

Utilizing *C. elegans* to Study Effects of Natural and Pharmaceutical Anti-Obesity Medications

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ABSTRACT

The purpose is to understand mechanisms associated with obesity and utilize natural or pharmaceutical anti-obesity medications on growth of nematodes. The hypothesis is anti-obesity medication, phentermine, would have the most significant effect on nematode population growth. The null hypothesis is there will be no significant difference between groups (blank, control, phentermine, bupropion, green tea, Mormon tea) tested. Five experiments were conducted examining population growth. Experiment one included 12 cultures, six each, non-genetically modified (WN2002) and genetically modified (RB1716) nematodes. Nematodes in experiment one were grown in (1:5) concentration. Experiment two-to-five, wildtype strain only and six cultures each, to pinpoint effects in model organism. Experiment two-to-three and four-to-five were grown in (1:10) and (1:12) concentrations respectively. Statistical analysis of variance (ANOVA) was conducted with [F (5, 30); $p < 0.05$] degrees of freedom and [F-critical= 2.53], [F (6, 42); $p < 0.05$] and [F-critical= 2.44]. Results: experiment 1 (WN2002) (F= 0.37), experiment 1 (RB1716) (F= 0.85), experiment two (F= 1.27), experiment three (F= 1.89), experiment four (F= 6.08), and experiment five (F= 4.00). T-tests were conducted to validate (ANOVA). Nematodes grown in (1:5) concentration show small population sizes and no observable significant effects. Nematodes grown in (1:10) concentration show small population sizes and no significant effects, but show effects comparing phentermine and green tea to blank (E3). Nematodes grown in (1:12) concentration show larger population sizes and significant effects.

KEYWORDS: *C. elegans*, Anti-obesity, HFCS, Population Growth

INTRODUCTION

Today, the growing concern surrounding obesity and complex weight-loss programs in the United States has led to an increased use of anti-obesity medications. Various Food and Drug Administration (FDA)-approved anti-obesity medications have been extensively clinically tested. This research project focuses on the effectiveness of various anti-obesity drugs on the excess lipid accumulation in genetically modified and non-genetically modified wild-type nematodes. Many different types of anti-obesity drugs are available through prescription, non-prescription, and commercial use which gives a wide range of supplements available to consumers, whether it be monitored under a licensed physician or self-supplemented. Nematode *Caenorhabditis elegans* (*C. elegans*) are utilized as invertebrate substitutes to more complex model organisms, such as mice, because of the availability, drastic price difference, and ethical responsibilities.

The increase in obesity rates in the United States may be associated with the excess sugar and caloric intake. Studies have been completed showing the adverse effects that various sugars, specifically high-fructose corn syrup (HFCS), can create when supplied in large, assessable quantities. HFCS is a complex polysaccharide with various forms and concentrations. The composition of HFCS is a combination of the monosaccharides (C₆H₁₂O₆), glucose and fructose, in varying concentrations for increased sweetness with lessened production and cost. The health effects associated with HFCS prompted concern to understand mechanisms of metabolic activity, excess lipid accumulation (obesity), and the utilization of natural and pharmaceutical anti-obesity medications. HFCS will be supplied as an independent variable additive per xenic culture to promote population growth and lipid accumulation.

C. elegans have a wide range of capability, due in part to the diversity, simplicity, and quantifiable analyses possibilities. *C. elegans* are non-parasitic, microscopic roundworms which have adapted to survive in all, or nearly all, biomes and environments on earth. Maintenance is efficient using personal protective equipment and safe laboratory techniques. *C. elegans* feed on *Escherichia coli* (*E. coli*) as their main food source, which facilitates the use of sterile laboratory techniques for inoculation and growth.

In order to fully analyze *C. elegans* in experimentation composed of population statistics, the understanding of the microscopic roundworm, as a single unit, must be achieved. Nematodes are characterized in the phylum Nematoda. The basic composition of the complex model organism, for research purposes, are as follows, (959) somatic cells with (300) being neurons controlling sensory activity (Edgley and Riddle, 2018). *C. elegans*' anatomical composition allows for research to be conducted to analyze anti-obesity medications with neuronal activity, "brain chemistry", as well as metabolic processes. The complex digestive tract is capable of metabolizing complex molecules, considering origin. In general, the wild-type *C. elegans* (N2) have an approximate lifespan of twenty-one days with novel generation approximately every three days (Zheng and Greenway, 2005). *C. elegans* are composed of two different strains when grown in populations, hermaphrodite and male. Hermaphrodites are more common, because of self-reproduction quality. *C. elegans* was the first multicellular organism to have its genome entirely sequenced, where "65%" of genes in the model organism have been shown to closely express human disease of medical and research interest (Zheng and Greenway, 2005, p. 186). Since the model organism *C. elegans* has the anatomical and genetic capabilities of expressing human obesity with accuracy, this experimentation will be conducted with wild-type (N2 and WN2002) strains and genetically modified (RB1716) strains.

The mechanisms and usages of the four treatments utilized in this research are as follows. Phentermine is an appetite suppressant that works metabolically and neurochemically in humans (Kim et al., 2006). A study utilizing phentermine as a medical weight loss supplement in Korean patients reported significant success in weight and waist circumference reduction with minimal reported side effects (Kim et al., 2006). Bupropion is an amino-ketone antidepressant normally used in conjunction with naltrexone for weight loss in brand name Contrave (Sherman et al., 2016). Bupropion, a weak dopamine and norepinephrine reuptake inhibitor, enhances [Pro-opiomelanocortin] (POMC) cell production and release of alpha-MSH and beta-endorphin in vitro", essentially working through neurochemical pathways

(Sherman et al., 2016). Green tea, a natural supplement believed to promote weight loss, will be utilized. Green tea has been shown to contain caffeine and flavonoid antioxidants speeding up the metabolic process (Kandola, 2018). Also, with the compounds present in green tea, the function has been shown to act through “inhibition of catechol O-methyl-transferase, and inhibition of phosphodiesterase” (Westerterp-Plantenga, 2010). Mormon tea, a natural supplement believed to promote weight loss, will be utilized. Mormon tea may provide aid for weight loss due to it containing tannins and alkaloids that may help increase metabolism (Staughton and Hegde, 2020).

The use of clinically tested and natural anti-obesity medications will be studied and quantified in several ways. Cultures grown on traditional agarose plates will be the main focus of the experimentation allowing the examination of population growth and statistical analysis. Other ways to facilitate research will include analysis on individual effects of anti-obesity medication on lipid accumulation of nematodes within an overall population. This can be accomplished through transfer of worms, (via platinum worm picker) onto individual agarose/microscopic slides fixed with chemicals that react to histochemical staining.

Analysis of effectiveness of the various anti-obesity medications/supplements may be accomplished through population counts and histochemical staining, quantifiable measures available in laboratory. Since raw data does not depict accurate population growth analysis, statistical analysis of variance (ANOVA) and statistical T-tests of the various experimentations will be conducted in order to pinpoint differences and effectiveness.

The purpose of this research is to understand various anti-obesity drugs and their effects on nematodes capable, similar to human processes, of weight gain. HFCS will be a controlled additive to all cultures to promote excess caloric intake. The variables will be the various anti-obesity medications and their ability to either reverse or heighten the effects of excess lipid accumulation.

The main goal of this research experiment is to understand if natural or pharmaceutical anti-obesity medications are a viable resource to combat obesity. This study can assist clinical researchers and medical professionals because of the similarities of the model organism, *C. elegans*, to humans and may give insight to potential treatment plans for patients. A study conducted with *C. elegans* can give valuable information for furthering a scientific suggestion and facilitate the use of more complex vertebrate organisms. The results may provide valuable insight to the overall effectiveness of anti-obesity medication and the potential beneficial and non-beneficial side effects accompanied by each remedial medication. The goal is to utilize the results gathered from this experimentation with nematodes and possibly create, or help support, correlations of model organism results to valuable human practicalities.

EXPERIMENTAL METHODS

Making Anti-Obesity Medication and Natural Anti-Obesity Remedy Solutions

All solutions were created following these procedures: Researcher equipped with personal protection equipment (PPE) for safety and sterility and solutions stored in sterilized centrifuge tubes.

Phentermine solution: Begin with a 15 mg capsule of phentermine, supplied from Parkhurst Pharmacy, and combine with 15 mL distilled water, weight of phentermine recorded with weigh boat and electronic scale, and solution held in a sterilized 15mL centrifuge tube. Aqueous solution is semi-soluble in distilled water so agitation with vortex mixer is crucial to solubility when pouring and creating experimental cultures.

Bupropion hydrochloride solution: Begin with a 10 mg immediate release (IR) tablet, supplied by Parkhurst Pharmacy, and combine with 10 mL distilled water, weight of the bupropion hydrochloride recorded with a weigh boat and electronic scale, and solution held in a sterilized 15 mL centrifuge tube. Aqueous solution is soluble in distilled water. Agitation with vortex mixer is completed for increased solubility for pouring and creating experimental cultures.

Green tea solution: Begin by weighing out one green-tea bag (2 g), measure 100 mL distilled water with a graduated cylinder and add water to 150 mL beaker, boil water in 30 second intervals; 2 minutes total, microwave covered with watch glass then remove from microwave with heat resistant glove, steep tea stirring with glass stirring rod, every 1-2 minutes for an overall time of 15 minutes, strain with #1 filter paper fitted in funnel placed on a funnel holding rack. Finally, place and store green tea in 50 mL centrifuge tube and let cool completely.

Mormon Tea solution: Begin by weighing out 2 g of Mormon tea, cut Mormon tea into smaller, homogenous pieces to be weighed and brewed, measure 100 mL distilled water with a graduated cylinder and add water to 150 mL beaker, boil water in 30 second intervals; 2 minutes total, microwave covered with watch glass then remove from microwave with heat resistant glove, steep tea stirring, with glass stirring rod, every 1-2 minutes for an overall time of 15 minutes, strain with #1 filter paper fitted in funnel placed on a funnel holding rack. Finally, place and store Mormon tea in 50 mL centrifuge tube and let cool completely.

Cultures made for Experiment One as follows: Two different strains, gathered from the Caenorhabditis Genetics Center utilized, strain WN2002 a wild-type strain and strain RB1716 a genetically modified strain. Six different cultures made and analyzed for each strain including: blank culture, control culture, phentermine culture, bupropion culture, green tea culture, and Mormon tea culture. Each culture in experiment one received ~15 mL nutrient growth medium, 2 mL of HFCS, and 1 mL of variable. The base use of one milliliter of variable solution was rationalized through dilution in preparation of solution and xenic culture. The concentration proved to be troubling, and without a specific constant classified weight of *C. elegans* as a single unit, alongside guidance from lab coordinator dilution decreases proved to be most reliable and probable for lab equipment available. Each culture received: *E. coli* arranged in a clockwise pattern (5 blots) as the main food source for the model organisms, 1 cm cube cut and transferred from master culture, from Caenorhabditis Genetic Center, to the culture. Nematodes were held in an incubator at 27-30 °C.

Cultures made for Experiment Two and Three with appropriate variable concentration adjustments as follows: One strain, gathered from Ward's Science, strain Wild Type (N2). Twelve different cultures made and analyzed for each strain including (six cultures for each experiment): blank culture, control culture, phentermine culture, bupropion culture, green tea culture, and Mormon tea culture. Each culture, in experiment two and three, received ~15 mL nutrient growth medium, 1 mL of HFCS, and 0.5 mL of variable. Each culture received:

E. coli arranged in a clockwise pattern (5 blots) as the main food source for the model organisms, 1 cm cube cut and transferred from master culture, from Ward's Science. Nematodes were held in an incubator at 27–30 °C.

Cultures made for Experiment Four and Five with appropriate variable concentration adjustments as follows: One strain, gathered from Ward's Science, strain Wild Type (N2). Twelve different cultures made and analyzed for each strain including (six cultures for each experiment): blank culture, control culture, phentermine culture, bupropion culture, green tea culture, and Mormon tea culture. Each culture, in experiment two and three, received ~15 mL nutrient growth medium, 1 mL of HFCS, and 0.25 mL of variable. Each culture received: *E. coli* arranged in a clockwise pattern (5 blots) as the main food source for the model organisms, 1 cm cube cut and transferred from master culture, from Ward's Science. Nematodes were held in an incubator at 27–30 °C.

Counting *C. elegans* for qualitative and quantitative analysis: After initial transfer, a start count is recorded utilizing dissecting microscope. After 24 hours, cultures are re-assessed to determine if an additional 1 cm cube from master culture should be re-transferred to ensure presence and growth of nematodes. Every 24 hours, depending on availability and access of lab on weekends, worm count of every culture is recorded. If population count exceeds ~150 nematodes culture split into four quadrants and count multiplied by four. For experiments one through three, 5 total days were recorded, and total "Day Count" for analysis is 6 days including start count. Yields of population size and days for experimentation is low, so adjustments are made to cultures to combat issue.

Statistical analysis for experimentation: Statistical Analysis of Variance (ANOVA) used to compare the means between different groups in an experiment completed by computing sum of squares between groups, sum of squares within groups, and total sum of squares to calculate an F-statistic and F-Critical Value. Statistical T-Tests completed by computing T-Tests used to compare individual culture with the population patterns observed in blank culture and control culture.

RESULTS

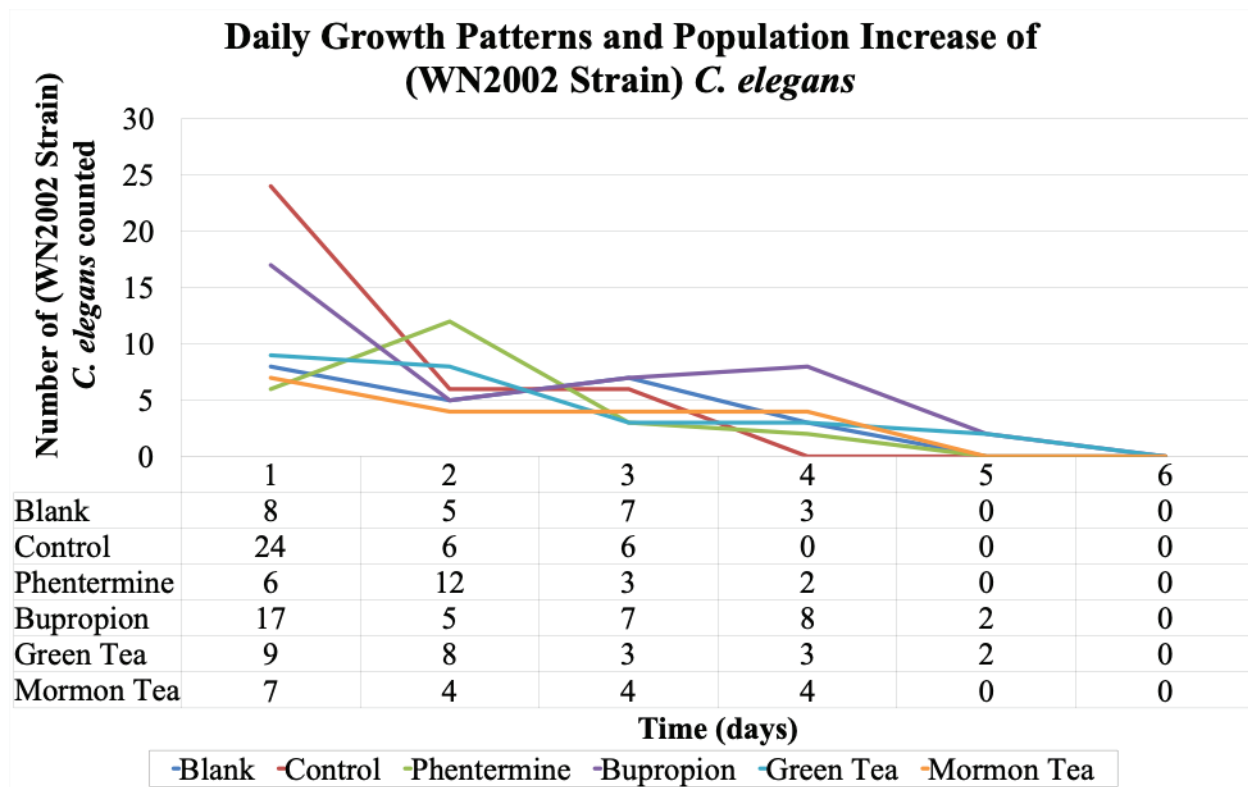


Figure 1.1. Daily Growth Patterns and Population Increase for Experiment 1 (CGC-WN2002).

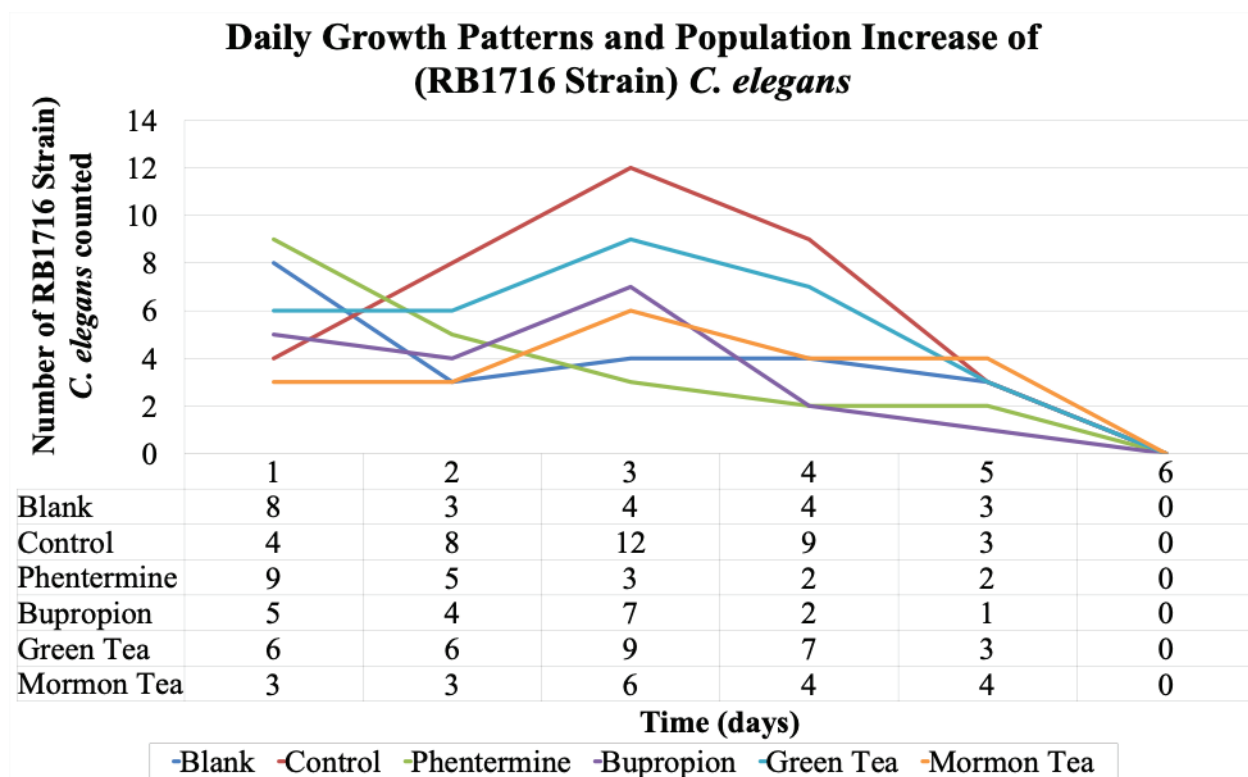


Figure 1.2. Daily Growth Patterns and Population Increase for Experiment 1 (CGC-RB1716).

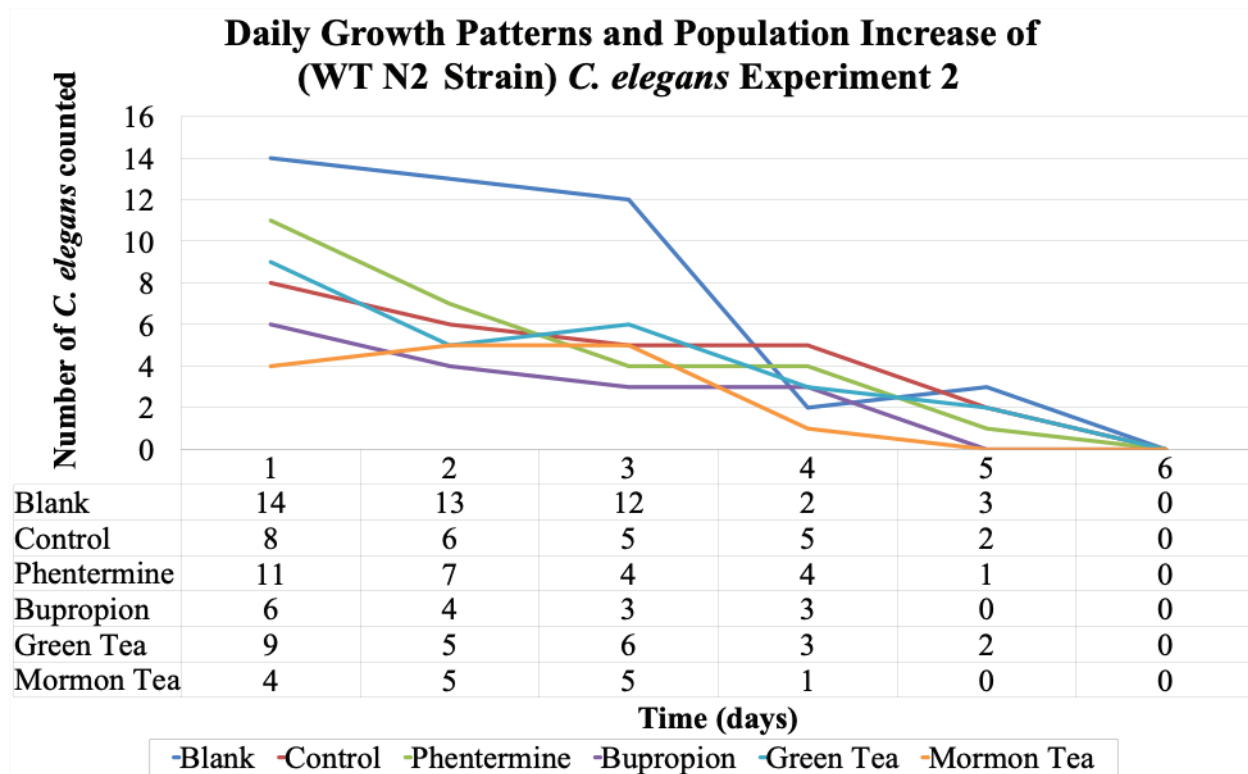


Figure 2. Daily Growth Patterns and Population Increase for Experiment 2 (Wild Type N2).

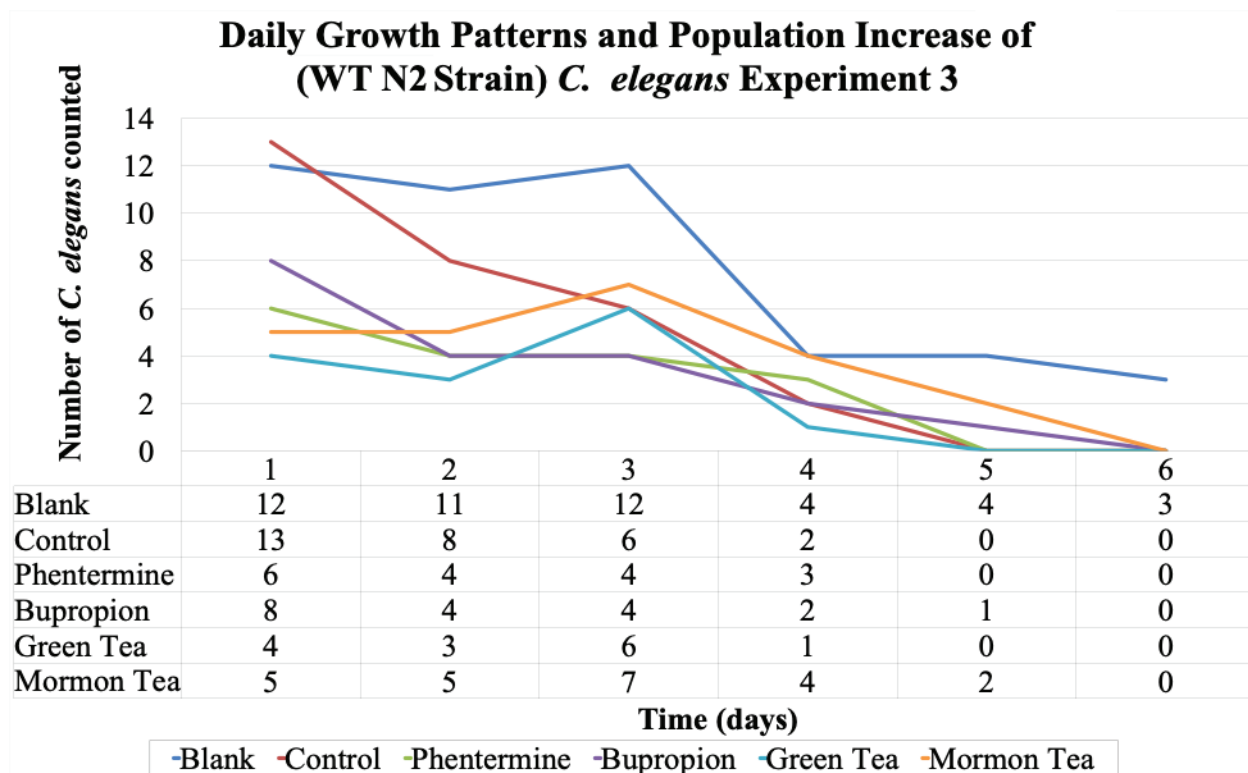


Figure 3. Daily Growth Patterns and Population Increase for Experiment 3 (Wild Type N2).

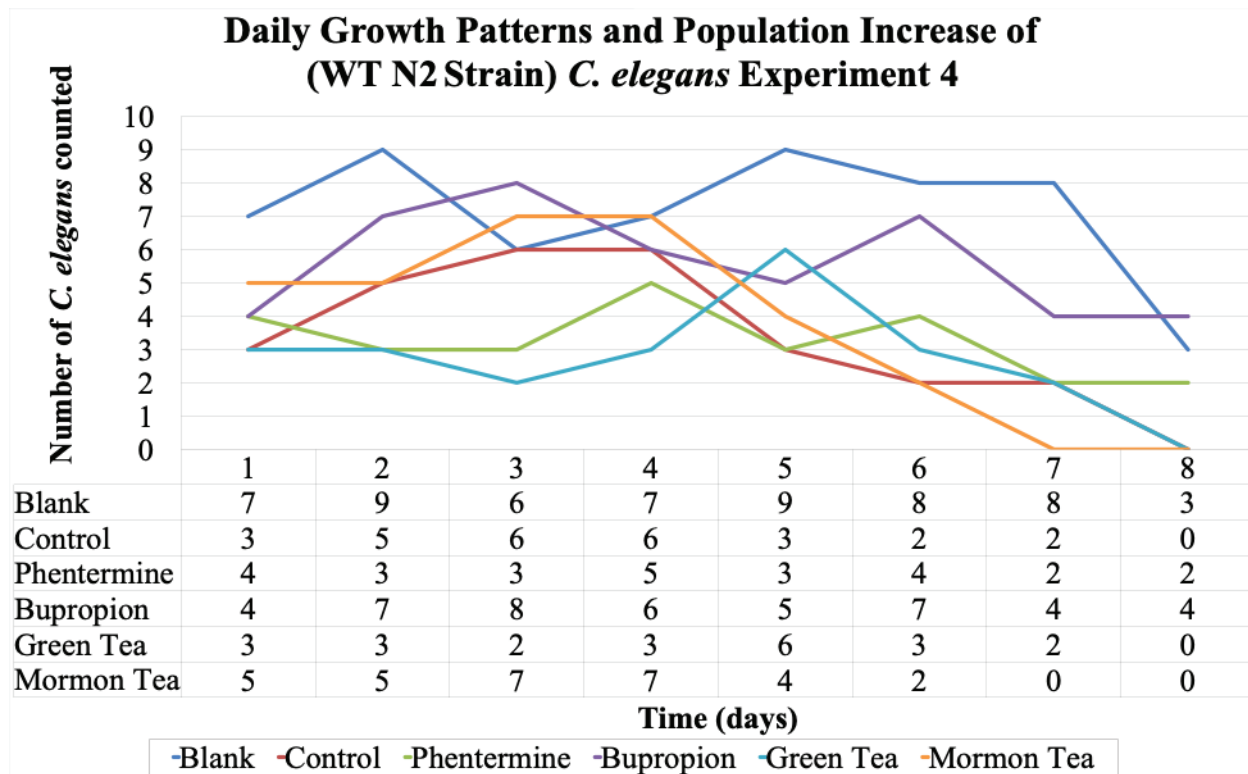


Figure 4. Daily Growth Patterns and Population Increase for Experiment 4 (Wild Type N2).

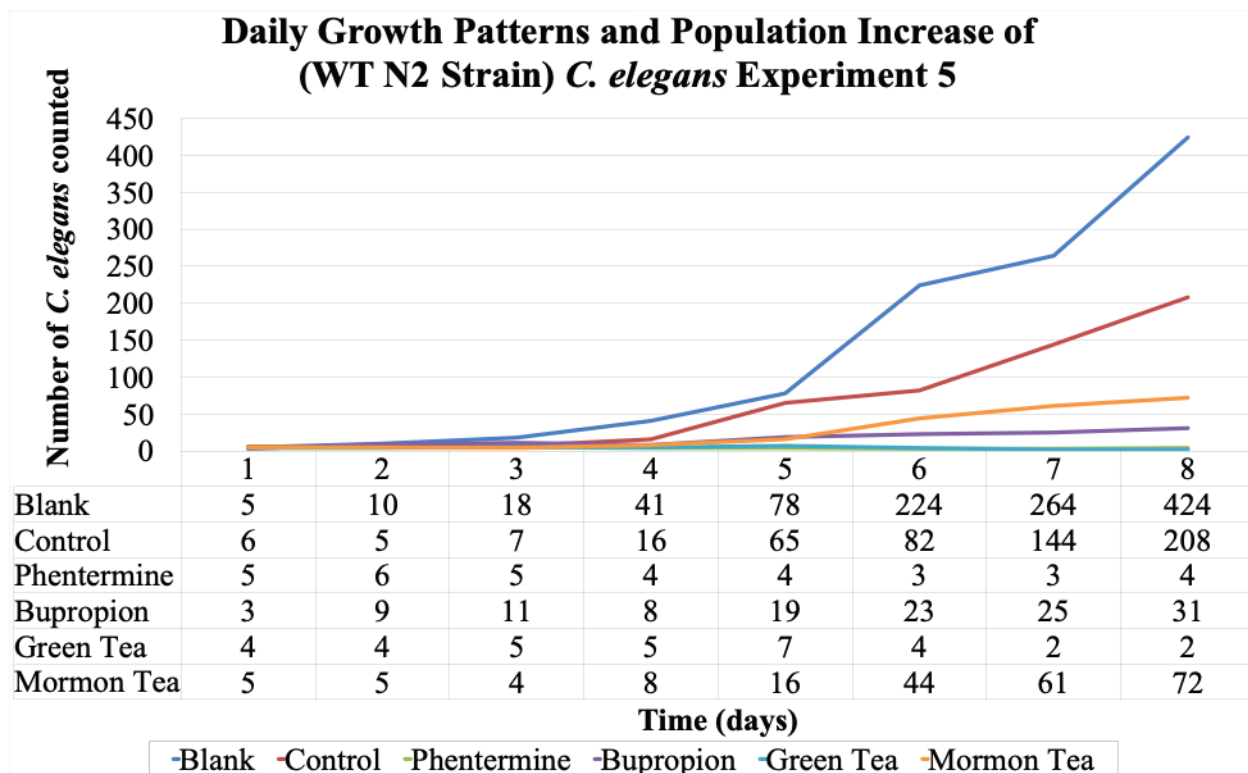


Figure 5. Daily Growth Patterns and Population Increase for Experiment 5 (Wild Type N2).

DISCUSSION

Experiment one tested genetically modified nematodes and non-genetically modified nematodes and the effects that natural and pharmaceutical medications had to combat effects associated with HFCS, and potentially, obesity. *C. elegans* were acquired through the Caenorhabditis Genetics Center, supplying a wild-type strain, WN2002, and a modified strain, RB1716, which expresses [gene *nhr-49*] which has been shown to promote lipid accumulation, making strain RB1716 desirable for experimentation. The overall population yield in experiment one, for both sets of cultures, was limited with short lifespans. The population size was observed and recorded over approximately six days, resulting in data that could be analyzed using mathematical and statistical analysis. Since interpreting population curve graphs poses a challenge to concrete answers about differences between groups, two statistical tests were conducted to understand results. The first conducted test was the statistical analysis of variance (ANOVA) to test differences of variances between groups. The (ANOVA) conducted was with raw data and single factor. First, for strain WN2002, the sum of squares within groups, sum of squares between groups, and total sum of squares was calculated along with degrees of freedom [F (5,30)] to find and interpret the F-value. The F-value is found by dividing the sum of squares between groups by their degrees of freedom, dividing the sum of squares within groups by their degrees of freedom, resulting in two numeric values. The formula will result in, $F = \frac{(\text{sum of squares between groups} / \text{degrees of freedom})}{(\text{sum of squares within groups} / \text{degrees of freedom})}$. Experiment one results from WN2002 strain was (F= 0.37) with (Critical value; 2.53) showing that there was no significant difference between the groups tested. Experiment one results from RB1716 strain was (F= 0.85) with (Critical value; 2.53) showing that there was no significant difference between the groups tested.

Statistical t-tests compare the variances between two different groups by computing mean, standard deviation, and variance to calculate a t-test value. The t-tests conducted were “two-sample assuming unequal variances” and “P (T≤t) two-tail”. Control/blank for experiment one WN2002 strain the t-test value was (p = 0.61), control/phentermine t-test value was (p = 0.62), control/bupropion t-test value was (p = 0.91), control/green tea t-test value was (p = 0.67), and control/Mormon tea t-test value was (p = 0.50).

Control/blank for experiment one RB1716 strain the t-test value was (p = 0.30), control/phentermine t-test value was (p = 0.29), control/bupropion t-test value was (p = 0.22), control/green tea t-test value was (p = 0.72), and control/Mormon tea t-test value was (p = 0.22). Since all the computed values did not satisfy the t-test, (P < 0.05), to show that variation was statistically (95% accurate), determine acceptance or rejection of null hypothesis.

After experiment one yielded small population sizes, experiment two setup was slightly altered. Nematodes wild type (N2) from Ward's Science were utilized being crucial of cost, and concentrations of variables were altered. Experiment two results show (F = 1.27) with (Critical value; 2.53) showing that there was no significant difference between the groups tested. The F-value was slightly higher in experiment two, indicating that there is a higher chance that there may be significant data between either the variables and the control or the variables and the blank. The t-tests conducted were “two-sample assuming unequal variances” and “P(T≤t) two-tail”. Control/blank for experiment two the t-test value was (p = 0.32), control/phentermine t-test value was (p = 0.94), control/bupropion t-test value was (p = 0.30),

control/green tea t-test value was ($p = 0.93$), and control/Mormon tea t-test value was ($p = 0.26$). These t-test results show that there is no significant statistical difference, individually, between the experimental groups tested.

Experiment three was structured exactly as experiment two to test if the data acquired was able to be replicated. Nematodes wild type (N2) was used, and concentrations of variables were kept the same. Experiment three results show ($F = 1.89$) with (Critical value; 2.53) showing that there was no significant difference between the groups tested. The F-value was slightly higher in experiment three but was similar to the F-value computed in experiment two. The t-tests conducted were “two-sample assuming unequal variances” and “ $P(T \leq t)$ two-tail”. Control/blank for experiment three the t-test value was ($p = 0.33$), control/phentermine t-test value was ($p = 0.42$), control/bupropion t-test value was ($p = 0.78$), control/green tea t-test value was ($p = 0.32$), and control/Mormon tea t-test value was ($p = 0.68$). These t-test results show that there is no significant statistical difference, individually, between the experimental groups tested. There was though, in experiment three statistical adverse effects when the phentermine and green tea were compared the blank.

After experiment two and three yielded small population sizes, experiment four and five experimental setups were slightly altered. Concentrations of variables were altered. Experiment four results show ($F = 6.08$) with (Critical value; 2.44) indicating that there was a significant difference between the groups tested. The F-value was slightly higher in experiment two, indicating that there is a higher chance that there may be significant data between either the variables and the control or the variables and the blank. The t-tests conducted were “two-sample assuming unequal variances” and “ $P(T \leq t)$ two-tail”. Control/blank for experiment four the t-test value was ($p = 0.003$), control/phentermine t-test value was ($p = 0.88$), control/bupropion t-test value was ($p = 0.03$), control/green tea t-test value was ($p = 0.53$), and control/Mormon tea t-test value was ($p = 0.77$). These t-test results show that there is significant statistical difference between some of the experimental groups tested.

Experiment five results show ($F = 4.00$) with (Critical value; 2.44) showing that there was a significant difference between the groups tested. The F-value was slightly higher in experiment two, indicating that there is a higher chance that there may be significant data between either the variables and the control or the variables and the blank. The t-tests conducted were “two-sample assuming unequal variances” and “ $P(T \leq t)$ two-tail”. Control/blank for experiment two the t-test value was ($p = 0.30$), control/phentermine t-test value was ($p = 0.05$), control/bupropion t-test value was ($p = 0.10$), control/green tea t-test value was ($p = 0.05$), and control/Mormon tea t-test value was ($p = 0.20$). These t-test results show that there is no significant statistical difference between the experimental groups tested.

CONCLUSIONS

Experiment one dealt with two different strains of nematodes and the concentration, (1:5) variable to agar concentration, seemed out of proportion and did not allow proper population growth. Experiment one yielded low daily population counts and an overall six-day experiment. The main focus of experiment one was utilizing the WN2002 strain as a control for the RB1716 strain which showed possible correlation to lipid accumulation and obesity.

The cultures were also designed to avoid discrepancies caused by experimental setup. In both strains, there was a control culture with only HFCS and no anti-obesity medication as well as a blank with only nutrient growth medium. The experimental design ensured that any adverse effects or non-adverse effects that would have occurred in the experimental variable cultures were due only to that added supplement.

The results from experiment one for statistical analysis of variance (ANOVA) and statistical t-tests shows that overall, there was no adverse effects between the groups at a (1:5) concentration. This may be due to the over-concentration and nematodes inability to sustain a population size adequate enough to analyze holistically. Since this was a concern, the experimental setup was altered in order to test and find out if the concentration of variable to agar was the cause of the low population numbers.

Experiment two and three were conducted with the wild type strain (N2), in order to examine the natural and anti-obesity medications with nematodes, remaining cost-conscious because of the costs associated with purchasing and shipping genetically modified nematodes. Experiment two and three also received a lower concentration of variable to agar, (1:10). This was an attempt to ensure that the variables were not becoming too toxic for the model organism to reproduce and sustain a large population size. Experiment two and three also yielded smaller population sizes and lasted for an overall six-day experiment. The cultures also all seemed to acquire minimal white fungus after approximately three days of growth. The fungus was common on all cultures surrounding, mostly, the 1 cm transferred block from the acquired master culture.

The results from experiment two and three were expected to be very similar, because of their exact experimental setup and treatment. The results from both statistical analysis of variances (ANOVA) and t-tests comparing variables to the control showed no significant statistical effects between the variables and the control culture at a (1:10) concentration. The t-test in experiment three comparing the blank/phentermine and blank/green tea showed ($p < 0.05$) showing that there was some statistical significance between these added supplemental variables and population growth. As of now, the effects seem to be adverse, but the natural and pharmaceutical medications may be providing the anti-obesity traits that would limit lipid accumulation in the nematodes. Further analysis and larger population sizes required for confirmation.

The results from experiment four and five were expected to be very similar, because of their exact experimental setup and treatment. The experiments yielded much higher population counts and overall, eight-day experiments. The results from both statistical analysis of variances (ANOVA) showed significant statistical effects between the variables and the control culture at a (1:12) concentration. The t-tests in experiment four comparing the control/blank and control/bupropion showed ($p < 0.05$) showing that there was some statistical significance between these added supplemental variables and population growth. In experiment five the results from both statistical analysis of variances (ANOVA) showed significant statistical effects between the variables and the control culture at a (1:12) concentration.

Due to lab non-availability post formal COVID-19 closings and restrictions, histochemical staining and individual analysis of composition of nematodes was unattainable. With further analysis or future research, assessing glucose, complex polysaccharide, and lipid concentra-

tion per single nematode using distinct histochemical stain could potentially grant more thorough insight on effectiveness of anti-obesity medications and quantifiable lipid accumulation data.

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ABBREVIATIONS

FDA, Food and Drug Administration; *C. elegans*, *Caenorhabditis elegans*; *E. coli*, *Escherichia coli*; HFCS, high-fructose corn syrup; CGC, *Caenorhabditis Genetics Center*; WN2002, *Caenorhabditis Genetics Center* wild-type nematode strain; RB1716, *Caenorhabditis Genetics Center* genetically modified nematode strain; Wild-Type N2, Ward's Science wild-type nematode strain.

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