen and anti-stress agent that provides many benefits to humans, including anti-inflammatory, antimicrobial, cardioprotective, and antidiabetic properties (Mikulska et al., 2023). With these effects, ashwagandha can reduce stress and relax muscles in the body to allow an increase in sleep (Mikulska et al., 2023). Bonilla et al. found that ashwagandha supplements had greater beneficial effects on physical performance compared to the placebo that was used in Bonilla et al.'s study in 2021. Ashwagandha is commonly used in Asian households, and ashwagandha is also sometimes used as a supplement to ease symptoms associated with diabetes and anxiety. Thus, ashwagandha supplements have been widely used as natural remedies for people with slight illnesses.

Ashwagandha is mainly known for its effects on humans, but ashwagandha also seems to have many effects when used on nearby plants. This is because human and plant cells are both eukaryotic and have very similar characteristics. There have been very few studies on ashwagandha's effects on plants, so the information on this topic is quite scarce, but in recent years, there has been a greater emphasis on studying all the functions of ashwagandha. Ashwagandha has been known to have allelopathic effects on plants. Allelopathy is a biological process where organisms release chemicals that affect surrounding organisms. These effects can either be positive or negative. The effects of ashwagandha have been studied on only two plants that have been tested: chickpea plants and wheat plants. The effect of ashwagandha on these two plants reduced growth and germination (Chandra et al., 2012). Then, leaf extracts of ashwagandha were used on wheat seedlings; this also led to a reduction in seedling germination and seedling growth (Mandal et al., 2018). These results are quite strange because, in nature, when ashwagandha is grown near other plants, the ashwagandha plant allows increased growth. This is because ashwagandha is in the Solanaceae plant family, which is a family that is known to have positive allelopathic effects on seed germination and seedling growth (Mushtaq & Siddiqui, 2018).

The motivations for this experiment are to understand ashwagandha's allelopathic properties by dropping a liquid organic ashwagandha root extract onto cilantro seeds, which are also known as coriander seeds. Ashwagandha's properties are not easily predictable because they are known to be allelopathic, but the variability of whether it will increase or decrease growth and germination is quite unpredictable. The effect of the ashwagandha supplement might depend on the type of plant used. Due to only two plant types, chickpeas and wheat, being used as samples in previous studies, it is very difficult to extend the results to other plants. So if cilantro, which originates from southern Europe and Asia, is used, the results might be able to be broadened to other plants.

The cilantro plant is often used as a food ingredient due to its fresh fragrance that attracts people. Cilantro is commonly used in Asian households for cooking purposes. Cilantro is low in calories and is made up quite evenly of carbohydrates, protein, and lipids (Mahleyuddin, 2021). The reason cilantro has been chosen for this study is because of the rapid germination and growth, which allows for convenience. This will show the short-term effects of liquid ashwagandha used on cilantro. A unique sample was created for experimentation on ashwagandha's allelopathy because the past two sample plants were chickpeas and wheat, which take a long time to germinate and grow. Cilantro has also been used for medicinal purposes, so it shares one similarity with ashwagandha's characteristics, unlike chickpeas and wheat. Cilantro has many similarities to ashwagandha's medicinal properties, including its anti-inflammatory, anti-diabetic, neuroprotective, and analgesic properties (Mahleyuddin, 2021). There have also been correlations between eating cilantro and increasing sleep quantity, which is similar to ashwagandha, but there has not been enough research on this effect. In conclusion, cilantro will possibly be better as a plant choice compared to chickpeas and wheat because it shares characteristics with ashwagandha.

Thus, the research question at hand is whether a liquid ashwagandha supplement will increase the rate of growth for cilantro. The alternative hypothesis is yes, liquid ashwagandha supplements will increase the rate of growth for cilantro due to the unique allelopathic properties of ashwagandha. While the null hypothesis is no, liquid ashwagandha supplements will not increase the rate of growth for cilantro. The goal of the experiment is to see the capabilities of ashwagandha's allelopathic behaviors. If ashwagandha can increase or decrease the rate of growth of cilantro, then we will have obtained a greater amount of knowledge about this ancient Indian plant, ashwagandha.

Methods and Materials

The materials for this experiment included water, organic liquid ashwagandha extract, organic neutral pH soil or potting and container soil, cilantro seeds, growth lights, a table, containers for the plants, and a ruler for measurement. All of these materials were ordered online through Amazon. The liquid ashwagandha served as the treatment. There were eight groups in this study. Group one received water. Group two received water and fertilizer. Group three received water and 0.25 mL of ashwagandha. Group four received water and 0.50 mL of ashwagandha. Group five received water and 1.00 mL of ashwagandha. Group six received water, fertilizer, and 0.25 mL of ashwagandha. Group seven received water, fertilizer, and 0.50 mL of ashwagandha. Group eight received water, fertilizer, and 1.00 mL of ashwagandha. Groups one and two acted as the control groups for this experiment because they did not receive the treatment.

The constants that all groups received included soil, an 80°F temperature maintained by a heater, growth lights, the container, and samples of 40 cilantro seeds. The container used in this experiment was a complete growing system for seedlings that provided an ideal environment for quick germination and vigorous root growth. The container included a greenhouse cover, an insulated growing tray, capillary matting, a two-quart water reservoir, and space for 40 seeds. This container was also considered a constant. Each group was placed in a separate container to prevent any errors. In total, this experiment had 320 samples of cilantro seeds. This sample size was justifiable because the cilantro seeds did not vary much.

The independent variable was the amount of liquid ashwagandha given. The dependent variables were the cilantro plant's height, number of leaves, and stem diameter. The experiment's conclusion depended on comparing cilantro with the ashwagandha supplement to cilantro without the ashwagandha supplement. The main statistical test used in this study was a two-way analysis of variance test, completed using JMP, as the means and data of multiple groups were compared.

The following steps were followed to experiment: First, the container was filled with soil. Then, the potting soil was compressed down by an inch to create a bed for the seeds. Next, the seeds were spaced in the container. After placing the seeds, they were covered with half an inch of soil. Then, 25 mL of water was added to each seed. Watering for the cilantro seeds occurred roughly every two days; however, if the soil appeared dry, 25 mL of water was added to all samples. The ashwagandha was added directly to the soil along with the water for the experimental groups that received the treatment. The groups receiving fertilizer were given ¼ cup of fertilizer. Fertilizer was provided every six weeks, as cilantro is an herb, and herbs do not require much fertilizer. Growth lights were placed at an angle above the plants to ensure all plants received light. The heater was positioned in a way that did not favor any specific group. The groups were labeled to prevent mixing up the treatments and variables. Each seed was labeled if necessary for the organization. The experiment was conducted in the upstairs guest room of the researcher's home. To ensure consistent heat distribution, window curtains and doors were closed to trap the heat.

This experiment involved very little risk to the experimenter; however, there were potential risks for the samples. When growing plants indoors, pests such as spider mites and aphids pose a threat by interfering with the growth of the cilantro. While this could be prevented, pests often returned. If this occurred, restarting the experiment was deemed the best course of action. Another possible confounding variable was contamination or infection of the plants. To mitigate this risk, gloves were worn when handling the plants.

Results

A two-way ANOVA test was run on the heights of the cilantro plants of each of the eight groups. The two-way ANOVA showed significant values, with a p-value below 5%. A Tukey HSD test was performed because the two-way ANOVA test showed significance. Each group contained samples of 40 cilantro plants, totaling 320 samples of cilantro. The groups treated with 1 mL of ashwagandha, fertilizer only, and ashwagandha plus fertilizer each resulted in a p-value less than 0.0001. Thus, the null hypothesis was rejected and the alternative hypothesis was accepted. There was a significant difference between the groups of ashwagandha, a significant difference between the groups of fertilizer, and ashwagandha influenced the effects of fertilizer.

Figure 1 shows the mean height for each cilantro group across four weeks of growth. None of the groups experienced growth in the first week. Only the control group experienced growth in the second week and showed the most growth for each additional week. The groups receiving treatments experienced less height growth than the control group. Cilantro plants that received 0.25 mL ashwagandha treatment had a mean height in cm (1.7 +/- 0.59) that was significantly lower ($p = <.00001$) than the control plants (5.27 +/- 0.78). The fertilizer and 0.50 mL ashwagandha-treated group did not experience any growth throughout the four weeks, resulting in a mean height (0 +/- 0). Cilantro plants that received 0.50 mL of ashwagandha treatment had a mean height (2.89 +/- 0.66) that was significantly higher ($p = <0.0001$) than the height of the group that received 0.25 mL of ashwagandha (1.7 +/- 0.59). Cilantro plants that received 1.0 mL of ashwagandha had a mean height (0.41 +/- 0.29), which was not significantly different ($p = 0.20$) from the mean height (0.47 +/- 0.27) of the group that received fertilizer and 0.25 mL of ashwagandha. The mean height of the control plants (5.27 +/- 0.78) was significantly higher ($p = <0.0001$) than the mean height of the plants that received fertilizer and 0.25 mL of ashwagandha (0.47 +/- 0.27). The control group was significantly higher ($p = <0.0001$) than all the other groups.

Figure 1

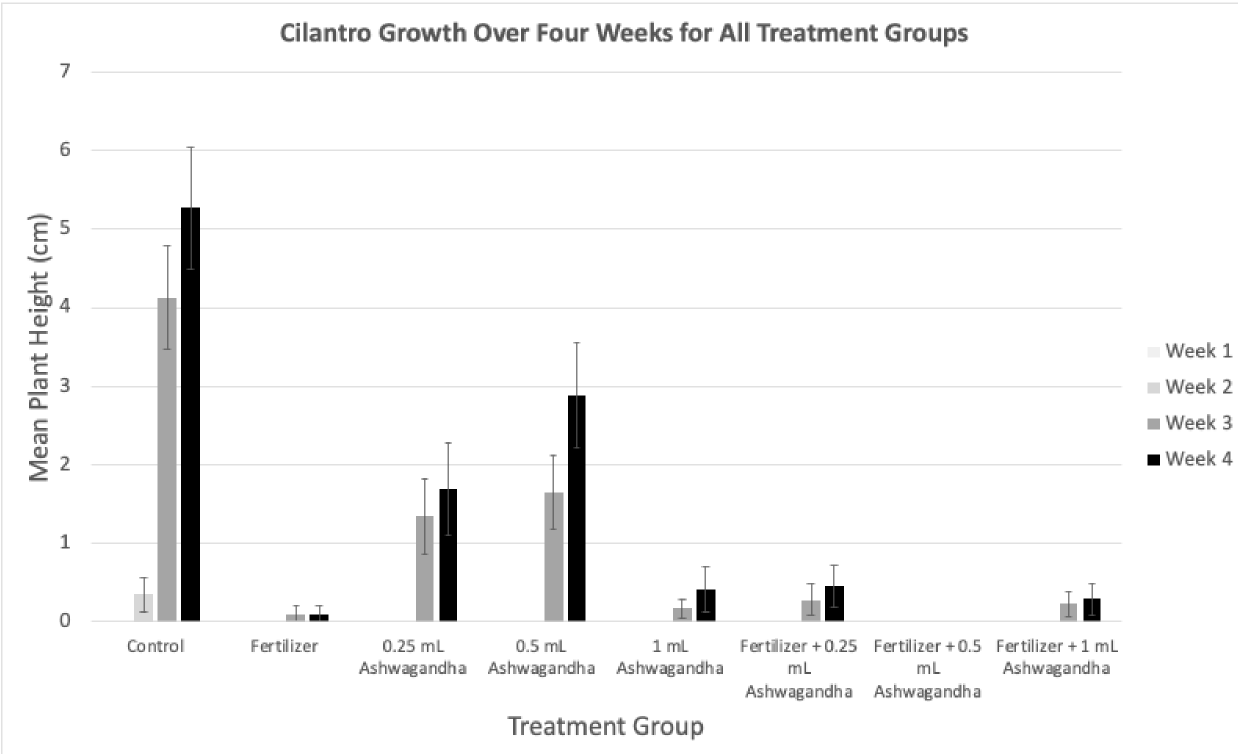

Figure 1. This graph shows the mean height of the cilantro groups for every week for every group. The x-axis represents the groups. The y-axis represents the mean height in centimeters of the cilantro plants (n = 40 per group). The different shaded bars represent weeks 1-4. The error bars represent the standard error of each mean.

Potential errors in this experiment include differences in proximity to light. The fertilizer group, 0.50 mL ashwagandha group, fertilizer and 0.25 mL ashwagandha group, and fertilizer and 1 mL ashwagandha group were closer than the control group, 0.25 mL ashwagandha group, 1 mL ashwagandha, and fertilizer and 0.50 mL ashwagandha. Some plants may have received more water than others unintentionally, even though water was measured for each sample.

Discussion

The null hypothesis of this experiment was $H_o = \mu_{Group\ 1} = \mu_{Group\ 2} = \mu_{Group\ 3} = \mu_{Group\ 4} = \mu_{Group\ 5} = \mu_{Group\ 6} = \mu_{Group\ 7} = \mu_{Group\ 8}$. μ was the mean height of the cilantro plants. The alternative hypothesis was that any μ value would not equal the other μ values. The p-values for ashwagandha (mL), fertilizer, and ashwagandha and fertilizer were all below 0.0001. When the ANOVA was conducted, the null hypothesis was rejected and the alternative hypothesis was accepted.

Ashwagandha has been a trending supplement for humans, but there have not been many studies on its allelopathic properties. This study was designed to see if ashwagandha could increase or decrease the growth of cilantro if ashwagandha were directly added to seeds upon planting. Based on height measurements, the groups that received any amount of ashwagandha grew less than the control group. The control group only received water with no treatments. Many cilantro seeds did not make it past germination when they were given ashwagandha and/or fertilizer. This phenomenon also occurred in chickpea and wheat plants (Chandra, 2012). The increased concentrations of ashwagandha in chickpea and wheat plants led to a reduction in germination and plant growth. Wheat plants experienced a greater reduction in germination and plant growth than chickpea plants. As mentioned before, this was because of ashwagandha's allelopathic properties that impacted plants. The direct cause of the reduction of growth and germination was unknown, but the best answer would be due to ashwagandha's alkaloid and withanolide contents (Chandra, 2012). This study and Chandra et. al's study both used different concentrations of ashwagandha and different groups. This study and Chandra et. al's study also resulted in reductions in germination and plant growth when ashwagandha was applied to the treatment groups. Ashwagandha has been shown to impact wheat seedlings' sugar, protein, and amino acid content (Mandal, 2018). This could be another reason why wheat and other plants experience reductions in growth when ashwagandha is applied to them. Mandal et. al's study was similarly set up to this study and Chandra et. al's study. All three studies used groups that received different concentrations of leaf extracts of ashwagandha to understand how concentrations influence growth and germination. Due to all three studies using three different plants (cilantro, wheat, and chickpea) in total and with a similar format, these results can most likely be generalized to other plants. This experiment used cilantro because cilantro originates in a similar area of Asia to where ashwagandha comes from. Despite this, the cilantro seeds were prevented from germination and growth compared to the control plants.

Group one, the control group, was expected to yield the highest mean growth because it was the ideal way to grow cilantro. Then, group two barely grew, which was unexpected; group two received only fertilizer. Moving forward, groups three and four grew significantly more than the other experimental groups, with group four growing more than group three. Group three received water and 0.25 mL of ashwagandha. Group four received water and 0.50 mL of ashwagandha. This could mean that a balanced combination of ashwagandha and other variables can allow more growth than an unbalanced combination of ashwagandha. In turn, all the samples in group seven did not make it past germination. Group seven received water, fertilizer, and 0.50 mL of ashwagandha. Fertilizers may have played a role in reduced germination, which was unexpected. Fertilizer did decrease the rate of growth according to the two-way ANOVA test. Ashwagandha also decreased the rate of growth according to the two-way ANOVA test. Fertilizer and ashwagandha together decreased the rate of growth according to the two-way ANOVA test. So, ashwagandha as a supplement did not increase growth when compared to traditional growing methods for cilantro.

This experiment could have been improved by making sure light was equally distributed, possibly even giving each group their own individual growth light. If this experiment were to be repeated, it would probably be beneficial to use natural light rather than growth lights. This will allow optimal results, as natural light would be better for plants than growth light due to the increase in chlorophyll content and photosynthetic rate (Jung, 2023). Natural light could potentially create different results.

Further research could be done to investigate if an ashwagandha dilution treatment would impact the growth of samples. In this experiment, ashwagandha and water were added separately and not in a dilution. A dilution could create a balance between water and ashwagandha. If this were to occur, it would be best to empty the ashwagandha concentration into water and then mix the two. The amount of ashwagandha concentration added to the water depends on the researcher. Afterward, apply the treatment to the samples. In this experiment, ashwagandha was treated as a fertilizer. Because of this, fertilizer was added only once, which was at the beginning of the study, and did not need to be added again because fertilizer would be added every four weeks. A dilution of ashwagandha could potentially create different results. If this experiment were to be repeated, a different plant sample should be used so it can be compared to previous plants that have been used; this can allow for generalization.

Citations Peer-Reviewed

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