AN INVESTIGATION OF THE UV LIGHT ABSORBANCE QUALITY OF ZINC OXIDE NANOPARTICLES IN PHOSPHOLIPID CARRIERS

By

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Abstract

Zinc oxide (ZnO) nanoparticles were studied for their lethality towards bacteria. However, ZnO also has a unique double-bond system that also allows them to absorb and pacify the carcinogenic effects of UV radiation. Under the intense sunlight of California, robust protection against UV damage may be achieved through ZnO nanoparticles delivered through liposomes. In the effort to incorporate zinc oxide nanoparticles into a phospholipid bilayer, the nanoparticles may be loaded into phospholipid-based nanoparticle carriers. After the fabrication of zinc oxide-containing particles, the success of the incorporation will be ascertained through observation of their UV absorbance. Following the procedure, the zinc oxide-loaded carriers will be tested on plant leaves through spectroscopy measurements that compare the wavelength of UV rays absorbed across leaves with and without Zinc Oxide Loaded liposomes. If loaded carriers provide a significant reduction in UV light absorbance upon UV light exposure, then, we would see a significant difference in peak intensity between the UV absorbance of plant tissues with zinc oxide-containing liposomes and plant tissues that did not. The success of the zinc oxide nanoparticles will not only provide a new way for anti-UV radiation methods to be introduced into plants but it may also be used in dermal applications to reduce UV light exposure in animal tissues.

Acknowledgment

Under the mentorship of Anvari Lab, I developed professional skills that contributed to the completion of my project. During my capstone experience, Doctor Bahman Anvari gave me crucial advice on both the procedures of my experiments as well as the methods through which the results are analyzed. In addition, I would like to give special thanks to Chi Hua Lee for introducing me to Anvari Lab as well as guiding me through the formation of my initial project ideas. Furthermore, I would like to thank Shimima Zamman for mentoring me during lab hours and providing oversight for all of the chemical procedures I used throughout my capstone project. Lastly, I would like to reiterate that this undergraduate endeavor was a fantastic experience that would not have been the same without the attentive support from all members of Anvari Lab.

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Introduction

Zinc oxide (ZnO) exhibits antibacterial and UV-absorbing effects due to a zinc and oxygen covalent bond that allows photocatalytic activities. Through the wide bandgap of the bond, the molecule can absorb UV radiation effectively at the wavelengths of 250 nm to 400 nm (Sirelkhatim, A., Mahmud, S., Seeni, A. *et al. 2015)*. Cosmetic companies have been taking advantage of "the positive benefit of engineered nanoparticles for use in cosmetics and as tools for understanding skin biology and curing skin disease," where the UV-absorbing effects of zinc oxide nanoparticles are incorporated into skin care products (De Louise L.A., 2012). A novel way of utilizing zinc Oxide nanoparticles for UV absorption that are not mentioned in previous studies is to include them within lecithin liposomes. As a simple platform based upon either animal or plant products, lecithin can be cheaply acquired and its liposome product, cheaply synthesized. In the study done by Cao D. and contributors, Zinc oxide nanoparticles were studied whilst encased in liposomes. Following the same principles, liposome is a promising carrier for ZnO nanoparticles that facilitates absorbance studies of ZnO within tissues. In an article presented by Bengalli, R. and contributors, the article describes an experiment that tests the effect that zinc Oxide nanoparticles have on the stability of red blood cells (Bengalli, R. et al., 2017). In the experiment, the study found that 97 percent of red blood cells remained intact after exposure to concentrations of zinc Oxide nanoparticles. Hence, proving that zinc oxide is a biologically safe substance to be used within tissues. Although ZnO nanoparticles may exhibit UV absorbance qualities within animal tissues, this study will focus on the absorbance effect of

lecithin liposomes encased around ZnO nanoparticles on plants. Previous studies on the effect that zinc oxide has on plants have been shown " to improve the growth and yield of Abelmoschus Esculentus," where the anti-microbial and growth-supporting properties of zinc oxide seemed to facilitate plant development (P., K., S., V., E., V., V.N, P., M., N., & PK, P. 2021). However, no further studies have been conducted on a similar basis that examines the UV absorbance qualities of ZnO nanoparticles within plant tissues. Encased within lecithin liposomes, ZnO nanoparticles are a nontoxic UV-absorbing compound that holds the potential of providing a safe way for plants to evade the harmful effects of UV radiation.

Using known synthesis pathways of both ZnO nanoparticles and lecithin liposomes, this study will examine the degrees to which ZnO nanoparticles change the absorbance spectra of Calabrese Broccoli. In contemporary studies, it has been found that ZnO absorbs UV light in the wavelengths of 250 nm to 800 nm, and in sizes of 5-6nm, the absorbance spectra have significant peaks from 360 nm-365 nm (Cao D. et al., 202). To examine the effect that ZnO has on the UV absorbance of light around the natural absorbance range of ZnO; leaves injected with ZnO nanoparticles were compared to control leaves in the absorbance range between 320 nm and 360 nm. In addition, a study on the growth rates of leaves that were treated with ZnO and leaves that were untreated was conducted. The absorbance data as well as the study on plant growth rates showed whether or not the ZnO nanoparticles and its liposome carrier can create a difference in the intensity of UV lights absorbed by the plant and determine if the modified UV absorbance intensity generates a .difference in plant leaf growth rates.

Design and Methodology

To synthesize the liposome carrier for the ZnO nanoparticles, egg lecithin was the first compound that was considered due to its cheap and easy synthesis procedure. First, fresh eggs were acquired from Costco. Next, the yolk was separated and combined with 15 ml of acetone inside a 100 mL beaker. After mixing and resting for 15 minutes, the resulting solution was centrifuged under 4000 rpm for 5 minutes. After centrifugation, the resulting precipitate was kept while the supernatant was discarded. The precipitate was washed with acetone again with enough acetone to submerge all of the precipitates. The precipitate with acetone was then filtered through a coffee filter that was folded into a cone. The residue was washed with acetone until it lost its color and turned white. After transferring the precipitate into an Erlenmeyer flask, a 2:1 ratio of chloroform and ethanol was added where 40 ml of chloroform was combined with 20 ml of ethanol. After mixing, the solution was left to rest for 3 hours. The resulting solution was filtered using coffee paper and the resulting precipitate was collected in a clean 200ml beaker and air dried. The completely dried filtrate was washed with petroleum ether and acetone. When petroleum ether was first added, enough petroleum ether was introduced to cover the precipitate. After the addition of petroleum ether, the solid precipitates were suspended from the bottom of the beaker. Next, acetone was added until the suspended solid was resettled to the bottom of the flask. During the procedure, a layer of oily substance settled on the bottom and the supernatant was discarded. This process of dissolving the precipitate and washing away the supernatant to expose the oil at the bottom of the flask had the function of purifying the oil. Thus, this purification step was repeated three times. After the oil resettled and dried, 0.0515g of the product was dissolved in 4.95 mL of methanol and the UV absorbance of the solution was

measured. Next, the egg lecithin residue was left to dry for one week in a petri dish. However, the oil cracked and seeped into the petri dish, causing the petri dish to crack. Thus sunflower lecithin in powdered form was used to form the liposomes instead.

To synthesize the ZnO nanoparticles, a protocol was adapted from a paper written by Penny S. Hale and contributors. To begin, Zinc acetate dihydrate was purchased from Walmart. The crystalline powder of 0.10g of zinc acetate dihydrate was combined with 25 mL of Walmart 75 percent isopropanol to create an initial solution in a 300 mL beaker that was heated to 65 degrees Celsius. A spinner was added and the solution was heated and spun for 15 minutes under 65 degrees Celsius. Next, a large tube of ice was used to chill 125ml of isopropyl alcohol and 0.05M of NaOH in 15 ml of isopropanol. The initially dissolved zinc acetate dihydrate was mixed with 125ml of isopropanol. After mixing, the solution was heated to 64 degrees Celsius and the 0.05M NaOH was added slowly to the mixture. The mixture was stored in a glass container, caped, and wrapped with parafilm.

To prepare the sunflower lecithin, 0.0515g of the powder was mixed with 4.95 mL of methanol. The particles were assumed to have charges similar to that of proteins. Thus, this parameter was used to determine the size of the liposomes, which was 1533 nanometers in diameter. After the size was determined, the liposomes were mixed with the ZnO nanoparticles in a 2:1 ratio in the same manner as the procedure carried out by Cao D. and contributors (Cao D. et al., 2020). Zinc acetate dihydrate has a molar mass of 219.49 g/mol. In the solution where 0.1 grams of zinc acetate dihydrate was used, the molarity of the solution was 0.00284 M. The vial that was used to store zinc oxide has a maximum volume of 300 mL. Thus, a 200 mL ZnO solution was used to make the injection solution. This yields 0.0462 g of ZnO and thus the mass needed for liposomes is 0.0231 g. The liposomes solution was made using 0.0515 g of powder

and 4.95 mL of methanol. Hence, to achieve 0.0231 g of ZnO, the volume needed is 2.22 mL. Thus, 200 mL of ZnO was used in combination with 2.22 mL of sunflower lecithin solution to achieve the initial injection solution.

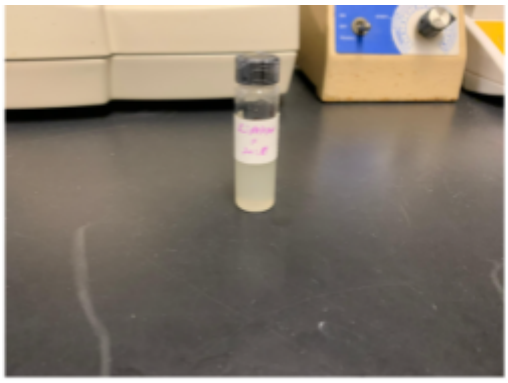


Figure #1 ZnO and Liposome solution in methanol and isopropanol

After sonication for 5 minutes at 2 seconds on and 2 seconds off, the solution was then centrifuged. 600 microliters of the solution were centrifuged at 2000 RPM for 10 minutes. After centrifugation, the supernatant containing methanol, and isopropanol was ejected. In its place, 600 microliters of nanopore water were added. The addition of nanopore water followed by vortex mixing for 30 seconds resulted in the final injection fluid that was used for absorbance testing of the solution itself as well as the UV absorbance qualities of the injected plants.

Brassica Oleracea was used as the platform upon which the growth study was conducted. First, three leaves were chosen where there existed three other leaves of similar sizes. At the start of the study, the leave sizes were grouped into 4 groups. The four leaf groups were labeled as Leaf 1, Leaf 2, Leaf 3, and Leaf 4. First, a contemporary leaf was used to examine the absorbance quality of untreated leaves. To accomplish this, a leaf was crushed in pesto and mortar with water added to ensure that the consistency of the solution was diluted enough for absorbance measurement. Next, using the insulin syringe, 300 mL of the ZnO liposome solution was injected into different leaves. Three injection techniques were attempted. First, the needle

was inserted into the blade of the leaf, where the injection site occurred right below the base of the largest vein adjacent to the midrib. This injection method was unsuccessful due to the ease with which the needle was able to over-penetrate the leaf. Next, the injection was done on the thickest part of the midrib. About one third of the way down from the base of the leaf, the injection ended in partial failure as the leaf experienced leakage further down the midrib. Finally, a consistent method was reached that involves both injecting at the middle of the midrib as well as the base of the petiole.

Figure #2: Here, injection site is at the base of the petiole

Prior to injection, the density of the ZnO and liposome injection fluid was determined through the average weight of the sample as well as the volume that was used. The average weight of the sample was 0.492 grams and the volume that the weights correspond to was 500 microliters. Thus, this gives a density of 0.9843 g per milliliter. When the leaves were injected, unsuccessful injection volumes were collected through a plastic weighing plate that is shown in the picture above. Subtracting the mass of the plate, the mass of the fluid that leaked out of the plant leaves during and after injection was collected and recorded. With the known density of the injection fluid, the volume of ZnO nanoparticles and its encasing liposomes was quantified. Using the proven method of injection, three leaves were injected and subsequently mashed for absorbance measurements. The first leaf that was injected was not subjected to the determination of the

amount of fluid that was actually injected. The following two leaves have the volume of fluid injected determined through the weight of the fluid that leaked from unsuccessful injections. The first leaf was recognized as the leaf that was left on the plant for 30 minutes after injection and the second leaf was recognized as the leaf that was cut off immediately after injection. In this case, the leaf that was left on for 30 minutes had an injection volume of 123 ml while the leaf that was immediately cut off had an injection volume of 221 ml. To determine the UV absorbance of the injected leaves, both the leaf that was left on for 30 minutes before it was cut off and the leaf that was cut off and measured immediately after injection were mashed and had its juices extracted.

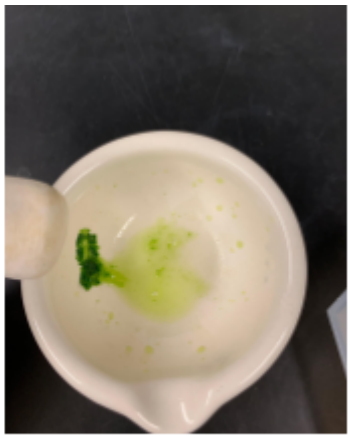


Figure #2.5 This is the method of mashing the leaves

During the procedure to extract the liquid from the leaves, a difference in extracted liquid concentrations was encountered. For both leaves, the amount of nanopore water used to mash the leaves was 100 microliters. In the case of the leaf that was cut off immediately after injection, the 250 microliters of plant juice that was extracted were mixed with 1500 microliters of nano-pure water to achieve a diluted solution that was suitable for UV absorbance measurement. The leaf that was left on for 30 minutes followed the same conditions. However, only 500 microliters of additional water were needed to achieve a similar amount of transparency in the solution that

allowed for an optimal level of measurement parameters. In this case, the level of transparency in the extracted leaf juice was qualitatively determined through the color of the leaf juice solution.

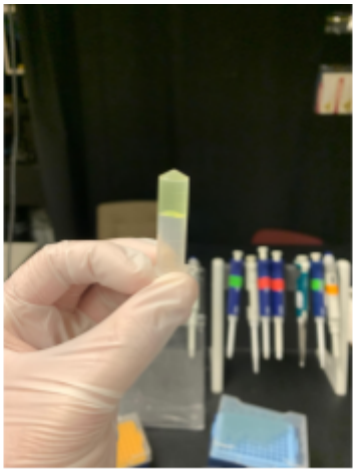


Figure #3 This is the concentration needed for absorbance measurement

After the initial injections, four leaves were chosen to conduct a growth test. Two leaves failed during the injection procedure. The two successful leaves have two other leaves that are of similar sizes which acts as the control. The two successfully injected leaves also had the amount of ZnO liposome solutions determined. After the injections, the leaves were left by the side of a window and received constant natural sunlight throughout the day. Data recording was done over the course of two weeks and measurements were taken every 12 hours with the exception of the first two data sets where measurements were taken every 2 hours instead.

Results and Discussions

The initial test of the ZnO nanoparticle solutions had the highest absorbance located at 358 nm. This falls within the normal range of literature values of ZnO, where 320 nm to 360 nm is the accepted value. To encase the ZnO nanoparticles, sunflower lecithin was used to make the liposomes. To ensure that sunflower lecithin is a good replacement for egg lecithin, literature values of sunflower lecithin were compared with the measurement taken for the synthesized egg lecithin.

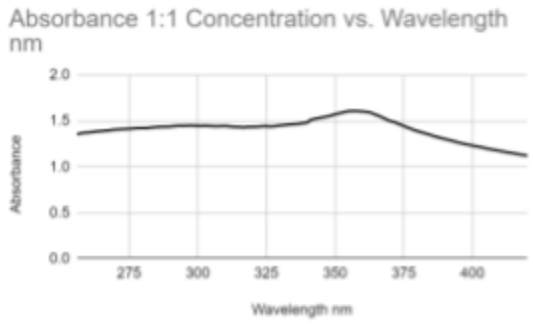
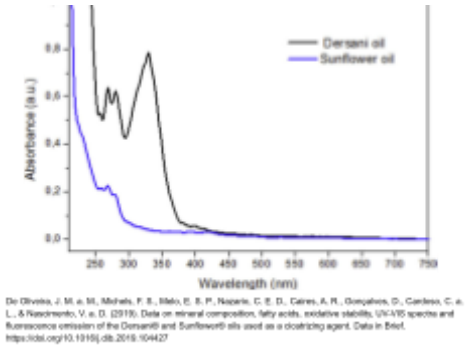


Figure #4 absorbance of ZnO in isopropanol



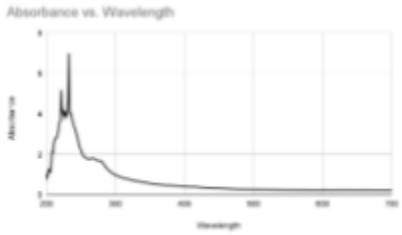


Figure #5 Egg lecithin liposome

Here, both the sunflower lecithin and the egg lecithin have strong absorbance peaks of just under 300 nm. Thus, this shows that sunflower lecithin is a valid substitute for egg lecithin. Once the ZnO nanoparticles are mixed with sunflower lecithin, the UV absorbance of the solution shows a slight peak in the region between 320 nm and 360 nm. However, there were a large number of extra peaks that saturated the area. The cause of the extra peaks could be impurities from the synthesis of ZnO nanoparticles or the sunflower lecithin powder. After centrifugation, the absorbance spectra became much more clear.

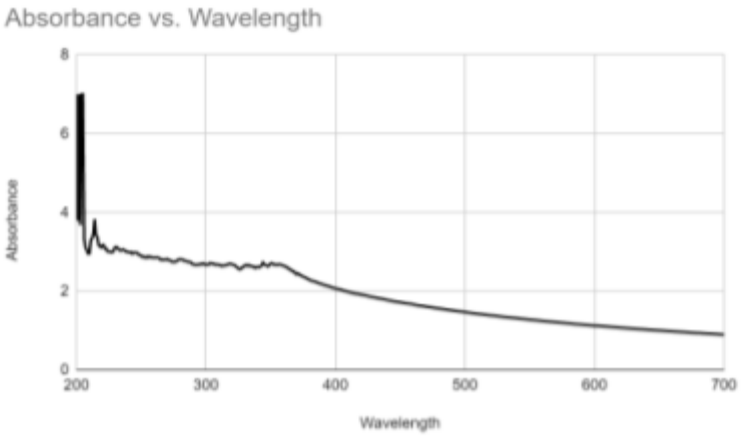


Figure #6a Absorbance of ZnO encased by liposome in water

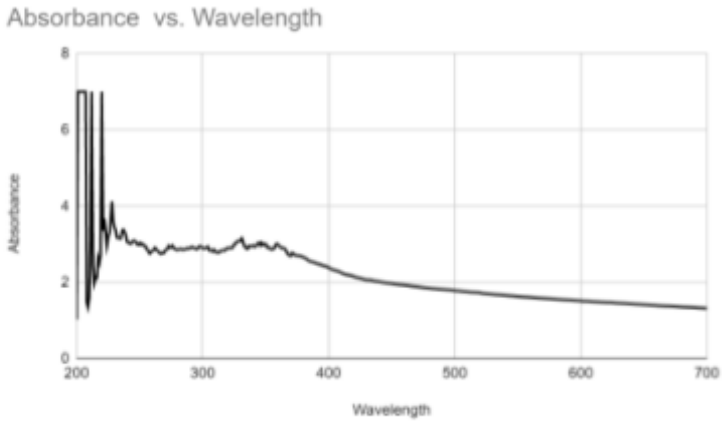


Figure #6b Absorbance of ZnO encased in liposome in original solution

Due to the centrifugation procedure and its ability to wash away impurities that are not the same density as the ZnO nanoparticles and their liposomes, the absorbance spectrum became much more distinct.

To assess the effectiveness of the ZnO nanoparticles and their sunflower lecithin liposomes, a control spectrum was created. This control spectrum was derived from a leaf that has not been injected with any treatment solution. However, since the leaf was crushed while hydrated with nanopore water, the subsequently extracted juice from the plant has the same condition as if water was injected into the leaf. Thus, this leaf acts as the perfect candidate to

investigate the effect that nanopore water has on the leaves through the direct mixing of crushed plant juices and the nanopure water that was used to achieve a concentration that was necessary for accurate UV absorbance measurements.

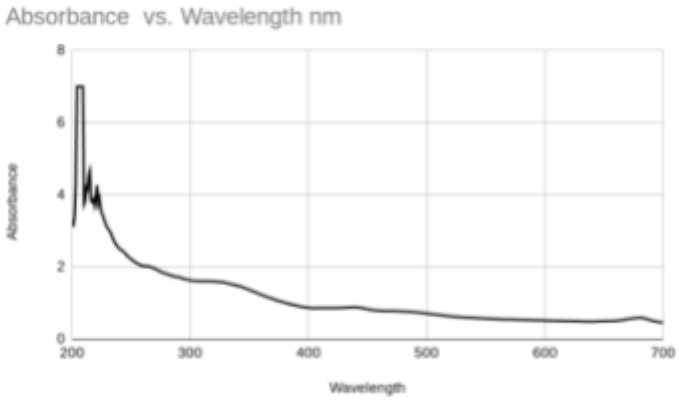
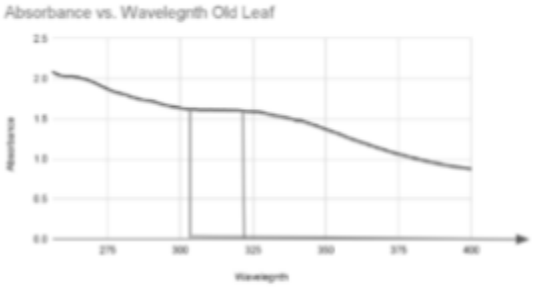


Figure #7 Absorbance of the old untreated leaves

Here, there is a slight peak in the regions between 300 nm and 350 nm. Since plants need some form of innate protection against the damaging effects of UV radiation, this slight peak can indicate the existence of innate UV absorbance within broccoli leaves. Upon a closer examination of the peak, where the region between 300 nm and 350 nm was focused upon, a comparison between peaks with the lowest absorbance value and the highest absorbance value reveals that the difference between them is 0.00081 in absorbance.



The highest peak occurred at 315 nm,1.60969 absorbance and lowest at 307 nm,1.60888 absorbance

Figure #8 This is the focused region of the old leaf used to examine difference in absorbance

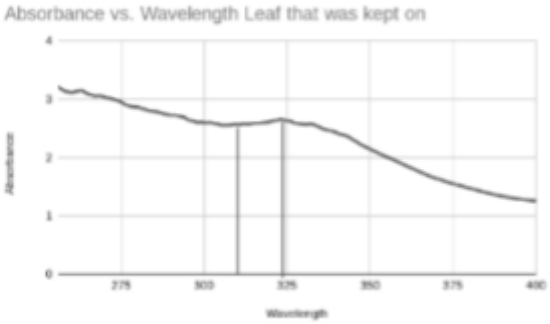


Figure #9 Focused absorbance of injected leaves

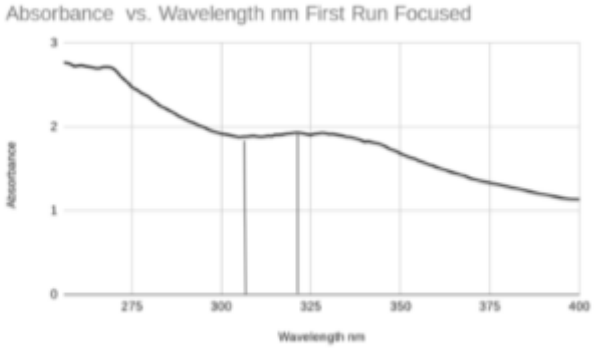


Figure #11 Absorbance spectra of injected leaves

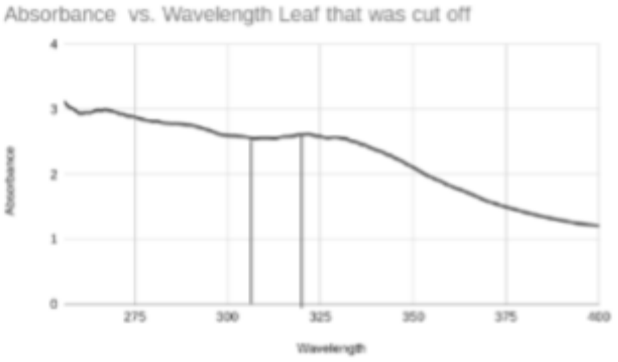


Figure #10 absorbance of injected leaves

The above figures indicate the ranges through which the differences in absorbance is measured. The lines indicate where the highest peaks were found and which wavelengths they correspond to.

	Leaf Without Zinc Oxide	Leaf & ZnO first run	Leaf & ZnO kept on	Leaf & ZnO cut off
Highest Peak	• 315 nm • 1.60969	• 321 nm • 1.9314	• 323 nm • 2.66228	• 322 nm • 2.62519
Lowest Peak	• 307 nm • 1.60888	• 305 nm • 1.87811	• 306 nm • 2.55679	• 307 nm • 2.55607
Differences in Absorbance	• 0.00081	• 0.05329	• 0.10549	• 0.06912

Figure #12 This compiles the difference in absorbance between the highest and lowest peaks for each tested leaf

Next, the three different absorbance measurements of leaves were conducted. To compare the peaks of these three leaves, the peak with the highest absorbance and the peak with the lowest

absorbance within the region of 320 nm to 360 nm were subtracted to find the difference in relative absorbance values. Between all four peak differences, the injected leaves have a larger difference in absorbance values than the leaf that was not injected. However, after the injections and the leaf comparisons, another leaf was used to make a control absorbance test where no injection of the ZnO liposome solution was made. In this sample, the leaf was greener and was therefore healthier than in the previous example. Through the same comparison methods, the difference between the highest peak and the lowest peak gave a value of 0.0424 in absorbance.

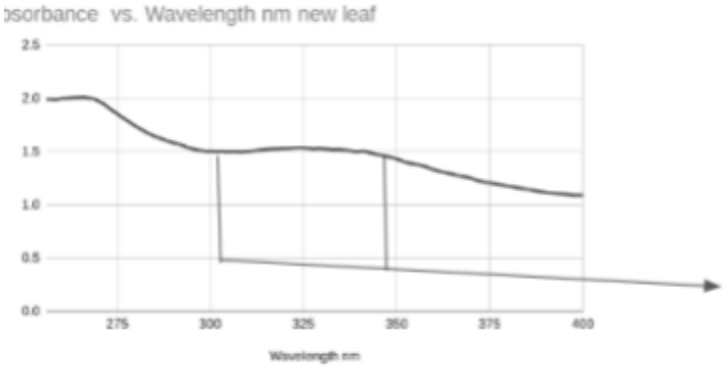


Figure #13 Highest peak absorbance-Lowest peak absorbance= 0.03773

Nonetheless, the peak difference is still the lowest when compared to the three leaves that received the injection. Thus, it can be concluded that the leaves that received ZnO nanoparticles and sunflower lecithin liposomes can absorb more UV light than leaves without treatment. To better demonstrate the effect of the ZnO nanoparticle treatment, the first control leaf and the subsequent leaves that received the nanoparticle injection were grouped into one graph. In this comparison, some of the leaf spectrums were too concentrated. The result of the difference in concentrations lead to a misalignment between the four different absorbance spectrums. However, this was remedied through observation of the innate absorbance qualities of plants. In plant cells, there exist two main types of chlorophylls, chlorophyll a and chlorophyll b (Guidi L. Tattini *et al., 2017*). When observing the literature values of both types of chlorophylls, the most

immediate link between the leaf spectra was revealed. In all of the spectra, from the control leaves to the leaves that received the injections, there always existed two peaks between 400 nm and 700 nm. The first peak appears around 678 nm and the second peak appears at around 438 nm. According to the literature spectra of both chlorophyll a and chlorophyll b, chlorophyll a has a peak right below 700 nm and another peak between 430 nm and 450 nm. Hence, the peaks between 400 nm and 700 nm that were found in all of the leaf absorbance spectrum measurements correspond to chlorophyll a. Choosing the control leaf as a basis for all of the other spectra, the absorbance spectra were all modified by multiplying a common absorbance factor based upon the lower absorbance peak of chlorophyll a that would align the shorter wavelength at 438 nm of chlorophyll a across all three of the injected leaves. For example, at 438 nm, the leaf that had not been injected with ZnO nanoparticles had an absorbance value of 1.19025. The absorbance value at 438 nm was then divided by the absorbance value at the same wavelength for all three other leaves. This achieved an absorbance factor that was used to multiply all other absorbance values at all wavelengths. Hence, achieving a normalized absorbance spectra that can better compare the peaks around 320 nm amongst all four leaves.

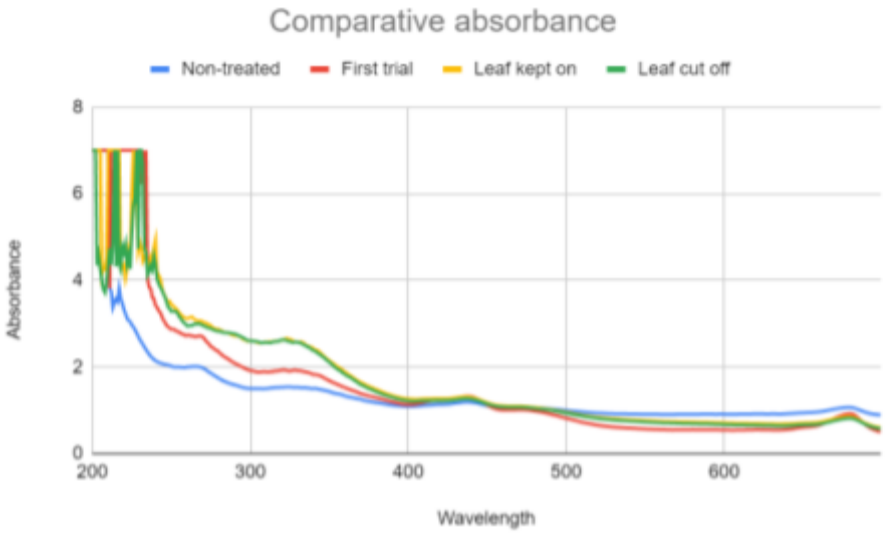


Figure #14 Combined absorbance spectra comparison

Once again, the graph shows a similar story to the difference in absorbance peaks displayed in the table. Compared to the absorbance spectra of the control leaf, all of the injected leaves display greater peaks within the expected regions.

Throughout all of the leaves that were tested, the highest peaks occur around 320 nm. The cause of this phenomenon could be a blue shift that occurred due to the interaction between the ZnO nanoparticles and the tissues around the plant. Since ZnO has a double bond that serves as the principal mechanism through which UV light can be absorbed, any alterations to the structure can cause the wavelength ranges to shift (Sirelkhatim, A., Mahmud, S., Seeni, A. *et al., year 2015*). Thus, it could be a possibility that the ZnO nanoparticles within some of the liposomes broke free from their carriers and interacted with compounds within the plants. In addition, the optical complexity of the plant leaves could have also contributed to the shift in wavelength absorbed. Nonetheless, the wavelength of UV light that was absorbed by the extracted leaf solutions falls within the ranges that ZnO nanoparticles can absorb. Thus, indicating that the nanoparticles played a role in absorbing some energy of UV lights that the

leaves were exposed to. After two weeks of growth, the experimental leaves with ZnO nanoparticles injected in them were taken off of their stems and crushed for absorbance testing. In the span of two weeks, the absorbance qualities due to ZnO nanoparticles have disappeared.

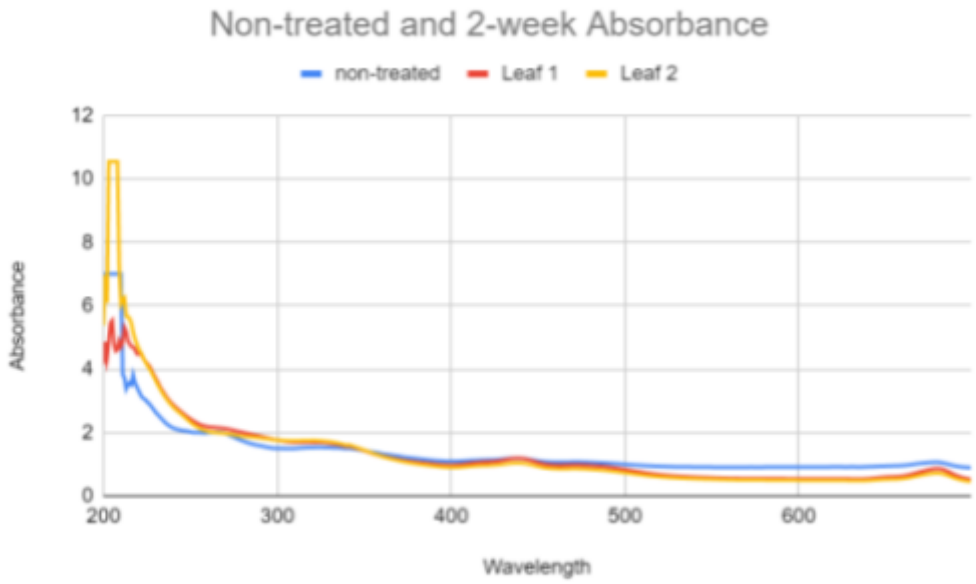


Figure #15 Combined absorbance spectra of injected leaves two weeks later

Regardless of the level of protection against UV rays that ZnO nanoparticles may offer, the absorbance effects of this compound can only be confirmed to last as long as 30 minutes after it is injected into plant leaves.

In the growth study that was also conducted, the amount of growth exhibited by the leaves was quantified by the percentage of growth beyond the starting length. From the starting length, each leaf was measured from the base of the petiole to the tip of the leaf. After two weeks of tracking the growth of the leaves, the data was collected and synthesized into a chart.

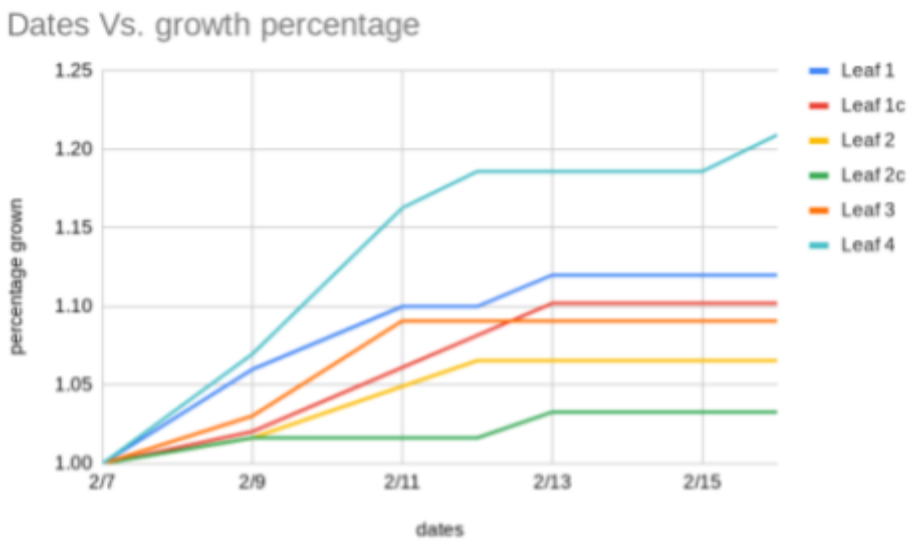


Figure #16 This chart describes the percentage of growth beyond the original length for all tested leaves

Based on the results shown, a Chi-square significance test with a degree of freedom of four was conducted on the leaves based on the growth of the injected leaves and the growth of the control leaves. With a P value of 0.4130, the data shown is not significant. Thus, it can be concluded that the treatment leaves did not exhibit any significant differences in growth rates as compared to the controls. The sample size in this study is very limited, hence, a larger sample size may indicate trends that the current study can not identify. Despite the shortcomings, two phenomena were observed throughout the growth test. First, the leaves ceased development after reaching a certain length. Hence, the age of the leaves and the intrinsic growth rates of each individual leaf may differ. The difference in growth rates based on the variation in the leaves themselves may be another factor that contributed to the result of the study. In addition to the pattern in growth rates, the study shows that both leaves 1 and 2 with ZnO had higher growth rates than the control leaves. Hence, it is possible that with more treatment groups that compare treated and untreated leaves of similar sizes, a clear difference in growth rates may surface.

Conclusion

The main scope of this study is to investigate the UV-absorbing qualities of leaves that have been injected with ZnO nanoparticles encased in liposomes. After synthesizing the appropriate solutions and injecting the ZnO nanoparticles into the leaves, it was found that the leaves exhibited an increase in UV absorbance qualities after injections. Although the injection procedures proved that ZnO nanoparticles within liposomes can contribute to an increase in plant leaf UV absorbance, expanded studies that look at the effect that the nanoparticles have on the leaves of other plants will provide more information on the feasibility of the methods in this study. In terms of the leaf growth rate study, the lack of significant findings is a result of the small sample size that the study used. In addition, the plants were subjected to normal levels of sunlight during winter. Since sunlight is the most intense during the summer, the protection offered by the ZnO nanoparticles may be more apparent under those circumstances. Overall, the study is very limited in terms of sample size as well as the methods used. In future experiments, it is possible to use a soilless solution as a growth medium and add ZnO nanoparticles that are encased in liposomes directly into the growth solution. Using the uptake of solutes via plant roots, more ZnO nanoparticles may be available inside the leaves for a stronger effect on the UV absorbance of these leaves. ZnO nanoparticles are compounds that are continuously being studied for their various properties. Based primarily on the UV absorbance quality of ZnO nanoparticles, there exist many opportunities to involve the compound and its UV-absorbing qualities to benefit plant nurseries and private plant growers. Utilizing the protection that ZnO nanoparticles give against the damaging effects of UV rays, this compound has the potential to become a useful tool for the cultivation of plants in environments saturated with intense sunlight.

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