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The effect of Caffeine on the rhizogenesis of Asparagus seeds.

Abstract

This experiment investigated caffeine's effect on asparagus root development and the absorption of minerals. Previous studies have tested different concentrations of caffeine in a nutrient medium; thus, obtaining data on caffeine's effect under optimal nutrient conditions becomes necessary. The influence of caffeine on plant yield and seed germination rates changes depending on different concentrations. To solidify the effects of different concentrations, this experiment has been conducted using concentrations of decaffeinated and caffeinated green tea. Given the prolonged germination period of asparagus seedlings, a pre-planting soaking procedure in water was done for 24 hours to enhance germination. After soaking the seedlings, they were placed in rockwool to germinate. Data was collected weekly on the root growth, and the fresh weight was collected after the experiment. By week 4, the root growth of 1% decaffeinated green tea exceeded threefold of what was observed in the control group. After week 4, all groups excluding 1% decaffeinated exhibited less root development than the control group. Concentrations of 2% or concentrations that contain caffeine restrict root growth and fresh weight. The fresh weight of seedlings that received caffeinated treatments is half that of seedlings treated with 1% decaffeinated solution. Notably, the sprouting ratio was most influenced by green tea concentrations rather than caffeine content, with 2% decaffeinated and caffeinated concentrations exhibiting the greatest sprouting ratio. With high root length and full sprouting ratio, asparagus seedlings treated with 1% decaffeinated green tea indicated the inefficiency of caffeine on the germination rate of asparagus seedlings.

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Introduction

This investigation aims to examine the impact of green tea, both with and without caffeine, on the growth of asparagus. It is postulated that the application of green tea, with and without caffeine, would increase asparagus seedlings' root development due to the beneficial factors related to green tea and caffeine. This experiment is done primarily to clarify caffeine's role on plants with nutrients. In the course of this experiment, solutions of green tea, both with and without caffeine, will be examined using a variety of concentrations. Despite the testing of green tea solutions on plants in previous studies, the distinctive focus of this experiment lies in examining the influence of caffeine on the absorption of minerals facilitated by green tea, as opposed to solely investigating the benefits of green tea on plant development.

Caffeine's effects have been tested on a variety of different plants. Caffeine may possibly stimulate salicylic acid production which results in pathogen-related genes being triggered (Koo, 2020). Pathogen-related genes in plants is a response built to protect plants against damage, specifically biotic and abiotic factors (Kim, 2008). Therefore, caffeine is conceived to help plants respond to pathogens (Kim, 2008). There have also been experiments of caffeine's effects on the rhizogenesis of a plant called rubus genus (Muratova, 2020). This experiment focused on the root's changes such as root frequency and the number of roots. There are several experiments testing caffeine's effects on a variety of plants at different concentrations. However, our experiment is distinctive to other experiments as it focuses on caffeine's effect on absorption of minerals that green tea may provide. Salicylic acid is also known to improve seed germination, thermogenesis, damaged DNA repair, and

increasing fruit yield (Koo, 2020). On account of this, caffeine was chosen to be tested with green tea.

Asparagus, a perennial herb, can grow back every spring, unlike annual plants, which only live for one season before dying off (Guo, 2019). Asparagus is a medical plant that contains a variety of biological properties, such as immunostimulants, anti-inflammatory, anti-hepatotoxic, anti-bacterial, anti-oxytocic, and reproductive agents (Sharma, D. 2021). Compared to normal vegetables, asparagus plants have five times the amount of protein, fats, vitamins, and minerals (Guo, 2019). Asparagus also contains bioactive components like polyphenols, saponins, dietary fibers, and anthocyanins (Guo, 2019). Polyphenols can protect the body's tissues from oxidative stress and cancers like coronary heart disease, and saponins can decrease the risk of cancer (Guo, 2019). It also contains flavonoids and other phenolic compounds, which possess strong antioxidant properties that are essential in providing vitamins and nutrients to humans or animals. (Nindo, 2003). In addition, asparagus plants have high repetitive DNA sequences and low gene density (Dufalt R., 1983). They also contain bioactive, healthy components like polyphenols, dietary fibers, and anthocyanin (Guo, 2019).

Green tea is widely used in various foods and beverages due to its health benefits (Perumalla, 2011). The properties of green tea can fend off microorganisms such as bacteria and mold or stop them from growing and becoming a major problem. With the increased demand for medicinal plants, several conversational groups are recommending the cultivation of wild medicinal plants. Green tea is one of them, as it's one of the most ancient and therapeutic beverages consumed around the world (Namita, 2012). Many of green tea's benefits are due to its antioxidant, antimicrobial, anticarcinogenic, and anti-inflammatory

properties (Perumalla, 2011). Green tea is a better and more natural alternative to chemical or synthetic antimicrobials and antioxidants due to its ability to produce them naturally, most possibly due to the thousands of its bioactive ingredients (Perumalla, 2011). Polyphenols, a compound apparent in green tea, play a large role in its ability to prevent and treat many diseases (Namita, 2012). These properties allow it to combat foodborne pathogens and inhibit lipid oxidation, meaning it has the ability to increase the shelf life of products such as asparagus (Perumalla, 2011). In addition, green tea has a significant impact on the gut microbial of humans by regulating its health (Perez M, 2021).

Caffeine, a compound from a group called methylxanthines, which is produced in almost 100 plant species. Methylxanthines or alkaloids negatively affect unwanted herbivorous insects. Caffeine creates stimulation in the nervous system that benefits the person's productivity, enhances alertness, refines the consumer's awareness, and increases urine output (Nawrot F, 2003). Because caffeine affects the plant by producing salicylic acid, it could increase in asparagus seedlings when they are exposed to caffeine (Koo, 2020). An increase in salicylic acid ultimately results in improved seed germination, thermogenesis, damaged DNA repair, and increasing fruit yield (Koo, 2020).

Methods

Table 1. There are 5 groups with different concentrations of green tea and caffeine.

Groups name	Caffeinated green tea	Decaffeinated green tea	Seeds per group	Amount of Green Tea dissolved in 1L of water (g)
JSC-I-(1-10)	0%	0%	10	0
JSC-II-(1-10)	0%	1%	10	2.73
JSC-III-(1-10)	0%	2%	10	5.45
JSC-IV-(1-10)	1%	0%	10	2.73
JSC-V-(1-10)	2%	0%	10	5.45

Preparation

In accordance with Table 1, each beaker was filled with 1 L of lukewarm water. 2.73g of caffeinated and decaffeinated green tea were mixed with 1 L of lukewarm water to achieve 1% concentration. The preparations of the 2% concentrations were created by mixing 5.45g of decaffeinated and caffeinated green tea with 1 L of water. Concentrations were stored in the fridge for up to a week. To enhance germination, a pre-planting soaking procedure in water was done for 24 hours. Each group was soaked in concentrations before planting according to the group. Seeds were then planted in rockwool and placed in a small tray filled with concentration. The seedlings are separated according to concentration, those being 1%

or 2% decaffeinated green tea or 1% or 2% caffeinated green tea. The seedlings' fresh weight was weighed in grams and the length of roots were measured in centimeters.

Data analysis

Roots were measured and weighed daily, concentrations and photographs were replaced and taken weekly. Rockwool were carefully cut with a pair of scissors to remove the asparagus. After the asparagus was rinsed and dried to remove excess debris the final weight and length of the roots were measured at the end of the experiment in g and cm. The measurements of the weight were then done with a scientific scale to have the precise weight of the seedlings. The length of the seedlings was measured with a transparent ruler. The measurements were then put into ANOVA to get the p-value which shows whether the results were significant or not. The data was then stored using excel into multiple sheets for each group. Standard deviation and the means of each group was calculated using functions from excel. Fresh weight was calculated at the end of the experiment after harvesting.

Results

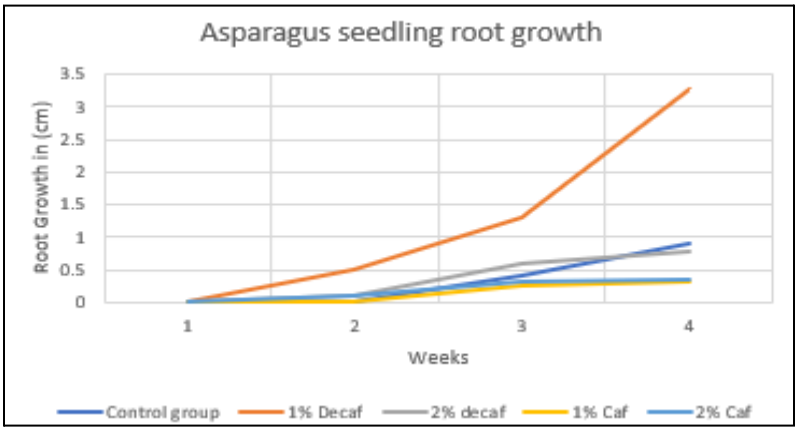

Fig 1. The average root growth for the Asparagus seedling in each group was measured over the course of 4 weeks. 1% decaffeinated green tea had the most significant effect on asparagus seed germination. 1% caf and 2% caf has the least significant effect on asparagus seed germination.

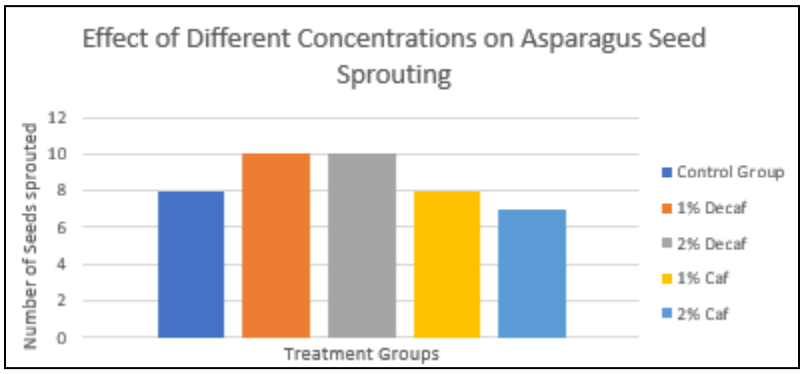

Fig 2. The effect of the concentrations has on the amount of seeds that have sprouted. Each group consisted of a sample size of 10 seeds. The amount of seeds that sprouted were noted and there was a trend that the 1% and 2% Decaffeinated groups had all of their seeds sprouted while the caffeinated and control groups did not.

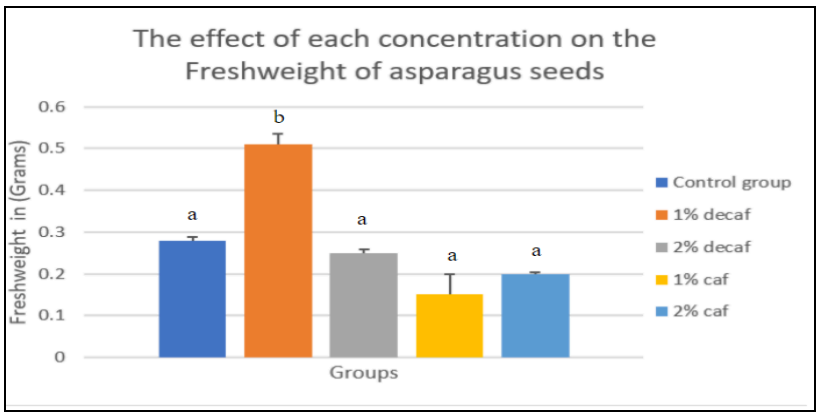

Fig 3. The effect of different concentrations on the fresh weight of asparagus seedlings. There was a sample size of 10 per group and the final freshweight was weighed for each group. After the experiment, the fresh weight of 1% decaffeinated was the highest at almost 0.5 of a g while the lowest was 1% caffeine with 0.15 of a g. The groups are labeled with a and b. If two groups have the same letter, that means they are insignificant with each other but if two groups have a different letter, they are significant to each other. The anova testing shows that the 1% decaf group is significant to every group while every other group is not significant with each other.

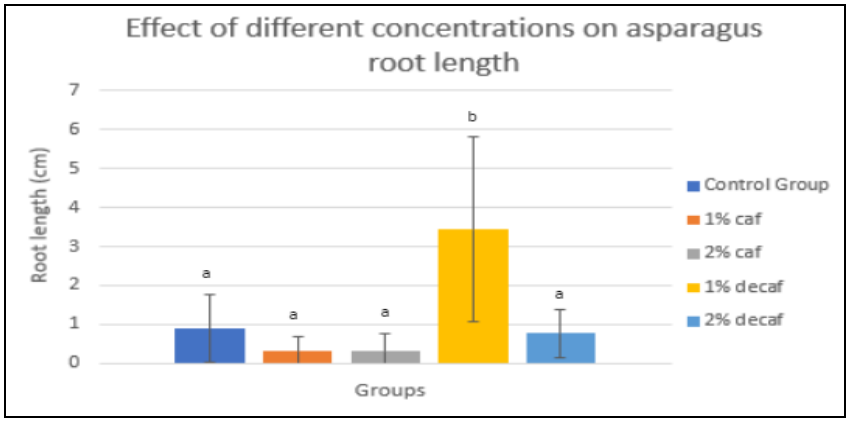

Figure 4. The root growth was measured under each concentration in centimeters. All the information was calculated on TUKEY HSD and ANOVA. Significance, found by using ANOVA, was indicated using letters a and b, different letters representing significance with one another. The information shows that 1% decaf was significant with the other groups, as it has a different letter from them. However, every other group had no significant relationship with each other since they had the same letter.

Discussions

Hypothesis and significance of data

In this experiment, green tea solutions with and without caffeine were tested in different concentrations on the germination of asparagus seedlings. Our initial hypothesis was that caffeine would improve plant growth due to the production of salicylic acid which speeds germination rates and yield (Koo, 2020). Our results are significant as it allows us to conclude that caffeine has an overall negative impact on asparagus seed germination rates.

Issues during the experiment

During this experiment, several challenges were encountered. Prior to planting seedlings into rockwool, a pre-soaking process was implemented for 24 hours. Although seedlings were soaked, they were not soaked in sufficient amounts of water. This caused the germination of the seedlings to become delayed, diminishing the fresh weight and root length in our data. To address this issue, adjustments were made in trial 2 to ensure that seedlings underwent a 24-hour soaking period in an ample supply of water.

Several different seedling species exposed to decaffeinated tea can be used for future research. Future research can also include how the plant absorbs certain nutrients or compounds such as caffeine. After analyzing where caffeine and nutrients are stored within a plant, conceptualizing how caffeine may stunt seed germination may become apparent.

Conclusions

These results allow us to conclude that caffeine has overall negative effects on asparagus seedling's development. Green tea, however, remains beneficial to asparagus seedlings. The group that was treated with 1% decaffeinated green tea is significant compared to the rest of the groups. However, decaffeinated green tea groups, 1% in particular, have the best results in the height and root growth. Conversely, the concentrations of caffeinated green tea demonstrated negative effects. Caffeine doses caused minimal root development, low biomass, and few emerging seeds in asparagus seedlings. Overall, the caffeine in green tea has negative effects, but the decaffeinated kind is beneficial to the seedlings and gives them the essential nutrients they need to sprout into asparagus plants.

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