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A PLANT BASED METHOD TO DECREASE PRO-INFLAMMATORY CYTOKINE PRODUCTION IN RESPONSE TO CYTOTOXIC VOLATILE ORGANIC COMPOUNDS: IMPLICATIONS FOR ASTHMA AND RESPIRATORY DISEASES

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ABSTRACT

Exposure to cytotoxic volatile organic compounds (VOCs) can cause both short and long-term adverse health effects. The purpose of this study was to investigate the ability of the *Chlorophytum comosum* (spider plant) to decrease the induction of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNFα), a precursor to respiratory inflammation, by VOCs. Several VOCs, including methanol, ethyl acetate, acetone, and formaldehyde, were isolated using a Vernier© gas chromatograph. Then, the absorption of these compounds by *Chlorophytum comosum* was measured by determining their decrease in mass over time in closed systems. On average, VOCs decreased by 78.3% in three days in bell jars with plants versus 23.1% in control jars with no plant (two sample t-test ($p < 0.001$)). Next, the capability of spider plant extract to decrease formaldehyde induced mRNA expression of the pro-inflammatory cytokine TNFα in U937 cells (a human cell line established from a histiocytic lymphoma) was investigated. Quantification of TNFα transcript levels utilizing RT-PCR showed that spider plant compounds exhibited efficacy in decreasing TNFα expression by 38.2% in cells exposed to formaldehyde (two sample t-test ($p < 0.001$)). A practical application of this research is in the prevention of disease, especially in populations who have increased incidence of asthma and respiratory diseases

Keywords: Volatile organic compounds; Asthma; Spider plant; Formaldehyde; Phytoremediation; Pro-inflammatory cytokine TNF-α.

INTRODUCTION

Volatile organic compounds (VOCs) are emitted from various solids or liquids as gases. Known VOCs include various alcohols, aldehydes, ketones, benzene, ethylene glycol, formaldehyde, methylene chloride, perchloroethylene, toluene, and xylene. Formaldehyde vapors are released from substances including: resins, lubricants, home furnishings, household cleaners, paints, textiles, landscape and yard products, wood decks, medicinal and personal care products, pesticides, polyoxymethylene plastics, 1,4-butanediol, and methylene diphenyl diisocyanate (1).

Formaldehyde is added to products because of its adhesive, fumigant, disinfectant, fungicide and fixative (varnish) properties (1, 2).

VOCs such as formaldehyde are associated with short term and long term health effects, ranging from eye, nose, and throat irritation, headaches, loss of coordination, nausea, liver damage, kidney damage, central nervous system damage, cancer and asthma (2). These effects have been called Sick Building Syndrome, which is now a widely recognized health problem (3, 4). In one study, it was estimated that over 30% of office workers in Germany have suffered from sick building syndrome (4). Another study found that children exposed to formaldehyde levels of 60 $\mu g/m^3$ are 39% more likely to develop asthma compared to children exposed to formaldehyde levels less than 10 $\mu g/m^3$ (5). People at the highest risk of developing these conditions usually work in industries that deal with the substances listed above, for example, paint or printing factories. Additionally, people who live in new or renovated homes are more likely to be exposed to substances that give off VOCs. A pro-inflammatory cytokine pattern with increased levels of IL-6 and TNFα was found in blood samples of children after indoor renovation activities, particularly in the presence of new floor covering (6). Children are also more likely to be negatively affected by VOCs because they inhale a greater volume of air than adults relative to their body size. Also, their organs, respiratory, immune, and neurological systems are still developing, and children are more likely to breathe in airborne chemicals because they are closer to the ground and/or flooring (7).

Research has indicated that certain plants have the ability to reduce the amount of VOCs in the air through a process called phytoremediation (8, 9, 10, 11). Phytodegradation, a specific form of phytoremediation, involves the breakdown of complex contaminant molecules into simple molecules which can then be used by the plant or incorporated into plant tissue. Unpublished mass spectrometry data from my laboratory (Figure 1) indicated that incubation of plant extracts with the VOC 2-phenyl ethanol decreases a peak at an m/v ratio of 6.46 to 6.49 by 60% over the course of five days, thus supporting the phytodegradation hypothesis. The findings provided validation of the experimental approach used in my project.

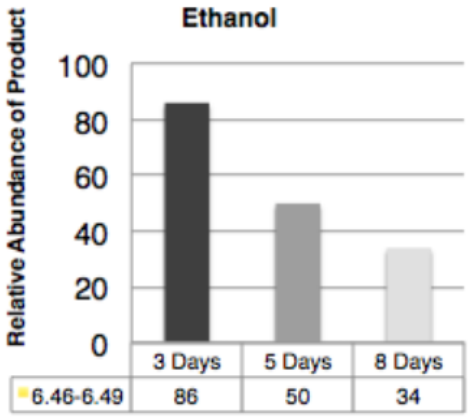


Figure 1. *GC-MS results support the mechanism of phytodegradation for the volatile organic compound 2-phenyl ethanol*. A peak at a m/v ratio of 6.46-6.69 decreases in abundance proportionally.

As plants are also living organisms, plants absorb these chemicals when the contaminants' solubility and hydrophobicity fall into a certain acceptable range (11). For example, formaldehyde can be metabolized to organic acids, amino acids, free sugars, lipids and cell-wall components, and benzene and toluene are generally removed from air and assimilated by plants and leaves by way of their spongy mesophyll (10). Researchers have also found that the formaldehyde-removal capacity of the plants depends on the dehydrogenase activity in the leaves

and root system or how efficiently the plant could metabolize formaldehyde (12). These plants can uptake the VOCs through their roots or leaves, and usually then convert the VOCs into molecules that can be used by the plants (10). I will be focusing specifically on the *Chlorophytum comosum* plant, more commonly known as a green spider plant because of its ubiquity and scalability in indoor environments. I first hypothesized that *Chlorophytum comosum* would eliminate various VOCs from the environment and sought to confirm the uptake of those VOCs by the plant. Secondly, my goal was to determine whether the plant decreased inflammatory markers in human cell lines exposed to VOCs. In summary, this study could help ameliorate the harmful effects of certain VOCs through phytoremediation.

METHODS

Determining the Retention Times of Volatile Organic Compounds

To determine that the VOCs were composed of just one species, A Vernier© Gas Chromatograph was controlled via computer using the proprietary Logger Pro program. Before injecting the chemicals, the chemicals were soaked in sodium sulfate for 12 hours to absorb all of the water. The needle was cleaned out using acetone, and then used to insert 0.2 µL of each chemical into the gas chromatograph. The chemicals used were acetone, methanol, ethyl acetate, and formaldehyde. Simultaneously, while the chemical was being injected, the computer started to collect data. The data collection was stopped after a peak had been detected. The syringe was then cleaned again with acetone and the next sample was analyzed. All reactions took place under a fume hood. Safety precautions included lab coat, gloves, goggles, and closed toe shoes.

Incubating Chlorophytum comosum With Methanol, Ethyl Acetate, and Acetone

To determine if plants could uptake VOCs into their tissues, dehydrated methanol, acetone, and ethyl acetate were incubated with and without spider plants. VOCs were placed in sealed labeled mason jars that contained a spider plant. The jars incubated for 72 hours in direct sunlight. Both before and after the 72 hours, the initial and final masses of the VOCs were weighed to quantify the amount of VOCs that were potentially absorbed by the spider plants.

Formaldehyde Clearance Assay

10 mL of 37% formaldehyde was placed in a closed plastic container for 1 hour to increase the concentration of formaldehyde in the container. A spider plant was placed in an airtight container sealed with paraffin film with dimensions of 60 cm x 41.6 cm x 33.7 cm with 0.30 mg/m^3 of formaldehyde, or 0.24 ppm of formaldehyde. The soil was removed from the spider plant to ensure that no soil microbes could account for the uptake of the formaldehyde. This amount was chosen because it is greater than 0.10 ppm, or the most common guideline for acceptable formaldehyde levels according to the Minnesota Health Department. Also, it has been found that some products and construction materials may emit formaldehyde at levels above 0.10 ppm especially when they are new. A handheld precision formaldehyde monitor from idiytool.com was placed on a stand inside of the container. The container was then sealed and paraffin film was wrapped around the container to ensure formaldehyde would not leak out of the environment. The concentration of formaldehyde was then measured at various intervals.

Procedure for Making Spider Plant Extract

12.32 grams of plant matter was homogenized with 200 mL of deionized water in a Nutrabullet blender for 60 seconds. The mixture was then centrifuged at 1,000 rpm for 10 minutes to separate the solid plant matter from the liquid (supernatant). The supernatant was kept frozen at -20°C before treating the U937 cells.

Treatment of U937 Cells

Experimental groups consisted of 3 µL formaldehyde, 3 µL formaldehyde with 10 µL or 100 µL of plant extract, and 10 µL or 100 µL of plant extract alone. U937 cells (ATCC® CRL-1593.2™) were placed in six well culture plates. These cells were incubated with DMEM media supplemented with 1% fetal bovine serum for 24 hours at 37°C with 5% carbon dioxide.

RNA Isolation

To homogenize the cells, 1 mL of TRI Reagent was added and the cell lysate was passed 15 times through a 1,000 µL pipette and then all of the homogenate was transferred into a 1.5 mL microcentrifuge tube. The homogenate was then stored for 5 minutes at room temperature to permit the complete dissociation of nucleoprotein complexes. The samples were covered tightly and vortexed for 15 seconds. The resulting mixture was stored at room temperature for 7 minutes and centrifuged at 12,000 rpm for 15 minutes at 4°C. Following centrifugation, the mixture was separated into a lower red phenol-chloroform phase, interphase, and the colorless upper aqueous phase. The RNA remained exclusively in the aqueous phase whereas the DNA and the proteins were in the interphase and the organic phase. The volume of the aqueous phase was about 60% of the volume of the TRI Reagent used for homogenization.

The aqueous phase was transferred to a fresh 1.5 mL microcentrifuge tube and the interphase and organic phase was saved at 4°C for subsequent isolation of DNA and proteins. RNA from the aqueous phase was precipitated from the aqueous phase by mixing the aqueous phase with isopropanol and then inverting the tube several times. 0.5 mL of isopropanol was added per 1 mL of TRI Reagent used for the initial homogenization. The samples were stored at room temperature for 5 minutes and were then centrifuged at 12,000 rpm for 8 minutes at 25°C. The RNA formed a gel like pellet on the bottom of the tube. The supernatant was removed and the RNA pellet was washed with 1 mL 75% ethanol by vortexing. Subsequently, the mixture was centrifuged at 7,500 rpm for 5 minutes at 25°C. The ethanol wash was removed and the RNA pellet was briefly air-dried for 5 minutes. The RNA pellet was dissolved in RNase free water by passing the solution several times through a pipette tip. The dissolved RNA was incubated for 10 minutes at 55°C and then placed immediately on ice. The RNA was saved at -70°C if not immediately used. A 1% gel was run to determine the quality of the RNA, while spectrophotometry was used to determine the quality of the RNA (measurements are recorded below in Table I).

Sample	260 Reading	280 Reading	Concentration of RNA (µg/µL)	RNA (µL)	H_2O Added (µg)
Control	0.549	0.306	0.439	8.66	1.3
3 µL Formaldehyde	0.639	0.342	0.511	7.44	2.6
100 µL Plant Extract	0.479	0.272	0.383	10.0	0
10 µL Plant Extract	0.555	0.270	0.444	8.56	1.4
3 µL Formaldehyde and 10 µL Plant Extract	0.887	0.518	0.710	5.35	3.6
3 µL Formaldehyde and 100 µL Plant Extract	0.824	0.465	0.659	5.77	4.2

Table 1. *RNA concentrations from U937 Cells.* Spectrophotometric data, indicating RNA purity was collected before proceeding to RT-PCR.

cDNA Synthesis- Reverse Transcription (RT)

Between 1 and 5 µg of RNA was added to a 0.2 mL PCR tube and then the volume was brought to 10µL with RNase/DNase-Free water. The RNA was then denatured at 95°C for 5 minutes in a Bio-Rad thermocycler and was then immediately placed on ice for at least 1 minute before it was used for the RT reaction. The following components were added to the 0.2 mL PCR tube: 8 µL dNTP's, 4 µL 5X First strand buffer, 2 µL 0.1M DTT, 1 µL Random primer, 1 µL RNase Inhibitor, and 1 µL Reverse Transcriptase. Then the samples were incubated in a Thermocycler programmed for 60 minutes at 40°C and then 10 minutes at 65°C. After the reaction was done the tube was placed on ice. The RT product was stored at -20°C for later use.

Polymerase Chain Reaction (PCR) for TNFα cDNA

The following components were added to a 0.2 mL PCR tube: 5µL 10X PCR buffer, 1.5 µL $MgCl_2$, 2 µL dNTP's, 1 µL Forward primer, 1 µL Reverse primer, 10 µL RT product (cDNA), 29 µL RNase Free water, 29 µL RNase Free water, and 0.5 1 µL Taq DNA Polymerase. Then, the samples were incubated in a thermocycler programmed: 95°C for 30 sec, 53°C for 30 sec, 72°C for 30 sec (30 cycles), next 72°C for 10 min (1 cycle), and then 4°C (soak cycle).

Gel Electrophoresis

A 2% agarose gel was made with Tris/Borate/EDTA (TBE) buffer containing 5µL ethidium bromide (2g agarose, 60 mL TBE buffer). 5 µL of 6X DNA loading buffer was mixed with 30 µL of the PCR product in a 0.5mL tube. 30 µL of this mixture for each sample and the molecular weight marker were loaded into the gel wells and the gel was run at 100 volts. The gel was viewed on a Transilluminator under Ultraviolet light and the size of the band observed was determined. Densitometry of cDNA bands was performed with ImageJ software.

RESULTS

Gas Chromatography Results

To determine the purity and identity of the volatile organic compounds, methanol. acetone, and ethyl acetate, the volatile organic compounds were injected into a Vernier© Mini-GC gas chromatograph to measure the retention times and compare them to known standards. The retention times in the column were in direct proportion to the boiling points for these molecules; 132.8°C, 148.5 °C, and 170.8°C for acetone, methanol, and ethyl acetate respectively (Figure 1). The retention times on the column were also as predicted; 60, 75, and 225 seconds for acetone, methanol, and ethyl acetate. Mixtures of the VOCs were also assayed using the gas chromatograph, which confirmed that the molecules separated at different times, in line with their boiling points and vapor pressures.

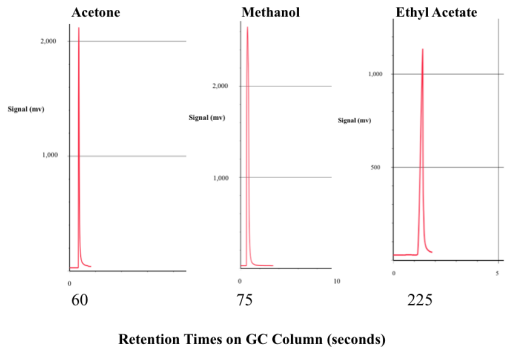


Figure 2. *After the volatile organic compounds were injected into the gas chromatograph, the vaporization time of the volatile organic compounds were measured*. This directly correlates to the composition of each molecule, specifically the intermolecular forces that hold them together. The gas chromatograph separates chemicals in a silica column based on their boiling points and the GC continuously measures an electrical potential (m/v) for fractions eluted from the column.

To test the first hypothesis, that that VOCs were cleared through uptake into the plant tissues, methanol, acetone, and ethyl acetate were placed in sealed mason jars with equal amounts of spider plant leaves (Figure 3). Jars contained calcium chloride as a desiccant to prevent water absorption by VOCs. The change in mass of the VOC after 72 hours was recorded.

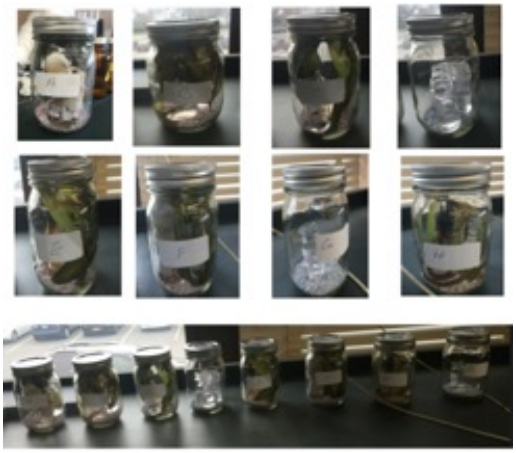
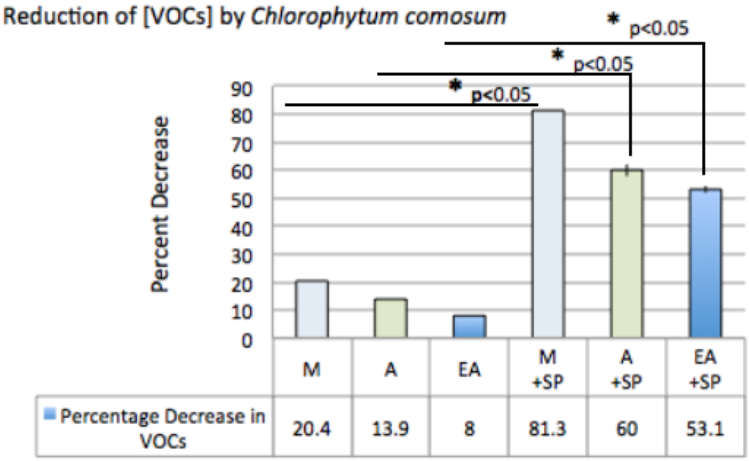


Figure 3. *Reduction of VOCs by Chlorophytum comosum*. Before the jars were sealed, the plants and the volatile organic compounds to be placed in the jars were weighed. After the jars had been sealed for three days, the volatile organic compounds were weighed again to determine the amount of volatile organic compounds that were taken in and reduced by the plant.

Figure 4. This graph quantifies the results of the experiment. M (methanol), A (acetone), EA (ethyl acetate), SP (spider plant). The labels in the figures below correspond to the following conditions; Jar A contained M+SP, Jar B contained EA+SP, Jar C contained SP+A, Jar D contained M alone, Jar E contained A alone, Jar F contained EF alone, Jar G contained M+A +EA, Jar H contained M+A +EA+SP.



As shown in Figure 4, VOCs decreased in mass from 8 to 20.4 percent over the course of 72 hours, due to either diffusion into the sealed jar or leakage from the jar. However, the percentage decrease in VOCs was significantly higher in the groups containing plant leaves. Methanol decreased by 81.3%, acetone decreased by 60% and ethyl acetate decreased by 53.1%. Ethyl acetate, which has the highest boiling point and lowest vapor pressure of the three VOCs, was reduced to a lesser extent, as expected, than the other VOCs. Interestingly, methanol was reduced to a greater extent than acetone (81.3% vs. 60% respectively). This may reflect a greater ability of the plant tissues to absorb or phytodegrade or absorb methanol relative to acetone.

Formaldehyde is an environmental toxin found in many home products. Therefore, the ability of spider plants to absorb formaldehyde was tested. Rather then measuring decline in mass of the VOC, a formaldehyde specific meter was used. This technique has the advantage of delivering accurate readings regarding the actual concentration of formaldehyde remaining in the air, after incubation with *Chlorophytum comosum*. Figure 5 shows the setup of the spider plant, formaldehyde meter, and formaldehyde source (originally 10 mL of formaldehyde in a graduated cylinder) in the sealed plastic container. While the spider plant was in the sealed container filled with gaseous formaldehyde, a formaldehyde monitor determined the concentration of formaldehyde in the atmosphere. The formaldehyde concentration was found to decrease exponentially with time (Figure 6). It took about 48 hours for the formaldehyde to decrease to concentrations that are not hazardous, and about 168 hours for the formaldehyde to be almost entirely consumed by the spider plant.

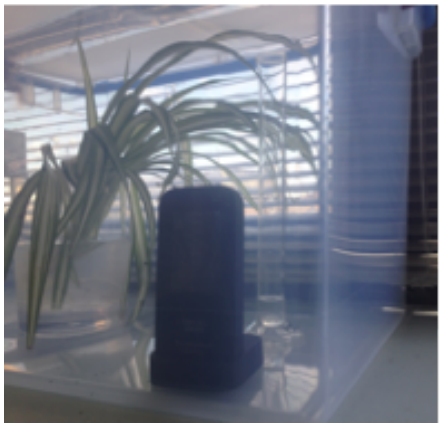


Figure 5. This shows the setup of the spider plant, formaldehyde meter, and formaldehyde source (originally 10 mL of formaldehyde in a graduated cylinder) in the sealed plastic container (inset). Graphical representation (bottom).

Concentration of Formaldehyde (mg/m^3)	**Time (hours)**
0.3	0
0.17	24
0.11	48
0.06	72
0.01	168

Table 2. *Formaldehyde Decrease as a Function of Time.* After 48 hours levels have reached what is considered by the EPA to be an environmentally safe level.

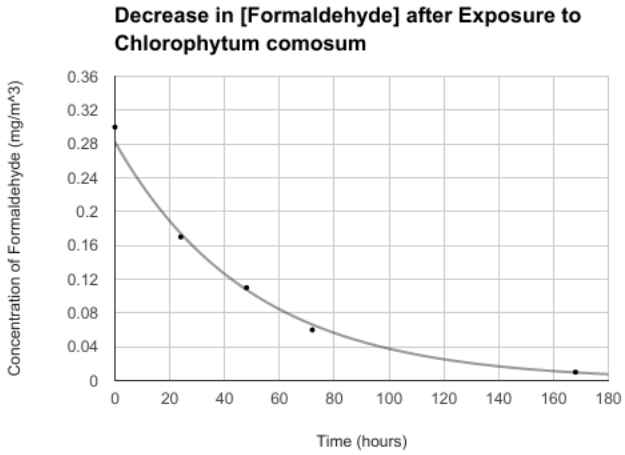


Figure 6. *Formaldehyde concentration decreases exponentially with time*. It took about 48 hours for the formaldehyde to decrease to concentrations that are not hazardous (under 0.10 mg per cubic meter), and about 168 hours for the formaldehyde to be almost entirely consumed by the spider plant.

RT-PCR for TNFα Gene Expression

To determine the effects of formaldehyde and the spider plant extract on inflammation in the human cells, human U937 cells were incubated for 24 hours with formaldehyde, which is known to induce tissue inflammation. The cells were also incubated with increasing concentrations of spider plant extract. Then, a reverse transcriptase polymerase chain reaction was conducted using TNFα specific probes to generate cDNA, indicating levels of transcription of the gene in response to formaldehyde. After using gel electrophoresis to determine the results, it was found that TNFα cytokine expression increased when U937 cells were incubated with formaldehyde compared to the control sample. Incubation with plant extract alone had no significant effect on TNFα expression. Conversely, TNFα expression decreased in a dose dependent manner upon addition of spider plant extract (Figure 7).

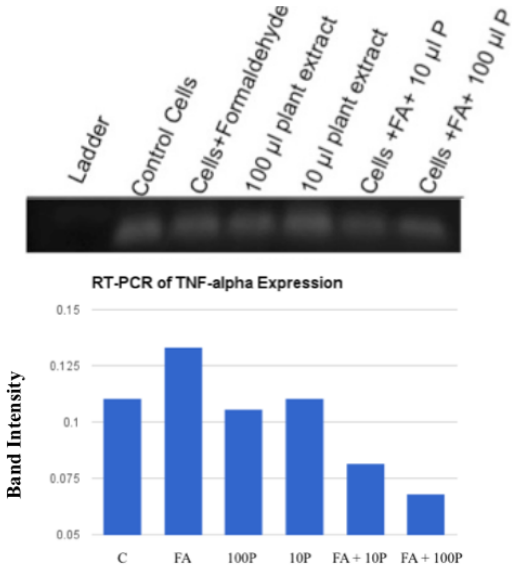


Figure 7. In this graph, C is the control sample, FA the sample incubated with 3 µL of formaldehyde, 100P the sample incubated with 100 µL of spider plant extract, 10P the sample incubated with 10 µL of spider plant extract, FA + 10P the sample incubated with 3 µL of formaldehyde and 10 µL of plant extract, and FA + 100P the sample incubated with 3 µL.

DISCUSSION

Gas Chromatography

Compounds that were more volatile, or had lower boiling points, had shorter retention times in the Vernier© gas chromatograph. VOCs with shorter retention times have higher vapor pressures, meaning that less VOCs with short retention times are found evaporating off of the substances that emit them.

Reducing the Amount of Acetone, Methanol, and Ethyl Acetate

The results from this experiment show that spider plants decrease the amount of VOCs in the environment by absorbing them and then breaking them down into compounds that can be used by the plants. The amount of methanol decreased by 74.81%, the ethyl acetate decreased by 53.19%, and the acetone decreased by 95.6% when placed in a mason jar with a spider plant. The mixtures of methanol, ethyl acetate and acetone decreased on average 82.05%. More acetone was absorbed and broken down by the spider plant because acetone has a short retention time, and therefore a high vapor pressure, which was determined using the gas chromatograph. This short retention time and high vapor pressure shows that the acetone evaporates faster and, therefore, more could be absorbed by the spider plant over the course of three days. Accordingly, less of the ethyl acetate, which had a longer retention time, was broken down by the spider plant. More methanol was absorbed than ethyl acetate, even though methanol has a longer retention time than ethyl acetate, because the spider plant used to absorb the methanol was approximately 20% larger than the spider plant used to absorb the ethyl acetate. Also, the polarity of methanol contributed to the longer retention time of methanol as determined by the gas chromatograph. The results, especially those from the three trials of the mixture of acetone, ethyl acetate and methanol, also show that the larger spider plants absorbed more VOCs. Therefore, it can also be concluded that the size of the spider plant contributed to the amount of VOCs that were absorbed because they had more surface area. Initially, all three substances were placed into one jar to see if the plant did indeed have a large effect on the amount of VOCs absorbed. When the jars were weighed, it was found that the methanol and ethyl acetate had actually increased and that the acetone had decreased by a significant amount. From this it was concluded that the methanol and ethyl acetate absorbed the acetone. The test was perfected by putting the three substances in individual mason jars to ensure that the acetone, methanol, and ethyl acetate were not just simply evaporating. The percent decreases for each jar were calculated by finding the difference between the original and final weights of the liquid chemicals, dividing that number by the original weight and then multiplying by 100. When no plant was present, acetone decreased by 13.70% as opposed to when it was in the presence of the spider plant and decreased by 95.6%. As for methanol in the trial without a spider plant, it decreased by 20.61% as opposed to with the plant when it decreased by 74.81%. Finally ethyl acetate decreased by 10.64% without the plant as opposed to 53.19%. It is important to note that even without the plant, the reason that the acetone, methanol, and ethyl acetate did decrease was due to the fact that it simply evaporated. In the trials where the plant was present it is very possible that some of the substances were lost due to evaporation as well. However, from these findings it can be concluded that spider plants do indeed absorb VOCs as noted by the fact that the trials where the plant was present saw a significantly higher absorption of VOCs as opposed to the trials where the plant was absent.

The data of percent decreases for the trials with methanol, acetone, and ethyl acetate is significantly different statistically. The statistically significant difference was calculated using a two sample t-test by first creating the null hypothesis that the difference between the percent decreases in the average of the samples with the plant and the average of the samples without the plant is 0. The alternative hypothesis was that the difference between the two percent decreases was not 0. Next, the mean percent decrease of the VOCs contained with a spider plant was found

to be 78.3%, while the mean percent decrease of the VOCs not contained with a spider plant was found to be 23.1%. Then, the standard deviations of these percent decreases were found to be 15.5 and 18.8, respectively. The t-statistic was then found to be 4.87. With a t-score so high, the p-value is less than 0.001. This p-value allows for the rejection of the null hypothesis and the conclusion that the spider plant made a difference in decreasing the VOCs.

Reducing the Concentration of Formaldehyde

When the quantity of formaldehyde in the air was monitored, the concentration of formaldehyde also significantly decreased when spider plant, compared to when no plant was present (two sample t-test ($p < 0.001$) when comparing formaldehyde concentrations after various time intervals). After a little over two days, the concentration of formaldehyde was down to a level safe to be continuously breathed in, or 0.1 mg/m^3. After three days, the amount of formaldehyde had decreased by 80%. By 168 hours, or one week, the formaldehyde was virtually gone, having decreased 97% to 0.01 mg/m^3.

The best relationship for the decrease in the concentration of formaldehyde over time was found to be an exponential relationship. The R^2 value, which measures the amount of variance, was calculated to determine how well the exponential relationship fits the data. Variance measures how far a set of data is from the mean. A variance of zero indicates that all the data points are identical. A small variance displays that the data points tend to be very close to the mean, which is the expected value or average, and therefore shows that the data points are close to each other. Contrarily, a high variance shows that the data points, or numbers, are very spread out around the mean and from the other data points. The variance of a set of data is able to describe the probability distribution of that data. The R^2 value was calculated to be 0.998 for the exponential equation $y = 0.282e^{-0.02x}$. This means that 99.8% of the total variation in Y can be explained by the exponential relationship between X and Y. Although the coefficient of determination is not a perfect 1, this coefficient is very close to 1, displaying that there is a high correlation in the data. The relationship was exponential because the spider plant, as a living organism, could only take in so much formaldehyde without poisoning itself. Also, as the concentration of formaldehyde decreased, there was less formaldehyde in the air for the plant to absorb.

The retention time of formaldehyde is not as short as some of the retention times of other VOCs, like alcohols. Because of this, it would be more feasible for a plant extract to be applied to a material containing formaldehyde to reduce the amount of formaldehyde vaporizing off of the surface of the material before those VOCs are inhaled.

TNFα RNA Expression

The cells were incubated for 24 hours to allow for the formaldehyde and plant extract to start taking effect on the cells, and in the plates where the two were together, each other. The increase in TNFα levels due to the addition of formaldehyde to the cells was expected because previous *in vitro* studies involving formaldehyde have suggested that formaldehyde causes inflammation, which leads to some of its adverse effects such as difficulties breathing (6). This experiment, however, found that the spider plant extract alone does little to decrease the inflammatory cytokine. The spider plant extract also was not toxic to the cells, which was seen in that the cells were able to grow normally when incubated with the extract and the amount of TNFα did not increase. When the spider plant extract was incubated with the formaldehyde, it was found that TNFα decreased when compared to all of the other samples (39% and 46% for 10P and 100P respectively when compared to FA, two sample t-test ($p < 0.001$ for both 100P and 10P added to FA). This means that the spider plant extract was able to reduce, even reverse, the inflammatory effects of formaldehyde. It can be concluded that the cells incubated with the 3 μL of formaldehyde and 10 μL of plant extract contained more TNFα than the cells that were incubated in 3 μL of formaldehyde and 100 μL of plant extract because the greater amount of plant extract was able to reduce more formaldehyde, mitigating the inflammatory results of formaldehyde.

There have been no previous studies concerning the reduction of formaldehyde using a spider plant extract *in vitro*. This research was novel in determining the effect of a plant extract on VOC induced inflammation.

CONCLUSION

These results ultimately demonstrate that spider plants can help to purify the environment by ridding it of toxic VOCs, decreasing the harmful effects of these compounds in the environment as well as *in vitro*. In the future, I would like to incubate the cells for longer lengths of time, for example one week, to try to simulate longer term exposure to VOCs like formaldehyde without killing the cells. I would also like to determine exactly why the TNFα levels decreased so much when the plant extract was added to the formaldehyde compared to when the plant was added alone to the cells. Additionally, I would like to see how well the spider plant extract would work when used to treat substances that give off formaldehyde, such as flooring.

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