

## Effect on the Growth and Nutritional Components in Two Red Lettuces (*Lactuca sativa* L.) Cultivated Under UV Light in a Mini Plant Factory

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### Abstract

Lettuce is one of the most suitable plant species to grow indoor or in a Plant Factory with Artificial lighting (PFAL) system, due to its short height and the relatively low light saturation point. Red lettuces are highly cultivated in PFALs, highly appreciated for its unique pigmentation and antioxidant activity, especially, the anthocyanin content that gives it the red color. However, red lettuces cultivated with PFAL in controlled environments face the challenge of having low anthocyanin content due of the lack of ultraviolet (UV) light that is necessary for its production. In this study, two red-lettuce cultivars ‘Annapolis’ and ‘Salanova’ were grown in a plant factory with LEDs (light-emitting diode) and UV light treatments to compare their growth trajectory and nutritional characteristics. Other characteristics such as Fresh Weight (FW) and total leaf area were measured to evaluate the influence of UV light in the lettuce growth. The nutritional quality of lettuces was assessed by measuring anthocyanins and polyphenols contents using a microplate reader and their ascorbic acid content measured via a reflectometer. Fresh Weight (FW), leaf area and leaf thickness under the conditions of this study, showed no effect ( $p > 0.05$ ) on the plants growth with the incorporation of UV radiation. However, the incorporation of UV light increased the functional components in ‘Annapolis’ and ‘Salanova’ lettuces. The polyphenol and anthocyanins contents in both lettuce cultivars showed significant differences ( $p < 0.05$ ). For ascorbic acid content, a significant difference was found only for ‘Annapolis’ lettuce. The incorporation of UV light can increase the functional ingredients such as polyphenols and anthocyanins without growth suppression for ‘Salanova’ cultivar. UV light increased polyphenol and anthocyanins contents in both cultivars, making them suitable cultivars for PFAL under the growth conditions used in this experiment. These results taken together can be used to improve the accumulation of functional ingredients in red lettuces without growth suppression incorporating UV light and that could be suitable for production in PFALs.

**Keywords:** Red lettuce (*Lactuca sativa* L.); UV light; Leaf area; Anthocyanins; Polyphenols.



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### 1. Introduction

The term Plant Factory with Artificial Lighting (PFAL) is referred as an indoor, advanced and intensive form of hydroponic production system [1]. A PFAL consist of a thermally insulated and nearly airtight warehouse-like structured with shelves with multiple tiers and equipped with hydroponic culture beds and lightning devices, air conditioners and CO<sub>2</sub> and nutrient supply unit [1]. In a PFAL, parameters such as temperature, relative humidity, light, CO<sub>2</sub> and nutrient solution are controlled within predetermined target ranges [2]. The plants more suitable to be grown in PFALs are in general those having short height, such as leafy greens and medicinal plants. This due the fact that the distance between the tiers in the shelves allows maximum space usage [3].

Lettuce is one of the most suitable plant species to grow in PFALs because the light saturation point is relatively low. There are many different lettuce cultivars that range in color from green and yellow to deep red as a result of different concentrations of chlorophyll and anthocyanin in their leaves. The primary difference between red and green lettuce is the anthocyanin content [4]. Anthocyanins are water-soluble pigments belonging to the phenolic group and are responsible for the red, purple and blue color that is present in some fruits and vegetables [5]. Due to the unique color and powerful antioxidant activity, red lettuces are used as decoration for the table and consumed to fight free radicals in the body, preventing some diseases such as cancer, heart disease and other degenerative diseases [6].

Management of light quality is important to achieve both fast growth and high functionality of lettuces. To be profitable, lettuce growth rates should be accelerated to the maximum possible. But red lettuce cultivar, especially dark red cultivar, grows slower than green cultivars. Red light stimulates the lettuce growth by increasing fresh and dry weight and leaf area, but inhibits the anthocyanin accumulation [7, 8], and blue or UV decrease or inhibit the growth, but stimulate the anthocyanin and/or some pigments accumulation [9, 10].

One of the challenges faced in red lettuce cultivations in controlled environments is the incorporation of UV light that is necessary for stimulating anthocyanin production [11]. Literature reveals that when red lettuce

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cultivation is carried out in a PFAL, the amount of anthocyanins will be insufficient compared with an open field production. Supplemental irradiation with UV-B or UV-A light alone during the dark period increased the anthocyanin content in the leaves of red leaf lettuce, compared with those receiving no supplemental irradiation [12]. Most research on anthocyanins has focused on their photoinduction by wavelengths in the UV, visible and far-red regions. The light stimulus is supported by the dark inhibition of anthocyanin synthesis, although too much radiation in the UV-B region inhibits anthocyanin synthesis [13].

Currently in Panama, the development of Plant Factories with Artificial Lightning (PFAL) under controlled environments is on its infancy. However, a few commercial Plant Factories exist and indoor production is booming. To enhance future dissemination and construction of more PFALs it will be necessary to identify highly efficient vegetables for production in hydroponics, such as ‘Salanova’, a red lettuce cultivar with a potential to be produced in Panama. The aim of this work is to study and compare the growth trajectory and nutritional quality of two different red-lettuce cultivars (*Lactuca sativa* L. ‘Annapolis’ and ‘Salanova’) in a small plant factory with white LED and UV light treatments.

## 2. Materials and Methods

### 2.1. Plant Material, Growth Conditions and Experimental Design

Two differently red-pigmented lettuce cultivars, ‘Annapolis’ (Mikado Kyowa Seed Co. Ltd., Japan) and ‘Salanova’ (Rijk Zwaan B.V., Netherlands) were used as plant material. The experiment was conducted in a chamber-type plant factory system with double tiered vertical shelves inside it. Each tier was equipped with white LED (light-emitting diode) lights (G-40N1-50KC-T8, Toshin Electric Co. Ltd., Japan). The room was maintained at a temperature of  $21 \pm 2^\circ\text{C}$  and  $\text{CO}_2$  concentration at  $1200 \mu\text{mol mol}^{-1}$ .

The seeds of ‘Annapolis’ and ‘Salanova’ were sown into an urethane mat containing the nutrient solution Chibadai Saradana formula (N  $18.6 \text{ me}\cdot\text{L}^{-1}$ , P  $5.0 \text{ me}\cdot\text{L}^{-1}$ , K  $10.4 \text{ me}\cdot\text{L}^{-1}$ , Ca  $6.4 \text{ me}\cdot\text{L}^{-1}$ , Mg  $2.9 \text{ me}\cdot\text{L}^{-1}$ ; Japan Inst. of Japan Institution of Horticulture [14] and urethane mat was put inside a polystyrene tray that was covered with a lid and kept in dark for 3 days to induce germination. After germination, the tray was uncovered and the seedlings were grown for 12 days with white LED lights and hand watered every 2-3 days with the nutrient solution.

The seedlings were transplanted into panels of 26 pits and were grown hydroponically for 10 more days after the first transplant was made. The lettuces were fed with a recycling nutrient solution system (Chibadai Saradana with EC  $1.45 \text{ dS}\cdot\text{m}^{-1}$ ) using the Deep Flow Technique (DFT) in the shelves. The system refilled the nutrient solution and water required automatically. The photosynthetically photon flux density (PPFD) at the top of the canopy of the white LED lights (Fig. 1) was maintained at  $200 \mu\text{mol m}^{-2}\cdot\text{s}^{-1}$  during the 18-hour photoperiod.

Then, the seedlings from the center with the biggest leaves and roots were transplanted once again into panels of 6 pits for 16 more days. In the last 5 days before harvest, lettuce plants received an irradiance of  $0.3 \pm 0.2 \text{ W/m}^2$  of ultraviolet fluorescent light (G40T10E, Sankyo Denki Co. Ltd., Japan) (Fig. 2) for a total time of 6h/day and with an intermittent irradiation of 30-minutes from light period start, alongside with the white LED lights. Trays with ‘Annapolis’ and ‘Salanova’ lettuces were left with only white LED lights as controls. The lettuces were finally harvested after a time period of 40 days since the sowed. The experiment was done twice.

### 2.2. Post-Harvested Measurements

Characteristics such as leaf fresh weight (FW) and total leaf area were measured for ‘Annapolis’ and ‘Salanova’ cultivars growth with and without UV light after harvest. Lettuces were separated from roots and were weighted to determine the leaf fresh weight in a scale.

For total leaf area, all the leaves from the lettuces were separated one by one and put placed over a white cardboard with a black reference square ( $100 \text{ cm}^2$ ) and photos were taken from above. The total leaf areas were determined using the LIA 32 image processing software. Afterwards, the lettuce samples were immediately stored in plastic bags at  $-20^\circ\text{C}$  in an ultra-freezer to keep them later for further analysis.

### 2.3. Lettuce Samples

Lettuce leaves previously stored, were lyophilized (Freeze Dryer FDU-12AS, EYELA, Japan) at  $-50^\circ\text{C}$  for 48 hours, weighed for dry weight (DW) and blended in a food processor to determine the anthocyanins, polyphenols and ascorbic acid concentrations of ‘Annapolis’ and ‘Salanova’ lettuces.

## 2.4 Determination of Total Polyphenols and Anthocyanins

### 2.4.1. Anthocyanin and Polyphenols Extraction

Approximately 20.0 mg of dry lettuce samples were placed in small individual plastic tubes and 600  $\mu\text{L}$  of 1% hydrochloric acid in methanol were added for extraction of anthocyanins. For extraction of polyphenols, 600  $\mu\text{L}$  of 80% methanol in water were added for extraction. The samples were covered with aluminum foil and left inside a refrigerator for 24 hours and centrifuged at 16500 rpm for one minute to separate and take the supernatant.

### 2.4.2. Anthocyanins Determination

In a reading microplate, samples were load 10  $\mu\text{L}$  with 190  $\mu\text{L}$  of 1% hydrochloric acid-methanol and measured at an absorbance of 530 nm using a microplate reader (Skanlt 4.0 for Multiskan GO: Thermo Fisher Scientific Co., Ltd., Japan). Cyanidinium chloride-3-O-glucoside was used for quantification.

### 2.4.3. Polyphenols Determination

In other small individual tubes, were added 300  $\mu\text{L}$  of the supernatant prepared previously. Then, were added 300  $\mu\text{L}$  of Folin-Ciocalteu's 1:1 phenol reagent in water. Subsequently, were added 300  $\mu\text{L}$  of 10% sodium carbonate in water. The samples were kept for one hour in the refrigerator. In a reading microplate, samples were load 10  $\mu\text{L}$  with 190  $\mu\text{L}$  of distilled water and measured at an absorbance of 760 nm using a microplate reader (Skant 4.0 for Multiskan GO: Thermo Fisher Scientific Co., Ltd.). Chlorogenic Acid was used for quantification.

### 2.4.4. Quantification of Anthocyanins and Polyphenols

The software Microsoft Excel 2016 was used to determine the regression equation and the correlation coefficient for the quantification of anthocyanins and polyphenols. Results are expressed as micrograms of anthocyanins per 100 g of fresh weight and micrograms of polyphenols per 100 g of fresh weight.

### 2.5. Ascorbic Acid

Approximately 50.0 mg of dry lettuce samples were placed in small individual plastic tubes with 1 mL of 5% metaphosphoric acid in water. The readings of ascorbic acid were done in the reflectometer equipment (RQflex 10, Merck, Germany) and calibrated for this test. Results are expressed as micrograms of ascorbic acid per 100 g of fresh weight.

### 2.6. Statistical Analyses

All measurements were evaluated for significance by two-way analyses of variance (ANOVA) using the statistical program MS Excel (Microsoft Office 2016). Differences at  $P < 0.05$  were considered to be significant. The results are presented as mean values  $\pm$  standard error.

## 3. Results and Discussion

### 3.1. Effect of UV on Growth

A two-way ANOVA did not revealed a significant interaction effect between light treatments and lettuce cultivars on the fresh weight (FW), leaf area and leaf thickness. Both red lettuces did not showed significant differences with UV radiation in the fresh weight when harvested. Results suggest that fresh weight of 'Annapolis' lettuces grew less with UV light and that 'Salanova' lettuces with UV light did not showed a growth decrease on the FW.

Leaf area presented similar results as FW. 'Annapolis' lettuces showed a small decrease in the leaf area with UV light. But, 'Salanova' lettuces did not showed significant differences on the leaf areas with UV light.

Leaf thickness was calculated as the ratio Leaf area/FW. The small mean values of leaf thickness indicate that the area of unit weight is small, that is, leaves were thicker; therefore, 'Annapolis' lettuce was the cultivar with the thicker leaves compared to 'Salanova' lettuce. Between both cultivars, 'Salanova' showed higher FW and leaf area when compared to 'Annapolis'. Under the conditions used in this study, the UV radiation treatment showed no effect on the plants growth. The results of FW, leaf area and leaf thickness for both lettuces are shown in [Table 1](#).

**Table-1.** The results are expressed as an average value ( $n=8$ ) with standard error

| Lettuce Type | Treatment   | FW (g/plant)     | Leaf Area ( $\text{mm}^2$ ) | Leaf Thickness       |
|--------------|-------------|------------------|-----------------------------|----------------------|
| 'Annapolis'  | Control     | 50.52 $\pm$ 2.97 | 102,708.10 $\pm$ 6,881.57   | 2027.90 $\pm$ 47.15  |
|              | UV light    | 41.78 $\pm$ 3.04 | 83,057.95 $\pm$ 7,496.82    | 1984.34 $\pm$ 101.03 |
| 'Salanova'   | Control     | 55.67 $\pm$ 4.89 | 127,110.76 $\pm$ 7,141.68   | 2344.79 $\pm$ 119.24 |
|              | UV light    | 53.12 $\pm$ 3.42 | 124,995.14 $\pm$ 5,083.03   | 2384.37 $\pm$ 84.80  |
| Significance | Cultivar    | *                | ***                         | ***                  |
|              | UV          | NS               | NS                          | NS                   |
|              | Interaction | NS               | NS                          | NS                   |

NS, \* and \*\*\* indicate not significant or significant differences of  $P < 0.05$  and 0.001, respectively, by two-way ANOVA. There were not significant differences between control and UV treatments in both cultivars.

The inhibitory effect of red lettuces confirms the findings of [Krzek, et al. \[15\]](#) regarding the fresh weight and the leaf size in red lettuces. The growth inhibition of the red lettuces by the incorporation of the UV light by the end of the growing stage could be due to damage in the photosynthetic apparatus and by the high metabolic cost of phenolic compounds for UV protection as suggested by [Krzek, et al. \[15\]](#) and [Tsormpatsidis, et al. \[16\]](#). Another possibility, according to [García-Macías, et al. \[17\]](#) is that the biosynthesis of anthocyanins reduces the photosynthetic capability of the leaves or that the increased production of secondary compounds acts in direct competition for assimilated carbon to the plants' growth process.

### 3.2. Effect of UV Light in Nutritional Components

For both 'Annapolis' and 'Salanova' lettuces, significant differences were found for total polyphenols and Anthocyanin contents. 'Annapolis' showed an increment of polyphenol and anthocyanin contents with the treatment of white LED+UV light treatment. In the same way, 'Salanova' lettuces showed an increment in their contents of total polyphenols and anthocyanins.

For ascorbic acid, ‘Annapolis’ lettuces showed a higher content with the UV light treatment. However, for ‘Salanova’ lettuces, there was not difference between the two light treatments.

A two-way ANOVA revealed a significant interaction effect between light treatments and lettuce cultivars for the anthocyanin and ascorbic acid contents, but not for total polyphenol contents. The results of total polyphenols, anthocyanin and ascorbic acid contents for both lettuces are shown in Table 2.

**Table-2.** The results are expressed as an average value ( $n=5$ ) with standard error

| Lettuce Type | Treatment   | Polyphenol (mg·100 g <sup>-1</sup> FW) | Anthocyanin (mg·100 g <sup>-1</sup> FW) | Ascorbic acid (mg·100 g <sup>-1</sup> FW) |
|--------------|-------------|--|---|---|
| ‘Annapolis’  | Control     | 248.43 ± 35.68                         | 83.50 ± 2.15                            | 243.30 ± 14.02                            |
|              | UV light    | 405.42 ± 27.45                         | 205.93 ± 14.28                          | 323.10 ± 17.92                            |
| ‘Salanova’   | Control     | 73.64 ± 22.97                          | 22.65 ± 2.49                            | 185.51 ± 10.34                            |
|              | UV light    | 150.68 ± 22.62                         | 56.01 ± 4.19                            | 195.44 ± 15.55                            |
| Significance | Cultivar    | ***                                    | ***                                     | ***                                       |
|              | UV          | ***                                    | ***                                     | **  |
|              | Interaction | NS                                     | ***                                     | *   |

NS, \*, \*\*, and \*\*\* indicate not significant or significant differences of  $P < 0.05$ ,  $0.01$ , and  $0.001$ , respectively, by two-way ANOVA. There were significant differences in the accumulation of polyphenol, anthocyanin and ascorbic acid contents in both cultivars.

The main differences by the exposure of UV light were most clearly seen in the color of the leaves, due to the accumulation of anthocyanins in the leaves. Lettuce leaves were less red in the control treatment in the case of ‘Annapolis’ lettuces (Fig. 3) and in the case of ‘Salanova’, lettuce leaves were all green with control treatment (Fig. 4).

The results of this experiment suggest that the incorporation of UV light by near the harvest of lettuces, increase the functional components in ‘Annapolis’ and ‘Salanova’ lettuces. ‘Annapolis’ cultivar showed higher contents of total polyphenols, anthocyanins and ascorbic acid than ‘Salanova’, this is mainly due that ‘Annapolis’ is an originally high-content cultivar. The results in the accumulation of polyphenols, anthocyanins and ascorbic acid by the different light treatments are shown in figure 5 for ‘Annapolis’ and ‘Salanova’ cultivars.

These results are in agreement with the results obtained by Tsormpatsidis, *et al.* [16], and García-Macías, *et al.* [17] who found that anthocyanins and total polyphenols were with the highest content with the incorporation of UV light to lettuce cultivars. It has been reported that these compounds absorbs UV radiation and accumulate in leaves, keeping UV radiation from reaching photosynthetic tissues and cause damage Stapleton [10].

## 4. Conclusion

Both cultivars growth in optimum conditions with the conditions established in the PFAL used in this study. The better accumulation of functional ingredients and intensive red color in lettuces were with the incorporation of UV light.

‘Salanova’ cultivar showed higher FW and leaf area than ‘Annapolis’ cultivar. Additionally, a faster growth might develop a bigger leaf area and therefore, a bigger light capture area, a very important characteristic for PFAL. ‘Salanova’ cultivar with the incorporation of UV light can increase the functional ingredients such as polyphenols and anthocyanins without growth suppression, making it a good option to cultivate under PFAL as a high-functional vegetable.

UV light increased polyphenol and anthocyanins contents in both cultivars and the highest accumulation of functional ingredients were reported in ‘Annapolis’ cultivar. The ascorbic acid contents increased with UV light in ‘Annapolis’ lettuce, but not in ‘Salanova’. The high-content of functional ingredients in ‘Annapolis’ lettuce makes it an excellent option for its multiple health benefits. The contents of polyphenols and anthocyanins of lettuces can be control with UV strength and irradiation time in PFAL conditions. Both lettuce cultivars are suitable for cultivation in PFALs.

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Fig-1. Spectral distribution of the white LEDs lamps

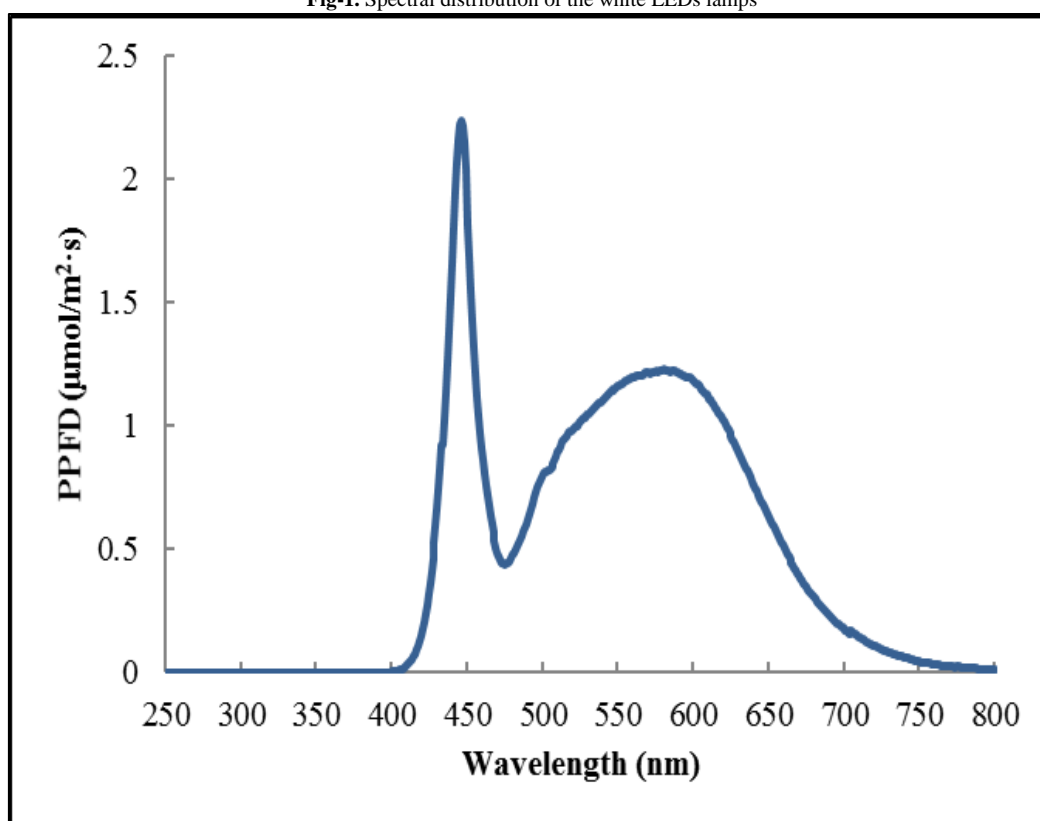


Fig. 2. Spectral distribution of UV fluorescent lamps

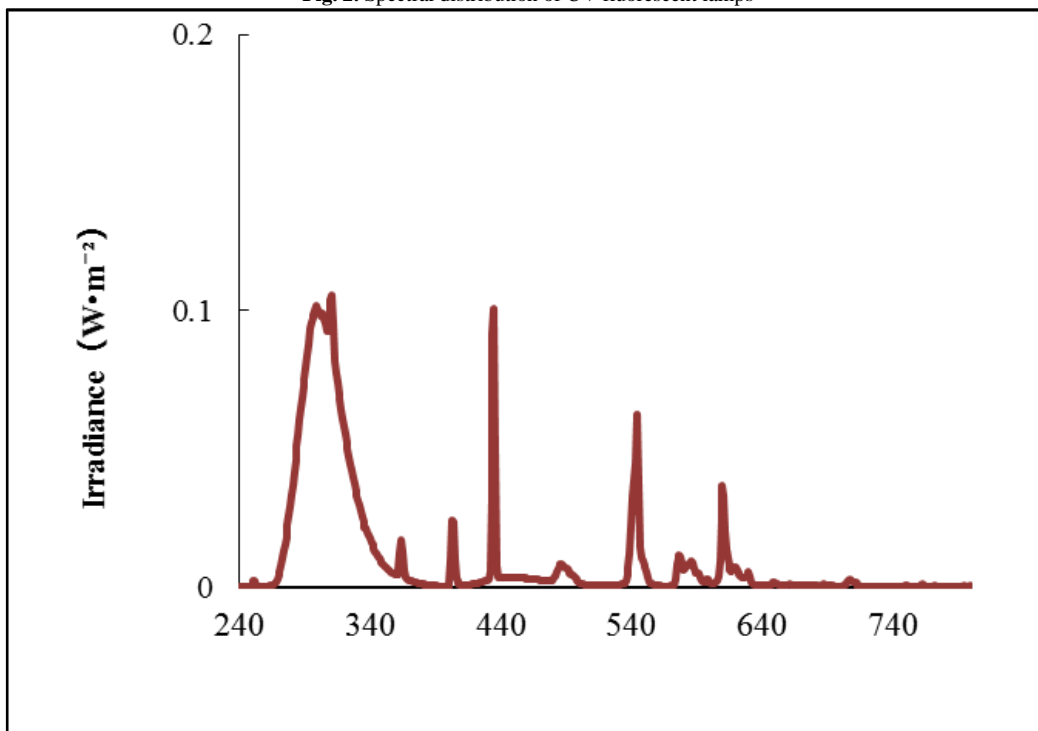
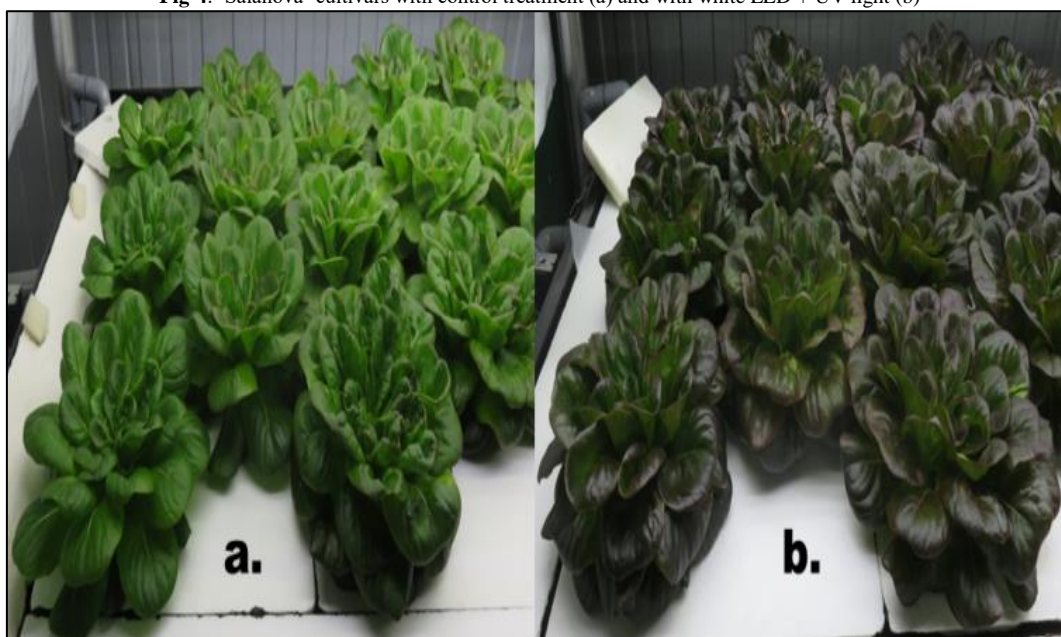


Fig-3. 'Annapolis' cultivars with control treatment (a) and with white LED + UV light (b)



Fig-4. 'Salanova' cultivars with control treatment (a) and with white LED + UV light (b)



**Fig-5.** Effect of UV light in the total polyphenol (A), anthocyanin (B) and ascorbic acid (C) contents in ‘Annapolis’ and ‘Salarova’ lettuces. The results are expressed as an average value (n=5) and vertical bars represent the standard error. Different letters on top of the bars indicate a significant difference between the light treatments (P<0.05)

