

The Effect of Air Fresheners on Planarian Regeneration

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Abstract

Purpose: Research has shown that air fresheners emit hazardous chemicals known as volatile organic compounds into the environment. In turn, new companies claimed to have discarded these chemicals by creating “safe and natural” air fresheners. There has been an absence of studies testing the effects of different air fresheners nor of varying concentrations on living organisms. The purpose of this study was to find the effects of different air fresheners and concentrations on planaria regeneration.

Method: The organisms planaria were used due to their regeneration properties. Thirty-five planaria were assigned to one of three air freshener conditions, then assigned to one of three different concentration groups (0.1%, 0.01%, or 0.001%). The planaria were then cut in half and allowed to regenerate for 14 days. Immediately following, data was collected and analyzed on the rate of regrowth that occurred for each planarian.

Results: This study showed that both the control and natural groups had similar percent regrowth with 87% and 86%, respectively, while the chemical group had a significant negative impact on their regeneration rates, with only a 64% regrowth. Findings additionally showed that increasing concentrations had a greater negative effect

on the regeneration rate for both chemical and natural air fresheners.

Conclusions: This experiment suggests that natural air-freshener is not as harmful as a chemical air freshener, which has many environmental and health implications. Also, higher concentrations of air fresheners could cause a greater amount of cellular and bodily health concerns.

Introduction

Explicit research about the dangers of air fresheners and their effects on organisms are unknown. Air fresheners emit volatile organic compounds (VOCs), such as formaldehyde, petroleum distillates, limonene, esters, alcohols, and more (Steinemann, 2017). Cleaning agents and air fresheners are considered toxic air contaminants (TACs), and a subset of these are regulated by the US federal government as hazardous air pollutants (HAPs) (Nazaroff & Weschler, 2004). After being constantly exposed to these VOC chemicals, humans are more susceptible to respiratory problems such as lung cancer (Oguma, et al., 2017). As well as causing adverse health effects to the population, these VOCs have been shown to impair the air quality of many indoor areas (Walsh, 2011). The federal and state governments are worried about VOCs being detrimental to our health, and the EPA recommends minimizing exposure to them (Palmer, 2010). To sway the public away

from these harmful effects of air fresheners, many companies have brought about alternatives and have been advertising how “natural”, “green”, and “healthy” their products are.

However, it has been shown that their so-called “green and organic” products can also emit potentially hazardous chemicals (Steinneman, 2017). According to the article by Schwartz (2016), a 2007 study conducted by NRDC found that of 14 home air fresheners, 12 of them contained phthalates— including the “all-natural” and “unscented” varieties. Phthalates, which are used to dissolve and carry fragrance, are linked to changes in hormone levels, poor semen quality, birth defects, and reproductive harm. To delve deeper into this topic, how air fresheners may affect living tissues and cells was investigated due to the lack of studies on the potential permanent effect of air fresheners on the health of cells. Furthermore, limited studies have shown the impact of increasing concentrations of air fresheners on the health of humans, as many have just shown the harmful effects of specific chemicals on humans. This is troubling since annually, air fresheners emit 7.5 tons of VOC emissions, which is the most out of other products, such as carpets, cleaners, waxes, and polishers commonly found in our homes. Also, air fresheners release the most VOC emissions per capita (230 mg per person) compared to any other household cleaning product (Nazaroff & Weschler, 2004). Planaria are excellent models for regeneration and have been used in stem cell research and surgical experimentation for over a century (Reddien & Sánchez, 2004). *Dugesia tigrina*, or Brown Planaria, were used in this study. The usage of *D. tigrina* was due to their fast regeneration rate of about 2 weeks.

In this study, the planaria were exposed to both chemical and natural air

fresheners to determine their effects on cellular regeneration. By illustrating how air fresheners affect the regeneration of planaria, the harmful effects of these air fresheners on our body could be modeled and determined. Therefore, the present study aimed to test the effects of natural (Grow air freshener) and chemical-based air fresheners (Febreze air freshener) on the regeneration ability of planaria. We hypothesize that when planaria are exposed to chemical and natural-based air fresheners, natural air fresheners will have less of an effect on the regeneration rates of planaria compared to chemical air fresheners, with the control having no significant inhibition on regeneration. Furthermore, we hypothesize that increasing concentrations of air fresheners will have a greater negative effect on planaria regeneration.

Method

To test our hypotheses, 35 brown planaria were obtained. Natural air freshener solutions of 0.1%, 0.01%, and 0.001% were prepared.. According to The Statistics Portal, in 2018, Febreze was the most widely used air freshener, which is why it was chosen as the chemical air freshener (The Statistics Portal, 2018). Due to the products popularity on amazon and the false propaganda of being “100% natural”, Grow Freshener was chosen as the natural air freshener. . Grow Freshener had also stated that it was “certified 100% plant-based” which made it key for utilization in this study as it did not contain any volatile organic compounds (Grow Freshener, 2019). Chemical air freshener solutions of 0.1%, 0.01%, and 0.001% were also prepared. For both experimental groups, their specific concentrations were modeled on a previous study, where specific substances were tested to find which concentrations were strong enough to affect the planaria but not kill them (Collins, 2015).

All six air freshener extracts were prepared using serial dilution, where each concentration was mixed (0.1mL, 0.01mL, and 0.001mL) into their respective amount of spring water to create a 100 mL solution, enough to house the designated amount of planarian in their respective concentration groups. The concentrations of the air fresheners were designed to be non-toxic concentrations, meaning that enough air freshener was used to affect the planaria, but was not designed to kill them. A control solution was also included for a basis of comparison at the end of experimentation.

Before the planaria were placed into their respective solutions, they were measured under a microscope, and then placed on ice to inhibit movement. The planaria were then symmetrically cut across the transverse axis and then re-measured again. The top half of the planaria was kept for further measurements while the lower half was carefully disposed, according to protocol. All 10 of the planaria were then distributed equally among both concentration groups. (see Figure 1).

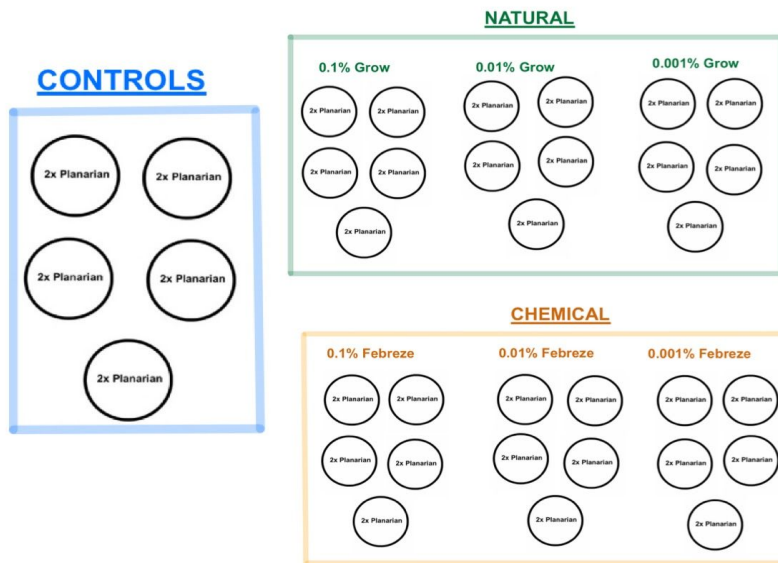


Figure 1. Planaria Experimentation Groups

The planaria were set to regenerate in their solutions for two weeks. Observations were taken and recorded on the progress of their regeneration daily. The purpose of the water change was to model what would happen if someone were to spray their room with air freshener frequently. Any dead planaria were recorded, extracted, disposed, and omitted from the data analysis at the end of experimentation. After the two weeks, the

final lengths of the planaria were measured and compared to the original lengths of the planaria.

Results

Over the course of the experiment, observations were recorded regarding the many effects of these air fresheners on the regeneration and health of the planaria.

Besides the effect of the air fresheners on the regeneration rate, the planarias' movements, colors, and sizes were also taken into account. Unfortunately, some planaria were able to swim to the lid of the Petri dish, causing them to dehydrate and die. The results for these planaria were omitted.

For control, the planaria exhibited a similar amount of movement throughout the

experiment compared to before they were cut, and they had a consistent dark brown color. Signs of regeneration were exhibited in around five days after cutting, with a 1-2 mm growth. The size compared before and after the 14 days were of similar size.

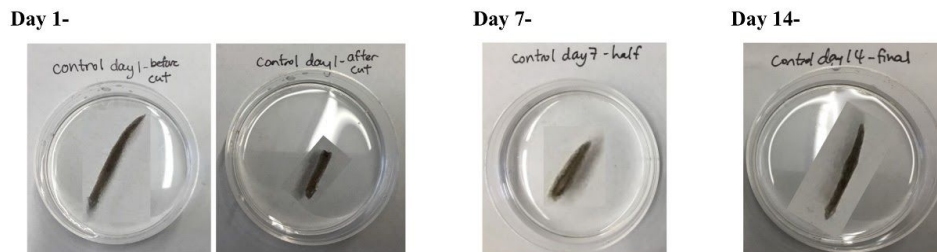


Figure 2. The progression of regeneration for control

For the natural groups, it was observed that the planaria in the three concentration groups exhibited a normal amount of movement throughout the 14 days. They began to show signs of regeneration around seven days into the

experiment, with a 1-2 mm growth. The planaria were lighter in color at the end of the experiment than before, especially in the higher concentrations. However, the planaria stayed a similar thickness and did not grow thinner.

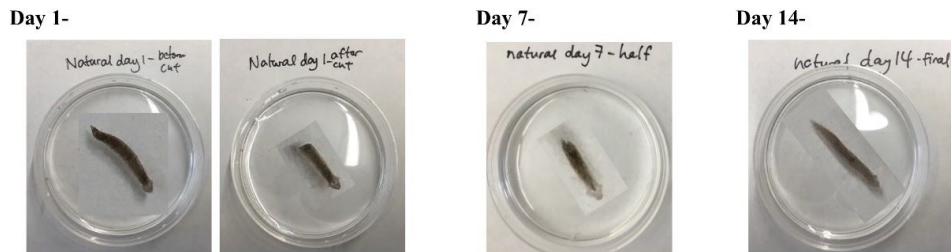


Figure 3. The progression of regeneration for natural

For the chemical groups, all the 0.1% concentration planaria were killed within a minute of being placed in the air freshener solution. The planaria in other conditions became much lighter in color and thinner. The planarian in both the 0.01% and 0.001%

groups were slow-moving and were observed to be lethargic. Many curled up in circles, which meant that they were extremely uncomfortable in the new habitat.

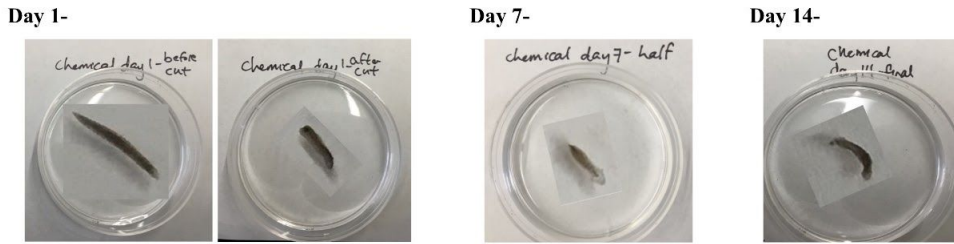


Figure 4. The progression of regeneration for chemical

The effects of different air fresheners were tested on planarian regeneration by a two-way ANOVA (univariate) and a Tukey-HSD Test. These tests showed that the type of air freshener significantly affected the regeneration amount and with a significance of $F(1, 21)=26.299, p<0.001$. The percent regrowth of control 87%, while the natural percent regrowth of 86%, which was similar to the control. However, both groups' conditions' percent regrowths were much larger compared to the percent regrowth of the chemical group lengths of 64% (p-value of less than 0.001). Furthermore, the percent regrowth of the natural condition (86%) is much larger than the percent regrowth of the chemical condition (64%), with $p<0.001$. Control

conditions within the spring water produced the best regeneration rates, but the results of the natural conditions (Grow Freshener) did not stray too far from the results of the control. In contrast, the chemical conditions (Febreze) produced the worst results with only a 67% regrowth (see Figure 5). Figure 6 shows the regeneration differences of length, with the control group exhibiting the least variation, while the chemical groups showed the most variation in length difference. In conclusion/ Overall the planaria exposed to natural air fresheners had less of an effect on the regeneration rates of planaria compared to chemical air fresheners, while the control group had the least inhibition on regeneration.

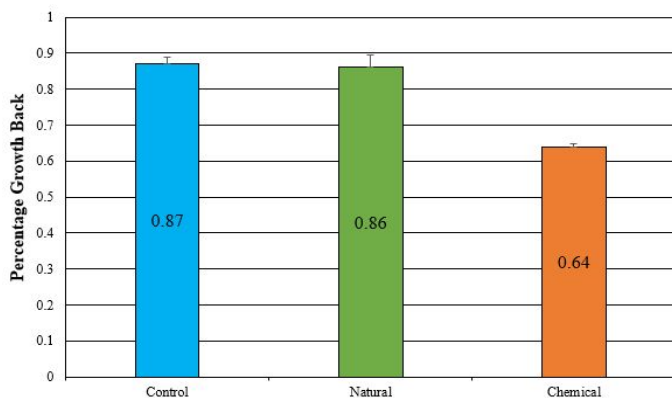


Figure 5. The Effect of Type on Regeneration Percentage

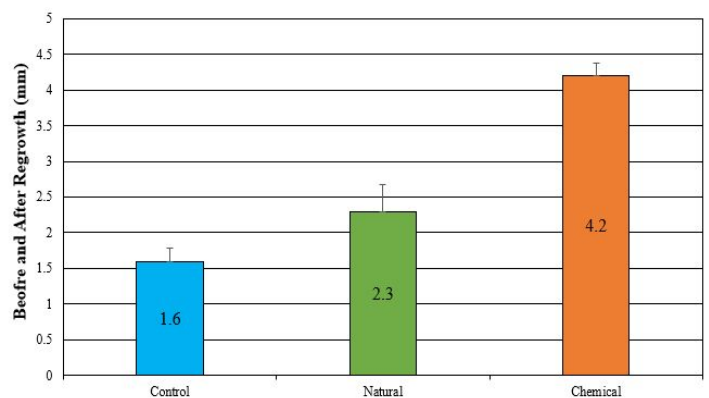


Figure 6. The Effect of Type on Total Planarian Regrowth Difference

To test the effects of concentration on planarian regeneration, a separate two-way ANOVA (univariate) and a Tukey-HSD Test was run. These tests showed that the concentrations of air fresheners affected the regeneration amounts of the planaria, with a significance of $F(2, 21)=8.413$, $p<0.002$. The control concentration (no added air freshener solution) versus the 0.01% concentrations had a significant difference with a p-value of 0.003. When the 0.01% concentrations were compared against the 0.001% concentrations, there was another significant difference with a p-value of 0.013. For the chemical group, the planaria among the highest concentrations (0.1%) were killed instantly, so the results for that concentration were omitted. Similarly, all planaria who died during the experiment were omitted from the data analysis and results. For the natural concentrations, all three produced similar

results when compared to one another. However, the natural 0.001% concentration regenerated more than the control concentration. According to Figure 7, the main effect of concentration with planaria percent regeneration is treated with a higher concentration. The higher the concentration of air freshener, the lower percent the planaria regenerate back. Figure 8 shows the regrowth differences before and after regeneration, in which the control had the least difference of 1.6 mm, then the natural conditions had an average regeneration difference of a little more than control, and the chemical conditions had a drastically higher regeneration difference overall, excluding the highest concentration. Therefore, the initial hypothesis is supported, as a greater concentration resulted in a greater negative effect on planaria regeneration rates.

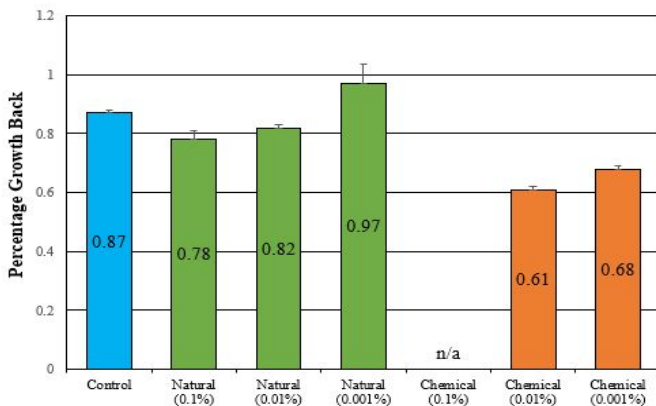


Figure 4. The Effect of Concentration on Regeneration Percentage

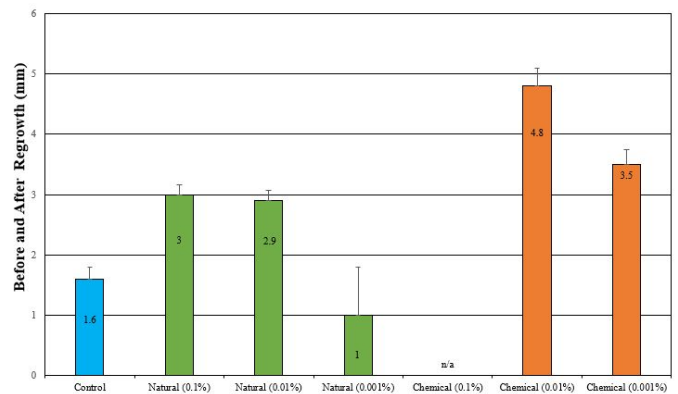


Figure 5. The Effect of Concentration on Total Planarian Regrowth Difference

Discussion

The natural air-freshener did not significantly inhibit regeneration, while the chemical air-freshener had a negative impact on the planaria regeneration rates. The

concentrations and percent regrowth had a negative relationship on planarian regeneration, as higher concentrations resulted in lower regrowth. Furthermore, planarian regeneration under chemical air freshener was inhibited in many ways including lighter and thinner bodies, slower

movements, and most importantly, lower regeneration rates. This information could be utilized to explain the effects these air fresheners have on our cellular and overall health. This is important because many people contain chemical air fresheners in their homes and are potentially at risk and unaware of the detrimental effects it could cause to their cells, as shown by the negative effects on the regeneration of the planaria. Promoting the use of natural or no air-freshener rather than chemical air-freshener would be another implication of this study. However, there are some limitations to this study. The control groups did not regenerate fully due to imperfect living conditions, lack of feeding, and human measurement error. Due to time constraints, only five planaria could be tested in each group, they couldn't be fed, and water couldn't be changed periodically. Also, we were only able to test one type of each air freshener due to time and resource constraints. The cuts to planaria were not done to precision and could have been potentially inaccurate as well as the measurements which are subject to human error. Additionally, an interaction was tested to investigate the relationship between the type of air fresheners and concentration, but there were no significant results ($p=0.492$). The planaria were kept in solutions for another two weeks before disposal to test for any further regeneration. There was no exhibition of any further significant regrowth, and some planaria even exhibited degrowth due to the evaporation water and air freshener. For further study, the presence of VOCs in planaria could be tested. The planaria could be tested with a VOC indicator after exposure to air freshener and after regeneration to determine the levels of VOC within the planaria. Planaria are perfect model organisms for humans on cellular regeneration as humans naturally regenerate

by replacing cells on both injured and non-injured tissues. If these chemical air fresheners are inhibiting the regeneration of planaria, they could be harmful to our cells and bodily health. Thus, the negative effects of these air fresheners on the regeneration abilities of the planaria, model the potential effects the chemicals have on cellular health and on our overall body health.

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