The Effects of Various Caffeine Concentrations on the Population Curve of *Tetrahymena thermophila*

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ABSTRACT

Due to the lack of literature on the effects of caffeine on humans, we aimed to further characterize its impact through this study. The experiment conducted investigates the impacts of varying caffeine concentrations on the population growth curve of the unicellular eukaryote, Tetrahymena *thermophila.* Through a series of experiments using stock caffeine concentrations of 10^{-2},10^{-3},10^{-4} M, the growth pattern of T. *thermophila* was monitored over 30 hours. We hypothesized that caffeine would influence the population curves of T. *thermophila*, either stimulating or hindering overall growth. Utilizing a hemocytometer for cell counting, growth rates were measured and various time points throughout the study. Results from the study were subjected to statistical analysis, using a one-way ANOVA TEST, yielding an F-statistic of 0.625793 and p-value of 0.60157. These results reveal no significant difference in growth curves among the caffeine concentrations and highlight the comparable variability between the four groups. However, observable trends were noted, including the overall decrease in population density of the organism for the four groups. Our study, like many others, includes several limitations including temperature variations, poor laboratory techniques, and variability in sampling. The statistical results of the experiments were inconclusive, suggesting further research to be carried out to address this study's limitations

INTRODUCTION

Throughout the history of biological and molecular research, the impact of various substances in *vivo has* solidified its place as a reliable method for scientific inquiry. In this study, we explored the effects of caffeine on the microorganism Tetrahymena *thermophila.*

T.*thermophila* is a unicellular eukaryote that belongs to the ciliated Protozoa, a family of diverse organisms that obtain a wide range of complex mechanisms and motility features (Laakso, Löytynoja, & Kaitala, 2003). This organism has been demonstrated to be an excellent model organism for studying the physiological response within eukaryotic cells due to the following characteristics: [1] The organism participates in inefficient nutrient uptake, mainly through phagocytosis and pinocytosis. As a result, this leads to extremely fast growth rates within the laboratory setting reflected by its doubling time of under 2 hours. [2] Inexpensive and simple culture co`nditions including basic storage methods, allow for accessibility by a variety of scientists. [3] T. *thermophila* has well-characterized genetics, physiology, and biochemistry, promoting easily identifiable characteristics for when cell lines are subject to testing. (Orias, Hamilton, & Orias, 1999).

Caffeine, a naturally occurring component in plant leaves, seeds, and other products, is the most commonly consumed compound by humans. This includes 90% of US adults who consume it regularly, constituting its high level of consumption worldwide (Wikoff et al., 2017). Although there is an understanding of caffeine as a stimulant and its effects on physiological performance, there is limited research on the understanding of its genetics in humans (Amponsah, T. O. et. al., 2013). This may be an area of concern, as demonstrated by Kaczanowski & Kiersnowska (2013) coincidentally with T. *thermophila,* caffeine can inhibit the S and G2 checkpoints within the cell cycle during replication. In addition, this compound is

speculated to cause or promote carcinogenesis, through the introduction of mutations and chromosome aberrations to the human genome (Eisen, J.D, 1960).

Motivated by the need to understand caffeine's specific influence on cellular dynamics, we conducted an experiment, testing the effects of various concentrations of caffeine on the unicellular eukaryote, Tetrahymena *thermophila,* hypothesizing that caffeine will affect the population curves of the eukaryote. Extrapolating from previous literature, it is predicted that the T. *thermophila* population curves will behave in one of two ways: [1] minimal concentrations of caffeine act as stimulants, fostering an increase in T. *thermophila* population size. [2] In contrast, we postulate that excessive concentrations of caffeine in growth media will hinder the population growth of T. *thermophila* .

METHODS

Using a hemocytometer-based counting of 10^{-2},10^{-3},10^{-4} M (s) of caffeine-induced T. *thermophila* incubated over 30 hours, we analyzed the trend in population growth of the cells and produced a Fuchs-Rosenthal hemocytometer correlation between the concentration of caffeine and growth curve.

To measure the growth rate of *T. thermophila* the concentration of cells in the stock solution must be determined. The first step in doing so is adding 10μL of an iodine fixative to 100μL of the sample. This immediately kills all *T. thermophila* cells in the sample, simultaneously terminating their growth and stopping their movement to make counting easier. 20μL of the fixed sample is then loaded into a hemocytometer. The chamber was then looked at under an Axiostar microscope under 10X magnification. Due to the sparsity of the cells, the entire 4mm x 4mm grid was counted, signifying a dilution factor of $3.125 * 10^2$. In this

experiment, the initial cell count is $5.9 * 10^3$cells/mL. As this value was below the planned initial concentration of $1 * 10^4$ cells/mL, no dilution was necessary and the experiment proceeded with the provided concentration.

The next step was diluting the caffeine solution to molar concentrations of 10^{-2},10^{-3}, and 10^{-4} using the *T. thermophila* growth medium. Serial dilution was used to maintain precision. The final growth samples contained 15mL of total solution with 10^{-2},10^{-3},10^{-4} and 0 molar concentrations of caffeine and $5.9 * 10^2$cells/mL*T. thermophila*. The growth samples were incubated at 30 degrees Celsius, where 100µL counting samples were taken at 3, 6, 21, 24, 27, and 30 hours of growth. These samples were mixed with 10µL of fixative to end cell growth and immediately refrigerated. To account for errors, three counting samples of each concentration were made, leading to a total of 12 samples. Finally, each counting sample was counted twice using the same method as the *T. thermophila* stock solution to obtain the cell number at each stage of growth.

RESULTS

For each transfer of *T. thermophila*, we followed the sterile protocol described by Orias et al.,(1999), to prevent contamination. At the start of the trial, as soon as the various treatments were established, we gathered data. We conducted a data collection process with two replicates of *tetrahymena thermophilia* with three readings each time to produce a total of six data points for the protozoan in the mentioned caffeine concentrations to assess population density and cell count in the presence of four different compound concentrations. Using a Fuchs-Rosenthal hemocytometer with 1mm grids, cell counts were observed under a compound microscope. The formula utilized for calculating the cell concentration per cubic millimeter (CMM) is as follows

Cells per CMM =(Number of cells counted×10^9) ÷3.2

This formula considers the counting of 16 one-square millimeter areas. We also observed any recognizable change in cell size which we found to be none.

Since the one-way ANOVA requires a normal data distribution, each sample's data was checked to see if it was normally distributed. To gain insights into the reliability of our results, a 95% confidence interval was calculated. The data was further visualized through a scatter plot to identify any discernible trends among the variables.

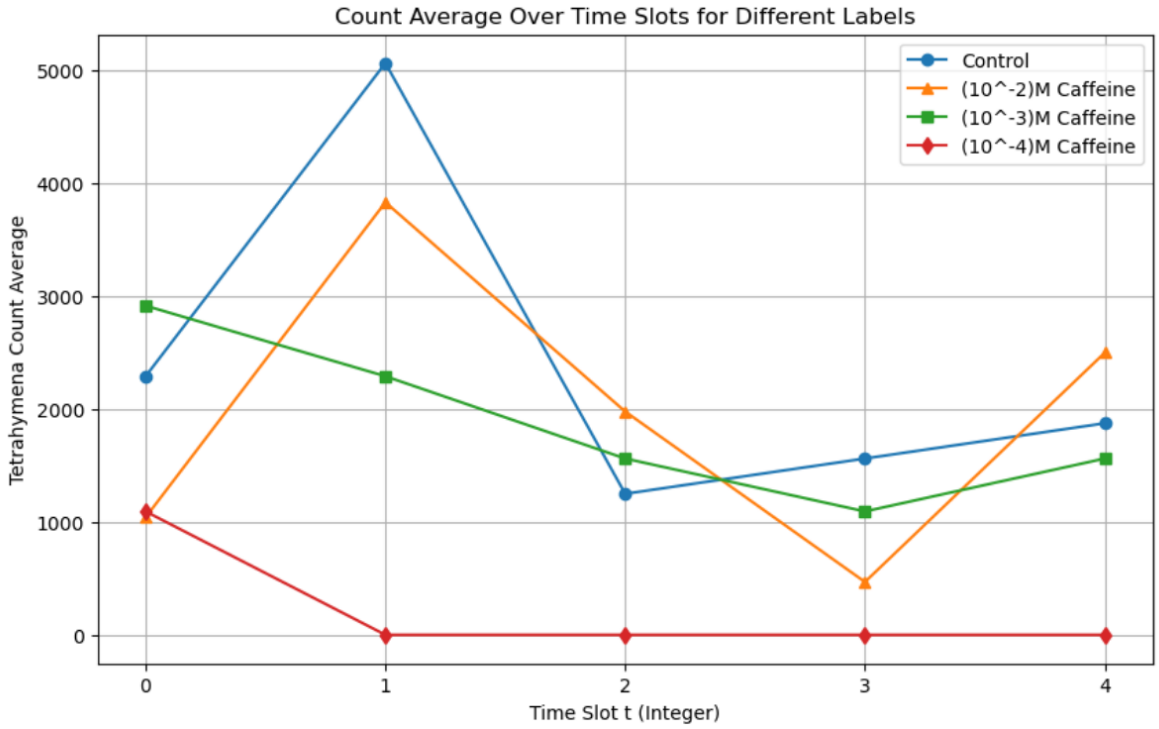

Figure 1: *Tetrahymena* Count Average Over Time

The key results of the ANOVA analysis lead to the following F-statistic value of 0.625793 and a p-value of 0.60157.

The F-statistic value of 0.625793 indicates that the variability within groups is comparable to the variability between the four concentration groups, including control.

The obtained p-value, 0.60157, surpasses the significance level of 0.05. A higher p-value suggests no significant difference in the growth curve of the mean concentrations among the tested concentrations of T. *thermophila* in the caffeine + SSP solution.

DISCUSSION

Based on the experimental results, the groups treated with caffeine at a concentration of 10^{-2} and the control group initially exhibited an increase in cell numbers. However, the increase in the 10^{-2} caffeine group was less pronounced than in the control group. Subsequently, there was a gradual decrease in cell numbers in the 10^{-2} M group, while the cell numbers in the 10^{-3} M and 10^{-4} caffeine groups continuously decreased from the beginning. This suggests that caffeine does indeed impact the growth rate of *Tetrahymena*. However, the obtained p-value in this analysis exceeds the 0.05 threshold, registering at 0.60157. This higher p-value strongly suggests that there is no significant difference in the growth curve of the mean concentrations among the tested concentrations of *T. thermophila* in the caffeine + SSP solution.

When an excessive amount of caffeine (10^{-2})M was added, the reduction in Tetrahymena growth rate was not significantly noticeable. On the other hand, with lower caffeine concentrations (10^{-3} and 10^{-4})M, the decrease in the growth rate was more apparent. Notably, the 10^{-4} caffeine group exhibited the most significant inhibition of Tetrahymena growth rate initially. However, overall, the 10^{-3} M caffeine group exerted the strongest inhibitory effect on Tetrahymena growth rate, aligning with our initial hypothesis. Over time, the cell numbers of

Tetrahymena in all groups continued to decrease, demonstrating that caffeine does indeed lower the growth rate of Tetrahymena.

The purpose of this study is to determine the effects of various concentrations of caffeine on the *Tetrahymena thermophila*. Several factors may impact the reliability and validity of the study. Firstly, the change in temperature during sampling may impact cell growth rates. To minimize the difference, at the time of sampling, our group added the 10uL fixative to all 12 counting tubes using the same tip. Then take out the cultures from the incubator and (changing tips each time) gently mix the test tubes before taking 100uL of culture and mix directly into fixative when counting our group resuspends the cells since all the “fixed” (dead) cells will be at the bottom. Additionally, the presence of caffeine-resistant mutants in *Tetrahymena thermophila* introduces potential variation. To address this, using the same stock solution of cells with the initial population count can make all subsequent treatments standardized. Lastly, small sample sizes may introduce bias, limiting generalizability. Caution is advised in drawing broad conclusions. Future studies could explore increasing sample sizes to enhance statistical robustness.

CONCLUSION

The results of our experiment did not provide enough evidence to reject the null hypothesis, suggesting no statistically significant change between the varying caffeine concentrations on the population growth of T. *thermophila.* Numerous error sources have been found in this research, which might affect the validity and dependability of the study's conclusions. The precision of cell growth rates may be jeopardized by temperature variations during sampling. To increase statistical robustness and the conclusions' generalizability, future research might concentrate on enlarging sample sizes. Researchers can contribute to a more thorough grasp of the subject matter by addressing these possible causes of mistakes and expanding on the foundation established by this study. This study opens up new avenues for investigation and improvement in subsequent research endeavors by offering insightful information on the intricacies of the experimental system.

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