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# Evaluating the Physiological Effect of Supplemental UV light on Malabar Spinach Grown Indoors

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## Abstract

The amount of usable farmland is decreasing worldwide as a result of an increasing global population as well as pollution. This means that we must search for ways to increase the efficiency of our agricultural systems. Industrial indoor farming has significantly augmented efforts to grow unique variants of crops and crops in places where the outdoor conditions do not permit natural field growth by growing crops with LED and fluorescent lights; however, this overlooks the possibility of applying UV light to indoor farming. The UV spectrum includes UVA ($\lambda$ = 315-400 nm), UVB ($\lambda$ = 280-315 nm), and UVC light ($\lambda$ = 100-280 nm). While the ozone layer blocks UVC light, UVA and UVB have been shown to have significant impacts on the development and morphology of various plant species. Red Malabar spinach (*Basella alba*) is an important crop in combating food insecurity due to its high nutritional value, high growth rate, and many harvestable parts. This research analyzed the effect of growing Malabar spinach under different UV radiation treatments in an indoor farming setting. Plants were grown for two trials in three chambers: white light (control), white light + UVA, and white light + UVA + UVB (sunlight conditions). For the second trial, the distance between the UV lights and plants was halved. Using an ANOVA and post-hoc Tukey test, statistically significant differences in plant height, leaf surface area, and leaf number were demonstrated between radiation treatments. The results of this experiment demonstrated that the UV treatments outperform the standard LEDs for the aforementioned characteristics with the UVA group showing the most growth. This study highlighted the stimulatory effect of UVA light on Malabar spinach growth and demonstrated the potential for incorporating UVA light into indoor agriculture systems to increase crop yields.

*Keywords: Ultraviolet, Malabar Spinach, Indoor Agriculture*

## 1. Introduction

Indoor agriculture has greatly advanced in the last 20 years in response to the decrease in usable farm land worldwide (Avgoustaki and Xyidis, 2020). The main advancements in indoor agriculture Have been centered in the central growth media such as water for hydroponics. In tandem, lighting technology has changed to improve the efficiency of LEDs, which are the most common in indoor agriculture, as well as to optimize the proper amount of light based on the targeted growth result. For these reasons, indoor growers continue to primarily use LEDs to supply light to crops. As a result, one area that has been largely overlooked is the application of ultraviolet (UV) light in indoor agriculture.

The UV spectrum is composed of UVA ($\lambda$ = 315-400 nm), UVB ($\lambda$ = 280-315 nm), and UVC light ($\lambda$ = 100-280 nm) light. UVC light, which is harmful to all living things, is absorbed by Earth's ozone layer and prevented from entering the atmosphere. On the other hand, UVA and UVB permeate the ozone layer and demonstrate significant morphological effects on Earth's plants (Neugart and Schreiner, 2018; Robson et al., 2014; Roeber et al., 2020). Most indoor grow lights, especially LEDs, either do not produce significant levels of UV light or have special UV filters for the UVA and UVB light they emit.

This experiment compares the effects of UVA and UVB light on notable crop characteristics such as plant height, leaf number, and leaf surface area of *Basella alba* (red Malabar spinach) grown indoors. Previous research indicates that increased UVB exposure can have inhibitory effects on the aforementioned characteristics whereas UVA can have stimulatory effects (Deckmyn et al., 1994; Jordan, 2002; Kataria et al., 2014; Nogués, 1998; Verdaguer, 2017; Wargent et al., 2009; Zu, 2010). Many of the studies that identify the specific pathways that are affected by UVA and UVB exposure were conducted on non-crop producing plants like *Dunaliella bardawil*, a type of algae (White, 2002). Additionally, there is little research on the impact of the specific effects of UVA and UVB light on leaf-bearing plants such as *Basella alba* (Malabar Spinach). As such, this study explores two novelties: applying UV light to indoor farming and the impacts of UVA and UVB light on Malabar spinach. By supplementing UV light in indoor growth chambers, this research hypothesizes that Malabar spinach exposed to UV radiation will demonstrate increased plant height, leaf surface area, and leaf number compared Malabar spinach exposed to only white light based on similar phenotypes observed in other plant systems.

## 2. Materials and Methods

### 2.1 Experimental Setup

This experiment used a growth chamber that was hand-built for this research in a garage. The construction began with the creation of a metal frame made using power conduits. Rods were run from end to end of the growth chamber and the lights were hung from these rods. This allowed for the lights to be adjusted horizontally and vertically as the plants grew to maintain the desired distance of the lights from the plants. Opaque black tarps were used to isolate each of the three chambers: white light (referred to as "control group"), white light + UVA (referred to as "UVA group"), and white light + UVA + UVB (referred to as "UVA + UVB group"). The tarps covered the entire growth chamber to create a separate internal environment.

A white light + UVB group was not tested as previous research has demonstrated that excessive UVB exposure to any plant can cause significant DNA damage (Frohenmeyer and Staiger, 2003; Jordan, 2002; Sharma, 2017; Strid, 1994; Suchar and Robberecht, 2015) and as such UVB was only used in tandem with UVA to mimic natural lighting conditions of outdoor farming. Additionally, Earth's atmosphere cannot prevent UVA light from passing through without some UVB as well. This makes white light + UVB an unrealistic group to test.

The control group had 10 pots while the UVA group and the UVA + UVB group both had 5 pots each (Figure 1). The fewer number of pots in the experimental groups was due to the power limitations of the UVA and UVB lights used which reduced the area that could be exposed while still maintaining significant light intensity at the desired distance.

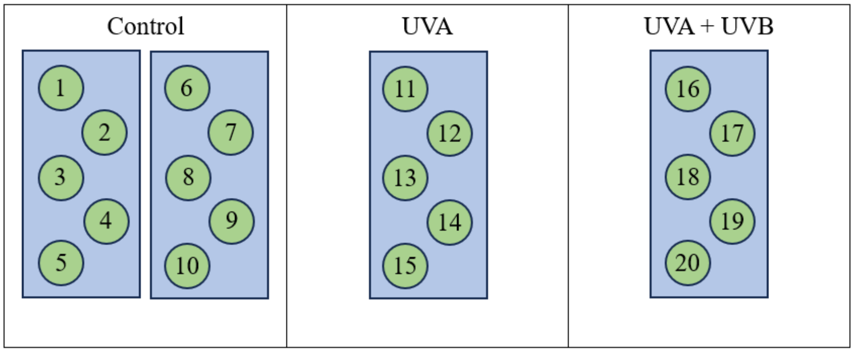

Figure 1: Experimental setup of the pots numbered by group: control group (pots 1-10), UVA group (pots 11-15), UVA + UVB group (pots 16-20).

Malabar spinach was used in this experiment because it is a heat tolerant, fast-growing plant with numerous harvestable parts and high potential as a super food. Typically, Malabar spinach is grown in humid regions in Asia near the equator. Malabar spinach is a fast-growing plant that can thrive in heat which made it an ideal choice for the temperature of the growth chamber which could reach ~29°C in the summer heat. Additionally, it is a highly nutritious plant which could greatly aid in the food crisis if its production could be augmented.

## 2.2 Data Collection

To begin trial 1 of this experiment, Malabar spinach seeds were germinated indoors for eight days (this duration was determined from a pilot experiment for optimal germination success) at room temperature (~21°C). The seeds were then planted in 14 cm in Miracle Grow Moisture Control Soil to allow enough room for sufficient root development. Additionally, to increase sample sizes, each pot had 3 seeds planted in it, and the seeds were arranged in a triangle spaced 5 cm from the center of the pot (Figure 2).

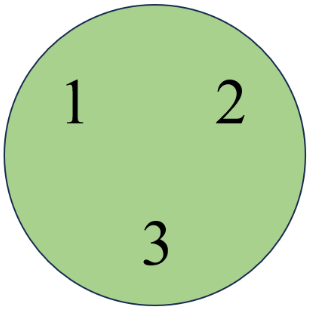

Figure 2: Arrangement of Malabar spinach seeds in each pot.

The seeds were given 12 mL of water daily and only exposed to white light that was 46 cm from the plant (1000 lumens per light strip x 2 strips per chamber with the optimal distance determined by the manufactures of the light) until the cotyledons (the embryonic leaves of the plant which help it begin growing) were grown. The exposure times were long day conditions consisting of 16 hours of light followed by 8 hours of darkness This was based on the optimal growing times for plants only receiving artificial light. After the cotyledons grew fully and the first set of true leaves began to emerge, the UV lights were turned on and followed the same 16 hours of light and 8 hours of no light. For the UV exposure in trial 1, UVA light was 25 cm away (~370 Lux) and UVB light was 15 cm away (~670 Lux). All lights were placed at the optimal distances described by the manufactures (Figure 3).

In trial 2, the plants were replanted in new soil of the same type and the germination, planting, watering, and data collection were the same as trial 1. However, the lights were moved closer to the plants: UVA was 13 cm away (~960 Lux), UVB was 8 cm away (~1410 Lux). The lights were placed slightly closer than the optimal distances determined by the manufactures. All white lights were still 46 cm away (1000 lumens per strip) (Figure 4). The lights were moved closer to determine if supplemental UV light at levels higher than the recommended amount could still increase overall plant growth as compared to plants not exposed to UV light. As such, the trial two plants were exposed to their respective UV lights at distances past their optimal levels. This was not for the purpose of creating two trials of data to be compared against each other, but rather to increase the number of samples to preform statistical analysis on.

Figure 3: Image of trial 1 light setup where UVA light is 25 cm and UVB light is 15 cm away.

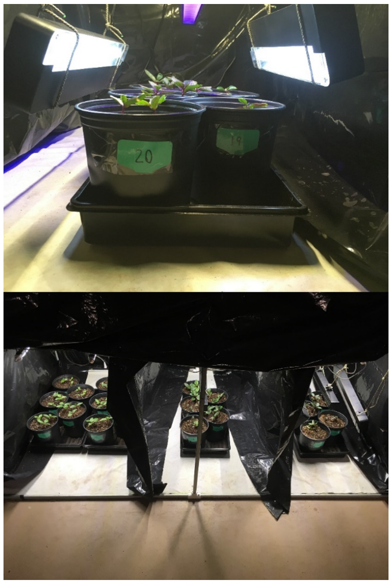

Figure 4: Image of trial 2 light setup where UVA light is 13 cm and UVB light is 8 cm away.

Measurements of plant height (cm), leaf number, and any abnormal features were documented every 6 days in both trials. This allowed for significant growth to occur in between data collection. During data collections, the height of the lights was adjusted accordingly to maintain the treatments predetermined distances. After the 5th data collection (30 days) in both trials, the largest leaf of the plant was removed and placed against a white background with a ruler for reference and overhead light to reduce shadows and a picture of the front and back of the leaf was taken to determine its surface area ($cm^2$).

### 2.3 Data Analysis & Evaluation

Throughout trial 2, because the growth chamber was in a garage, the temperature inside the chamber was subjected to variance from the outside temperature. This did not affect trial 1 significantly because the average temperature outside was ~29°C which resulted in the temperature of the growth chamber being ~23°C. During the second trial, the average outside temperature was ~15°C. An insulating material was added to the outside of the growth chamber which helped to maintain an internal temperature of ~20°C. The temperature was measured with a thermometer during data collection to ensure there was enough insulation to maintain an acceptable temperature. The average internal temperatures for both trials were within the optimal range for Malabar spinach growth (15-27 °C). To ascertain whether the change in temperature between the two trials impacted the distributions of each measured notable crop characteristic, the trial 1 values were plotted against the trial 2 values in a Quantile-Quantile (Q-Q) plot. These plots were used to measure the degree of similarity of the distributions of two datasets. Next, each Q-Q plot was compared with a linear regression to obtain an $R^2$ value between [0,1]. Higher $R^2$ values in this range indicated a better correlation (and in this case, a more similar distribution). If the distributions of the plant height, leaf surface area, and leaf number measurements were similar, the values from both trials could be synthesized for the following statistical analyses. The pictures of the biggest leaves from both trials were analyzed using the ImageJ software which examined the surface area of the leaves by using the number of pixels in a premeasured reference area in the picture. All leaf borders were digitally traced on the leaf pictures to eliminate errors in surface area measurements from shadows in some pictures. The final data from each trial for plant height, leaf surface area, and leaf number was analyzed in RStudio. This was done to report findings on the most significant effects that can be seen on the plants, which would be in the final measurements of each trial. These characteristics were analyzed first using an ANOVA summary followed by a TUKEY test. This yielded 3 *p*-values, one for each lighting group's comparison against the other. Each p-value was compared to a significance threshold of 0.05 for all statistical tests, meaning if the p value is greater than 0.05, then measured characteristic was not deemed to be significantly different.

## 3. Results

### 3.1 Data Synthesis Validation

The measurements of each notable crop characteristic from both trials were plotted in Q-Q plots and compared with a linear regression to obtain an $R^2$ value between [0,1]. $R^2$ values closer to 1 indicate that the data from both trials have similar distributions. The $R^2$ values for each characteristic are summarized in Table 1. As such the temperature was also not considered a restriction in combining the data between trials 1and 2.

Table 1: $R^2$ values of the comparisons of the distributions between trials 1 and 2 for each crop characteristic.

| **Crop Characteristic** | **$R^2$ value** |
|---|---|
| Plant Height (cm) | 0.951 |
| Leaf Surface Area ($cm^2$) | 0.896 |
| Leaf Number | 0.944 |

The $R^2$ values observed were 0.951 for plant height, 0.896 for leaf surface area, and 0.944 for leaf number. These values demonstrate that the distributions between the trials 1 and 2 data are highly similar and can be synthesized for the subsequent statistical analyses.

### 3.2 Notable Crop Characteristic Analysis

After synthesizing the data from both treatment groups, an analysis of variance (ANOVA) test was conducted. The following *p*-values were a result of the ANOVA test which examines all three treatment groups to test if any of them are significantly different – a significance code of 0.05 was used for the ANOVA test as well as all other statistical tests.

Table 2: P values of the ANOVA test for each crop characteristic.

| **Crop Characteristic** | ***p*-value** |
|---|---|
| Plant Height (cm) | 0.0309 |
| Leaf Surface Area ($cm^2$) | 2.5e-7 |
| Leaf Number | 2.11e-6 |

This experiment compiled all data in RStudio using Tukey HSD tests to determine significant differences between treatment groups. All measurements from both trials were analyzed to track the overall growth of the plants. When synthesizing data for analysis, the last measurements from each trial were taken.

Each notable crop characteristic was graphed using boxplots with a compact letter display to represent the results of the Tukey HSD test. Graphically, each boxplot has a letter assigned to it based on its statistical significance determined by the Tukey HSD test. The Tukey HSD test compared each treatment against each other to determine significant differences. The compact letter display uses this test and assigns a unique letter to each group that is statistically different from the others. If two groups share a letter, they are not statistically different. If a group has multiple letters, it is not statistically different from any other groups that share a letter with it.

Table 3: TUKEY Test results which show each crop characteristic's p value for the comparison of each treatment group against one another with significant p values being bolded.

| Measured Characteristic | Treatments Compared | *P Value* |
|---|---|---|
| Plant Height | **UVA - Control** | **0.023** |
| | (UVB + UVA) - Control | 0.812 |
| | (UVB + UVA) - UVA | 0.194 |
| Leaf Surface Area | **UVA - Control** | **1.00 e-7** |
| | (UVB + UVA) - Control | 0.084 |
| | **(UVB + UVA) - UVA** | **5.06 e-3** |
| Leaf Number | **UVA - Control** | **2.07 e -5** |
| | **(UVB + UVA) - Control** | **2.06 e-4** |
| | (UVB + UVA) - UVA | 0.907 |

The plant height measurements from both trials were graphed by lighting treatment using boxplots and the results of the Tukey HSD test are shown (Figure 5). The average plant heights observed for the UVA group, UVA + UVB group, and control group were 4.72 cm, 3.91 cm, and 3.81 cm, respectively. The UVA group generated the largest plant height on average. Based on the compact letter display, the UVA + UVB group is not statistically different from either the UVA group ($p = 0.194$) or control group ($p = 0.812$). However, the UVA group is statistically different from the control group ($p = 0.023$).

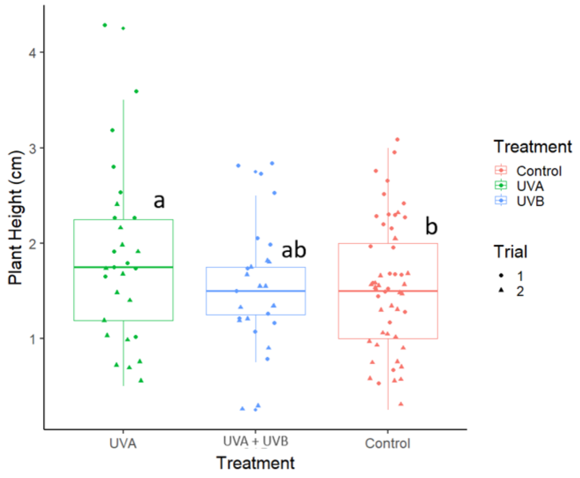

Figure 5. Plant height (cm) by treatment group graph with compact letter display to show significance results of Tukey HSD test.

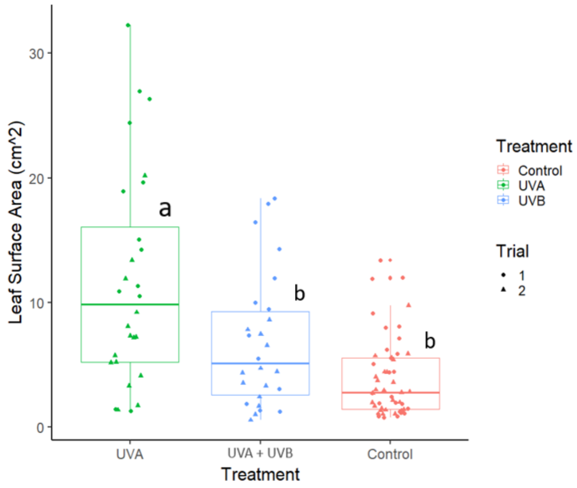

Figure 6. Leaf Surface Area ($cm^2$) by Treatment Group graph with compact letter display to show significance results of Tukey HSD test.

Similarly, the leaf surface area data were graphed with boxplots based on the lighting conditions and show the compact letter display (Figure 6). The average leaf surface areas observed for the UVA group, UVA + UVB group, and control group were 11.58 $cm^2$, 7.04 $cm^2$, and 3.86 $cm^2$, respectively. The UVA group induced the largest average surface area growth. Based on the compact letter display, the UVA + UVB group and control group are not statistically different from each other (*p* = 0.085). Although these two groups were not statistically different, the UVA + UVB yielded a higher average surface area. On the other hand, the UVA group is statistically different from both the UVA + UVB group (*p* = 5.06e-3) and the control group (*p* = 1e-7).

The leaf number values were also graphed by lighting conditions using boxplots with the results from the Tukey test shown (Figure 7). The average leaf numbers observed for the UVA group, UVA + UVB group, and control group were 5.10, 4.96, and 3.67, respectively. While the UVA group had the largest average leaf number, both UV groups notably outperformed the control group. Based on the compact letter display, the UVA group and UVA + UVB group are not statistically different from each other (*p* = 0.906), but both are statistically different from the control group (*p* = 2.11e-6 and *p* = 2.07e-5, respectively).

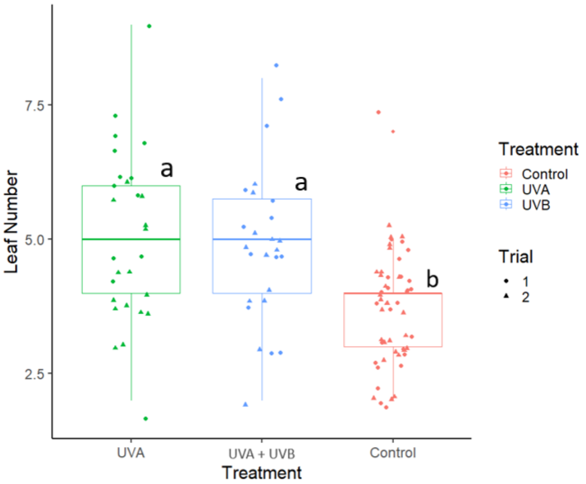

Figure 7. Leaf Number by Treatment Group graph with compact letter display to show significance results of Tukey HSD test.

## 4. Discussion

This research investigated the hypothesis that Malabar spinach exposed to supplemental UV radiation will have a greater plant height, leaf surface area, and leaf number than Malabar spinach exposed to only white light in indoor growth chambers. The degree of similarity between trial 1 and 2 as found using the Q-Q analysis reveals that the temperature was not a confounding variable for the Malabar spinach, furthermore that trials 1 and 2 were not significantly different which also means that exposure to UV light can still have significant effects on Malabar Spinach growth within the plant's viable temperature range.

For plant height, the UVA group outperformed the other lighting conditions on average but was only statistically different from the control group (*p* = 0.023). While the UVA group and UVA + UVB group were not statistically different (*p* = 0.194), the lower average plant height of the UVA + UVB group compared to the UVA group was likely associated with the previously discussed inhibitory effects of UVB light.

For leaf surface area, the UVA group had a higher average than both the UVA + UVB group and control group and was statistically different from both (*p* = 5.06e-3 and *p* = 1e-7, respectively). Additionally, the UVA + UVB group had a higher average value than the control group, but these groups were not statistically different (*p* = 0.085). These measurements show the improved growth of Malabar spinach leaves in indoor farming when exposed to supplemental UV light as opposed to just traditional LEDs. Again, the average of the UVA + UVB group being lower than the UVA group is likely linked to the previously mentioned inhibitory effects of UVB light and stimulatory effects of UVA light.

For leaf number, the UVA group and UVA + UVB group had higher averages than the control group. While the UV groups were not statistically different from each other (*p* = 0.906), they were both statistically different from the control group (*p* = 2.11e-6 and *p* = 2.07e-5, respectively). The leaf number data demonstrate the increased growth of Malabar spinach indoors when exposed to supplemental UV light compared to just LED light.

The results from this study demonstrate that growing Malabar spinach indoors with supplemental UV light yields increased plant height, leaf surface area, and leaf number compared to traditional LED lights. Specifically, the stimulatory effects of UVA light demonstrate an increase in all aforementioned crop characteristics. Albeit the UVA + UVB group performed lower on average than the UVA group in plant height and leaf surface area (with a statistical difference observed in the latter), no conclusions about the effects of UVB light specifically can be made since no white light + UVB group was tested.

### 4.1 Limitations

One limitation during this experiment was the intensity of the light sources used. The UVA and UVB lights used were near the lower ends of their respective UV ranges, so lights with wavelengths further into the range could yield more statistically significant results or, as seen in some plants, promote certain growth factors. Solutions to this are to use lights that are of higher intensity or to create an experimental setup that can incorporate more lights. Additionally, a problem that arose when conducting trials 1 and 2 was that the outside temperature began to decrease which resultingly lowered the temperature inside of the plant chambers. While this did not hinder the significance tests in this experiment, one way to overcome this is to run the experiment in an indoor environment that has temperature control or to conduct the study during the same season to ensure a constant average temperature.

### 4.2 Future Work

This research can be continued to identify the most efficient use of UVA light in indoor growth structures. Specifically, by testing different wavelength ranges of UVA light and measuring pigment concentrations to understand its effects on photosynthetic efficiency (Basahi et al., 2014; Grammatikopulos et al., 1994). Additionally, to maximize the potential applications of UVA light, it is necessary to further experiment on the optimal exposure times as well as UVA wavelength. Another avenue for future research is to measure terpene and flavonoid concentrations, both of which are a general label for plant molecules that influence our perception of taste and smell of a plant. The application of UV light can be used to affect terpene and flavonoid concentrations to adjust taste and scent for different market applications. Moreover, certain terpenes and flavonoids can be isolated for medicinal use such as cancer-combative medications (Ni et al., 2020; Ullah, 2020).

## 5. Conclusion

This experiment supports the hypothesis that exposing Malabar spinach to UV light indoors will increase plant height, leaf surface area, and leaf number compared to traditional LED light used in indoor farming. Specifically, this research demonstrates the stimulatory effects of UVA and not UVB light on Malabar spinach for the aforementioned characteristics in an indoor setting. Furthermore, it helps demonstrate the potential of using supplementary UV light in indoor agriculture to promote growth in crop relevant characteristics for Malabar spinach as well as other crops. Hopefully, this research can be used to improve indoor agricultural efficiency and help pave a path toward combating the global food crisis.

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